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Lethal and sublethal synergistic effects of a new systemic pesticide, flupyradifurone (Sivanto®), on honeybees

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The honeybee (*Apis mellifera* L.) is an important pollinator and a model for pesticide effects on insect pollinators. The effects of agricultural pesticides on honeybee health have therefore raised concern. Bees can be exposed to multiple pesticides that may interact synergistically, amplifying their side effects. Attention has focused on neonicotinoid pesticides, but flupyradifurone (FPF) is a novel butenolide insecticide that is also systemic and a nicotinic acetylcholine receptor (nAChR) agonist. We therefore tested the lethal and sublethal toxic effects of FPF over different seasons and worker types, and the interaction of FPF with a common SBI fungicide, propiconazole. We provide the first demonstration of adverse synergistic effects on bee survival and behaviour (poor coordination, hyperactivity, apathy) even at FPF field-realistic doses (worst-case scenarios). Pesticide effects were significantly influenced by worker type and season. Foragers were consistently more susceptible to the pesticides (4-fold greater effect) than in-hive bees, and both worker types were more strongly affected by FPF in summer as compared with spring. Because risk assessment (RA) requires relatively limited tests that only marginally address bee behaviour and do not consider the influence of bee age and season, our results raise concerns about the safety of approved pesticides, including FPF. We suggest that pesticide RA also test for common chemical mixture synergies on behaviour and survival.

1. Introduction

Pollinators provide ecosystem services that are crucial for crop production and wild plant biodiversity [1]. The honeybee is a major pollinator [2] whose global decline in health raises concerns about ecological impacts, including food security and human welfare [3]. Multiple studies have focused on honeybees because their general biochemistry and neurophysiology are better known than other pollinators and since bees can be used to model pesticide harm to other insect pollinators [4]. Pesticides are among the most important stressors affecting bee health [5] and pose heightened risks when bees are exposed to multiple pesticides for extended periods [6]. Attention has focused on the neonicotinoid pesticides [7], but their use has been progressively restricted because of their adverse effects on bees [8], and growing pesticide resistance [9]. New pesticides, such as flupyradifurone (FPF, Sivanto®, Bayer CropScience AG [10]), have therefore entered the market [9].

FPF is a newly developed systemic insecticide [11] that shares multiple similarities with the neonicotinoids. Its chemical structure partially overlaps with neonicotinoids, but FPF is a butenolide insecticide because of its different pharmacophore [12]. Nonetheless, they share the same target site (agonists of

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insects nAChRs, Insecticide Resistance Action Committee (IRAC) group 4), are both systemic [12] and control a wide variety of pests [12] on diverse crops through multiple application methods [10,12]. FPF metabolites include 6-chloronicotinic acid, which is also a metabolic by-product of most neonicotinoids [9], and can cause adverse oxidative stress in organisms such as freshwater amphipods and algae [13].

Because FPF is relatively new, fewer pest species are resistant to it as compared with the neonicotinoids [11,12,14]. FPF is also thought to have a favourable ecotoxicological safety profile [12] and is defined as relatively 'bee safe' [15]. Consequently, while neonicotinoids can only be used to treat crops in the absence of bee foraging, FPF can be used on flowering crops when bees are actively foraging.

Relatively few studies have investigated the impacts of FPF on bees. Hesselbach & Scheiner [16] showed that acute exposure to a high, non-field-realistic FPF dose (1.2 μg bee^{-1}) impaired bee taste and cognition. Tan *et al.* [17] demonstrated that chronic exposure to FPF impaired olfactory learning in larval (0.033 μg larvae d^{-1}) and adult (0.066 μg adult bee d^{-1}) Asian honeybees (*Apis cerana*) at field-realistic doses. Campbell *et al.* [18] tested the effects of FPF in a USA field study and observed no significant side effects on bee colony strength. This latter study, however, shows the difficulty of performing ecotoxicological field trials [19]: bee-collected nectar and pollen sampled from control fields were also contaminated with FPF.

Synergistic effects occur when combined exposure to two factors results in an effect that is significantly greater than the sum of individual effects. Such synergistic effects can occur between pesticide and poor nutrition, reducing bee survival, food consumption and energy levels [20]. Synergy can also arise between a pesticide and disease [21] or from exposure to multiple pesticides [22]. Chemical mixtures can have sublethal effects that do not immediately reduce survival [23,24]. For example, pesticide mixtures can synergistically alter behaviour such as mobility in aquatic organisms [25,26]. The combination of diseases (fungi) and pesticide (imidacloprid) can synergistically alter beetle movement [27]. However, there is, to date, no evidence that pesticides have significant synergistic effects on pollinator behaviour.

Neonicotinoids and SBI (sterol biosynthesis inhibitor) fungicides have synergistic effects on bees because SBI fungicides can inhibit detoxification [28]. FPF and the fungicide, tebuconazole, decreased FPF LD_{50} of in-hive bees by 6-fold [15]. The SBI fungicide propiconazole (PRO; chemical group: triazole; MoA code: G1, DeMethylation Inhibitors (DMI), SBI class 1; Fungicide Resistance Action Committee (FRAC) code: 3) is one of the most commonly used fungicides and is found in the environment and in bee food [29–32]. Both FPF and PRO are used on the same common crops [33]. As FPF is a relatively new pesticide, no monitoring studies have yet tested its co-occurrence as an environmental contaminant with other pesticides. However, FPF is approved for many of the same crops as the neonicotinoids [33], and neonicotinoids co-occur with SBI fungicides in bee pollen [6]. We therefore investigated the potential synergistic effects of two systemic pesticides, FPF and PRO.

We also examined the effects of seasonality. Although summer bees are typically more sensitive to pesticides than winter bees [34–37], some studies have reported different results [35,38]. Baines *et al.* [38] showed that early spring bees (March) were more susceptible to pesticides than summer bees. Decourtye *et al.* [35] found that exposure to a neonicotinoid pesticide (imidacloprid) reduces survival of winter bees, but reduced learning performances of summer bees. Seasons influence the floral and food resources available, thereby altering pesticide resistance [20], toxicokinetics and bee immunity [39]. Pesticide effects are also temperature dependent and alter thermoregulation [40], and season could alter bee detoxification abilities [39].

The effects of pesticides can be influenced by the age and body weight of an organism [39,41,42]. In-hive bees are typically heavier and more resistant to pesticides than foragers [43]. Bee responses to toxins also change as they age [44–48], and older bees may be more sensitive to pesticides [34]. However, to date, no studies have examined how the toxicity of FPF varies across worker type and season. We thus tested the effects of FPF over seasons and between worker types, assessing its interactive effects with a common SBI fungicide, PRO, on bee behaviour and survival.

2. Material and methods

We used six healthy honeybee colonies (*Apis mellifera ligustica* Spinola, 1806, located at the UCSD Biology Field Station apiary, La Jolla, USA), studied forager and in-hive honeybees, and followed standard collection and rearing methodologies [49]. To test the effect of season, we collected bees at two different colony developmental stages: early spring (February–March 2016) and summer (July 2016). We tested the synergistic and individual effects of FPF exposing bees to five acute oral doses of FPF or FPF + PRO. Based on current guidelines [50,51], we tested FPF doses (375 and 750 ng bee^{-1}) considered field-realistic, since bees can ingest higher FPF doses while foraging (see electronic supplementary material for the worst-case scenario estimations).

Following previous studies [22,28,52], we used a relatively high PRO dose that nonetheless, on its own, has no impact on bee survival (7000 ng bee^{-1} [22,52]). PRO is one of the most commonly used fungicides that contaminates bees and the environment [31,32]. Bees can be simultaneously exposed to FPF and PRO (or another SBI fungicide with similar mode of action) because they are used on the same crops and ornamentals, including fruits (e.g. citrus), oilseeds (e.g. soya bean, peanuts), cereals (e.g. corn, sorghum) [10,12,15,53–55], although guidelines state that flupyradifurone should not be directly tank-mixed with azole fungicides when applied to flowering crops [10]. These pesticides can be used multiple times over a year in the same crop (and over different seasons) and applied in multiple ways (i.e. aerial, chemigation or ground application). In addition, bees can also be exposed to pesticides that drift from different crops (i.e. buffer zones) or are stored in the same hive [56,57]. FPF and PRO are easily taken up by plants and thus contaminated soil and water may lead to unintended absorption. This can result in prolonged, multi-year contamination [56,58,59]. Bees can therefore be exposed to pesticide combinations that are contraindicated in tank mixes [6].

We tested a control dose (0 ng bee^{-1}), a total of six doses of FPF (375, 750, 1500, 3000, 6000, 12 000 ng bee^{-1}, respectively corresponding to 37.5, 75, 150, 300, 600, 1200 ppm), and five doses of the positive control dimethoate (DIM; 50, 100, 200, 400, 800 ng bee^{-1}, respectively corresponding to 5, 10, 20, 40, 80 ppm). In the combined FPF + PRO treatment, each FPF dose was tested in combination with a single sublethal dose of PRO (7000 ng bee^{-1}, corresponding to 700 ppm). We used technical grades of all active ingredients. The test solutions (sucrose 50% w/w, 100 μl cage^{-1}, 10 μl bee^{-1},) were provided inside each cage using an Eppendorf cap [60], contained acetone as a solvent (0.7%) and were completely consumed 60 min after oral administration [60].

We measured the effects of treatment on bee survival (1–48 h) and the frequency of bees exhibiting abnormal behaviours (1–4 h, see below).

Detailed methods are reported in the electronic supplementary material.

(a) Abnormal behaviours: synergistic and individual effects

We measured the percentage of bees exhibiting abnormal behaviours (i.e. the number of abnormally behaving bees per cage) across time (1, 2 and 4 h after treatment) depending on pesticide dose, season and worker type. We quantified the following behaviours: motion coordination deficits, hyperactivity, apathy, curved-down abdomen or moribund (electronic supplementary material, table S1) [4,15,51,60–62]. These abnormal behaviour categories are based on official ecotoxicological guidelines [60,63]. The unit of replication was the cage, and we observed each bee for 6 s (a maximum of 60 s for a cage with 10 bees). To improve the standardization and repeatability of our behavioural assessments, we refined the accuracy of the definitions of the behaviours of the official ecotoxicological guidelines [60,63] through videos (electronic supplementary material) and descriptions (electronic supplementary material, tables S1 and S2). We measured abnormal behaviours up to 4 h because high mortality at later time points severely reduced sample sizes in certain treatments and because behavioural abnormalities primarily occurred less than 4 h after treatment. The experimenters were blind to the treatments and were trained using standard descriptions (electronic supplementary material, tables S1 and S2) and videos (electronic supplementary material) of the behaviours. Before being allowed to score behaviours, experimenters needed to score standard videos with greater than 95% consistency.

(b) Statistical analysis

To test for synergy, we determined if the difference between the expected and the observed effects (either mortality or presence of abnormal behaviour) of the combined treatment could arise by chance alone or was larger than the simple additive effect of both pesticides.

We used the concentration addition (CA) reference model to define biologically significant synergy of chemical mixtures [64]. Based on each worker type LD_{50}, we calculated the model deviation ratio (MDR) to determine if the FPF + PRO interaction caused synergistic (MDR > 2), additive ($0.5 \leq MDR \leq 2$), or antagonistic (MDR < 0.5) effects [25]. To estimate the MDR, we calculated the toxic unit (TU) of each individual pesticide (FPF, PRO) and of the binary chemical mixture (FPF + PRO) [51].

We calculated the risk ratio (RR) and the risk difference (RD) to quantitatively express both relative (RR) and absolute (RD) size of the interactive effect of the chemical mixture on bee survival (frequency of dead bees; electronic supplementary material, table S3) and behaviour (frequency of abnormally behaving bees; electronic supplementary material, table S4) [65,66]. The RR was determined by dividing the observed effects by the expected effects and therefore cannot be calculated when the expected effect is 0 [65,66]. The RD is the difference between the ratio of observed and expected effects.

3. Results

(a) Pesticides synergistically increased mortality

The combination of FPF and PRO (FPF + PRO) synergistically increased mortality of both in-hive and forager bees (binomial proportion test, Holm correction; electronic supplementary material, table S3; figure 1*a*,*b*). The synergistic effect of FPF + PRO significantly reduced in-hive bee survival at 750 ng bee^{-1} (1–48 h after exposure, $RR_{Max} = 9$, $RD_{Max} = 44$; electronic supplementary material, table S3), 1500 ng bee^{-1} (1–48 h after exposure, $RR_{Max} = 5$, $RD_{Max} = 37$) and 3000 ng bee^{-1} of FPF (1 h after exposure, $RR_{1h} = 5$, $RD_{1h} = 23$). The synergistic effect of FPF + PRO significantly reduced forager survival at 750 ng bee^{-1} (1–48 h after exposure, $RR_{Max} = 5$, $RD_{Max} = 64$; electronic supplementary material, table S3), 1500 ng bee^{-1} (1 h after exposure, $RR_{Max} = 5$, $RD_{Max} = 27$) and 3000 ng bee^{-1} of FPF (1–2 h after exposure, $RR_{Max} = 11$, $RD_{Max} = 33$).

These synergistic effects were weaker at higher doses (electronic supplementary material, tables S3 and S4): FPF alone caused higher mortality at increasing doses, approaching the upper threshold of 100%, and thus reducing the difference between combined and individual treatments. Because synergy is better captured when the mortality of individual treatments is low, the synergistic effect was longer lasting for in-hive bees (i.e. 1–48 h at 1500 ng bee^{-1}), than for foragers (i.e. 1 h at 1500 ng bee^{-1}).

The LD_{50} of FPF + PRO ($TU_{FPF,summer\ in\text{-}hive} = 0.25$; $TU_{FPF,summer\ foragers} = 0.19$; $TU_{PRO} = 0.07$, $TU_{FPF+PRO,summer\ in\text{-}hive} = 0.32$, $TU_{FPF+PRO,\ summer\ foragers} = 0.26$) was also significantly lower than that of either compound alone for both in-hive (4-fold toxicity increase, MDR = 3.1) and foragers (5-fold toxicity increase, MDR = 3.9). Because the MDRs are higher than 2, the FPF + PRO interaction was synergistic for both worker types [64]. PRO alone did not cause any significant effect on survival ($\chi^2 = 0.6376$, d.f. = 1, $p > 0.42$).

The lower field-realistic FPF dose (375 ng FPF bee 1 h^{-1}) caused a 73% mortality in bees when combined with PRO. Thus, the FPF + PRO LD_{50} was lower than 375 ng FPF bee^{-1}.

There was a significant effect of FPF dose on bee survival (Fit proportional hazards, $p < 0.0001$; electronic supplementary material, table S5). Pesticide-free bees survived significantly longer than bees exposed to 1500 ng bee^{-1} (Kaplan–Meier^{DS}, $p < 0.049$; electronic supplementary material, table S6), 3000 ng bee^{-1} ($p < 0.0001$), 6000 ng bee^{-1} ($p < 0.0001$), 12 000 ng bee^{-1} ($p < 0.0001$) of FPF.

The FPF LD_{50} of our study (2995 ng bee^{-1}) is 2.5 times higher than the value reported by the US EPA (1200 ng bee^{-1}) [15] when we compare toxicity on standard individuals (in-hive summer bees [60], figure 2). However, we had a slightly different protocol than the US EPA [15]. We used 1 h exposure to 10 µl, not 6 h exposure *ad libitum*. Our positive control (DIM, reference toxin) met validity criteria because the 24 h LD_{50} of DIM was within the standard limits (0.10–0.35 µg bee^{-1}) defined by official international guidelines [60]. There was no significant effect of the colony on bee survival ($p > 0.05$; electronic supplementary material, table S5).

(b) Pesticides synergistically increased abnormal behaviours

FPF + PRO synergistically increased abnormal behaviours of both in-hive and forager bees (binomial proportion test, Holm correction; electronic supplementary material, table S4; figure 3). The synergistic effect of FPF + PRO significantly increased in-hive bee abnormal behaviours at the lower FPF doses of 750 ng bee^{-1} (1–4 h after exposure, $RR_{Max} = 10$, $RD_{Max} = 63$; electronic supplementary material, table S4), 1500 ng bee^{-1} (1–4 h after exposure, $RR_{Max} = 2$, $RD_{Max} = 33$)

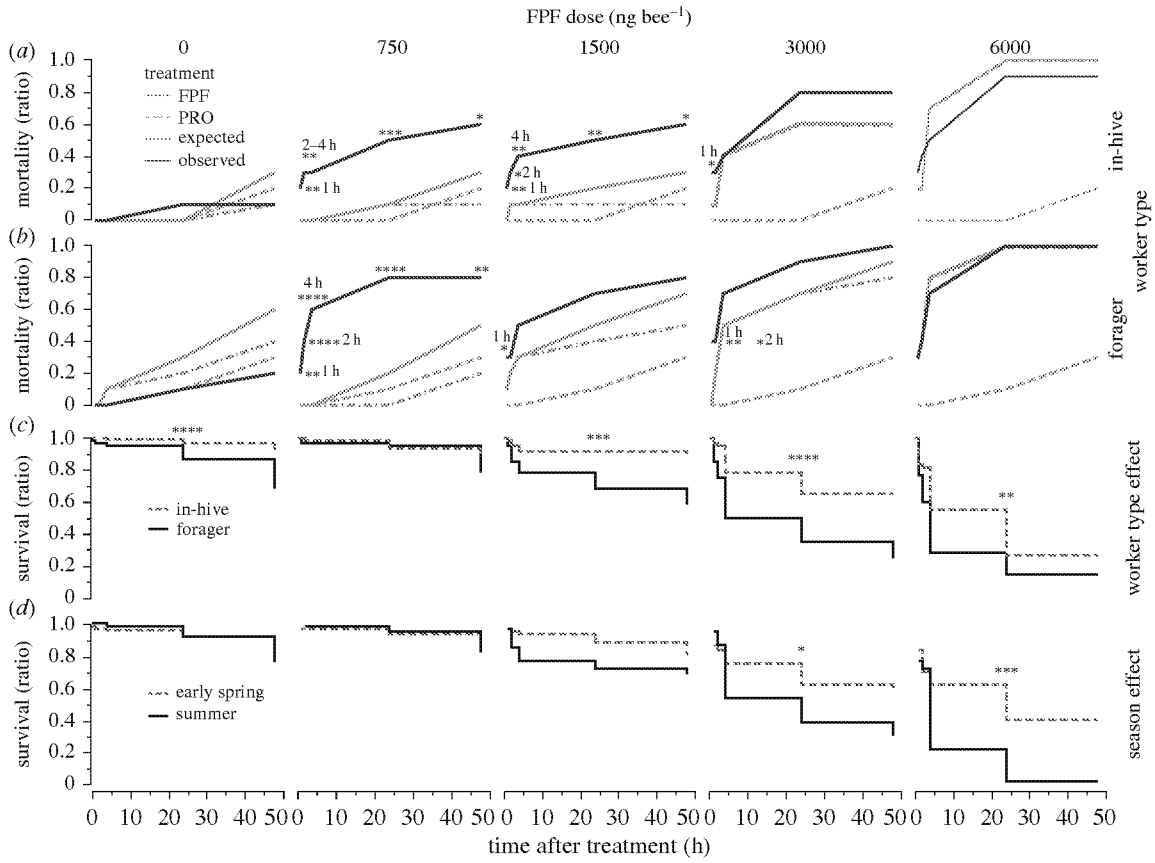

Figure 1. Lethal effects of FPF vary based on (*a,b*) combination with another pesticide (PRO), (*c*) bee worker type and (*d*) season. In (*a,b*), lethal synergistic effects of FPF + PRO on bee survival across time and worker type ((*a*) in-hive bees; (*b*) foragers). We tested the individual effects of FPF (blue dashed lines) and PRO (green dashed lines) and compared their expected (orange full lines) and observed (red full lines) combined effects, on bee mortality. In (*c,d*), we show the influence of (*c*) worker type and (*d*) season on bee sensitivity to FPF doses, assessed as survival across time. Asterisks indicate significant (*a,b*) synergistic (significant difference between mortality of expected and observed combined treatment; binomial proportion tests, Holm corrected; $n = 390$; electronic supplementary material, table S3) or (*c,d*) individual (Kaplan–Meier[DS]; $n = 1440$; electronic supplementary material, table S6) effects of FPF at specific time assessments (*$p = 0.05$, **$p = 0.01$, ***$p = 0.001$, ****$p = 0.0001$).

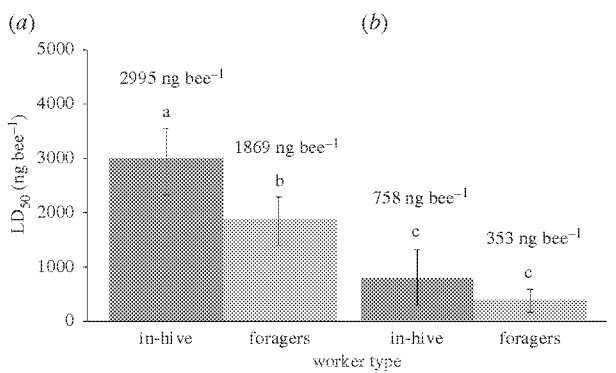

Figure 2. The LD_{50} (48 h) of bees exposed to FPF (*a*) and FPF + PRO (*b*) across worker types (in-hive bees versus foragers) in summer. Above each bar, we show the LD_{50} values. Different letters indicate significant differences. We show the 24 h LD_{50} of foragers (light grey bars), because high summer forager mortality at 48 h prevented the accurate estimation of their 48 h LD_{50} (standard LD_{50} estimation time, dark grey bars). Error bars represent 95% confidence intervals ($n_{overall} = 1080$). The LD_{50} of FPF across season and worker type is reported in electronic supplementary material, figure S1.

and 6000 ng bee^{-1} of FPF (4 h after exposure, $RR_{4h} = 2$, $RD_{4h} = 27$). The synergistic effect of FPF + PRO significantly increased forager abnormal behaviours at 750 ng bee^{-1} (1–4 h after exposure, $RR_{Max} = 15$, $RD_{Max} = 47$; electronic supplementary material, table S4), 1500 ng bee^{-1} (2–4 h after exposure, $RR_{Max} = 2$, $RD_{Max} = 30$) and 6000 ng bee^{-1} of FPF (1 h after exposure, $RR_{1h} = 2$, $RD_{1h} = 23$). PRO alone did not cause any significant abnormal behaviour. As with survival (see above), the synergistic effects on abnormal behaviours were more evident at lower doses.

The lower field-realistic dose of FPF (375 ng FPF bee 1 h^{-1}) significantly increased the number of bees exhibiting abnormal behaviours ($RR_{Max} = 36$, $RD_{Max} = 80$, $p < 0.0001$) when combined with PRO (electronic supplementary material, table S7; figure 3). These adverse behavioural effects started rapidly and remained consistent after treatment (RR $Range_{1-4h} = 34$–36, $RD_{1-4h} = 74$–80, $p < 0.0001$; table 1; electronic supplementary material, table S7).

There was a significant effect of dose 1, 2 and 4 h after treatment with FPF (Mixed $Model_{REML}$, $p < 0.0001$; electronic supplementary material, table S8; figure 3), DIM ($p < 0.0001$) and FPF + PRO ($p < 0.0001$). After treatment, bees showed coordination problems fairly consistent across time. Specifically, they mostly showed hyperactivity and curved-down abdomen in the shorter term after treatment (1 h) and apathy later on (4 h).

(c) Pesticides were more toxic to foragers than in-hive bees

There was a significant effect of worker type on the survival of bees exposed to FPF and DIM (fit proportional hazards,

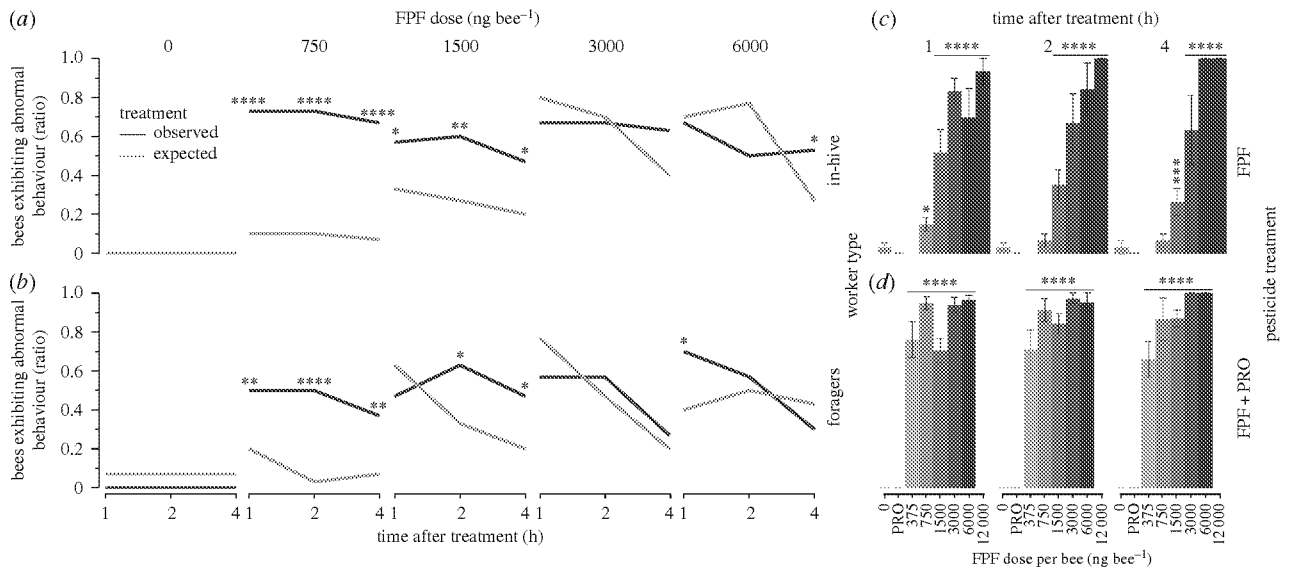

Figure 3. Sublethal (frequency of bees exhibiting abnormal behaviour) effects of FPF in combination with another pesticide (PRO). On the left (*a*,*b*), the synergistic effects of FPF and PRO across time (1–4 h after treatment) and worker type (in-hive bees: (*a*); foragers: (*b*)). We tested the individual effects of FPF and PRO and compared their expected (orange) and observed (red) combined effects (*a*,*b*, binomial proportion tests, Holm corrected; $n = 390$, electronic supplementary material, table S4). Because PRO alone did not alter bee behaviour alone, we only show the expected and observed results (*a*,*b*). On the right (*c*,*d*), sublethal effects of (*c*) FPF (750–12 000 ng FPF bee^{-1}) and (*d*) FPF + PRO (375–6000 ng FPF bee^{-1}, 7000 ng PRO bee^{-1}) as compared with each respective control (mixed model$_{REML}$, contrast test^{DS}, Dunn–Sidak corrected; $n = 342$; electronic supplementary material, tables S7 and S8). We show the effects observed in summer, pooled by worker type in (*c*,*d*). Asterisks indicate significant effects at specific time assessments (*$p = 0.05$, **$p = 0.01$, ****$p = 0.0001$).

Table 1. The frequency of bees exhibiting abnormal behaviours after exposure to the lower field-realistic FPF dose tested (375 ng FPF bee^{-1}), combined with PRO. We report the effect size as the risk ratio (RR, observed/expected) and the risk difference (RD, observed–expected), which compared the effects of FPF + PRO (375 ng FPF bee^{-1} and 7000 ng PRO bee^{-1}) versus either control (0 ng bee^{-1}) or the lower dose of FPF tested alone (750 ng FPF alone per bee). Combined exposure to FPF + PRO (375 ng FPF bee^{-1}) resulted in a higher frequency of bees exhibiting abnormal behaviours, even when compared with higher doses of FPF administered alone (750 ng bee^{-1}; $RR_{range} = 7–34$; $RD_{range} = 43–75$). Because of the low effect of FPF alone at 375 ng FPF bee^{-1}, we did not test the effects of this dose alone. We report 'n.a.' because the expected mortality was 0, and RR calculation is not possible when the denominator is 0.

				synergistic increase			
				versus control		versus FPF alone	
treatment	worker type	time after treatment (h)	bees exhibiting abnormal behaviour (%)	RR	RD	RR	RD
FPF + PRO (375 ng FPF bee^{-1})	in-hive	1	76	n.a.	76	34	66
		2	67	n.a.	67	30	56
		4	50	n.a.	50	22	43
	foragers	1	76	34	74	8	56
		2	74	33	72	7	71
		4	82	36	80	12	75

$p < 0.0001$; electronic supplementary material, table S5). Foragers are older than in-hive bees, and thus it is not surprising that in-hive control bees lived longer than control foragers (Kaplan–Meier^{DS}, $p = 0.0001$; electronic supplementary material, table S6; figure 1*c*). As the FPF dose increased, the survival of both bee castes decreased and, at each dose, the difference between the survival of each caste tended to increase (interaction FPF dose × worker type, $p = 0.001$; electronic supplementary material, table S5; figure 1*c*,*d*). FPF was significantly more toxic to foragers (compared with in-hive bees) at almost all doses tested: 1500 ng bee^{-1} (4-fold increase at 48 h, Kaplan–Meier^{DS}, $p = 0.0002$; electronic supplementary material, table S6), 3000 ng bee^{-1} (2-fold, $p < 0.0001$), 6000 ng bee^{-1} (1.2-fold, $p = 0.006$) of FPF. At 12 000 ng bee^{-1} of FPF, there was no significant effect ($p > 0.26$) of worker type on survival, because the mortality of in-hive and forager bees was very high.

The LD_{50} assessments confirmed that FPF and DIM toxicity was influenced by worker type (figure 2; electronic supplementary material, figure S2). Foragers were significantly more susceptible to pesticides in both early spring (FPF: 2-fold toxicity increase; DIM: 4-fold) and summer

(FPF: 2-fold; DIM: NS), as compared with in-hive bees. High forager mortality in summer prevented the estimation of the 48 h LD_{50} of summer foragers. Foragers were more affected by the synergistic effects caused by FPF + PRO (5-fold increased mortality), as compared with in-hive bees (4-fold).

There was a significant effect of worker type on bee abnormal behaviours 1 h after treatment with FPF (mixed $model_{REML}$, $p = 0.046$; electronic supplementary material, table S3), and 1 and 2 h after treatment with DIM ($p < 0.005$). There was no significant effect of worker type at any other time point ($p > 0.57$). There was no significant effect of worker type on abnormal behaviours after exposure to FPF + PRO ($p > 0.05$).

There was a significant effect of the interaction dose × worker type after treatment with FPF (1–2 h: $p < 0.049$), DIM (1–4 h: $p < 0.025$) and FPF + PRO (4 h: $p = 0.003$; electronic supplementary material, table S3; figure 3) on bee abnormal behaviours. Foragers were more susceptible to FPF (1500 ng bee^{-1}: $p < 0.0001$; electronic supplementary material, table S9) and FPF + PRO (375 ng bee^{-1}: $p < 0.006$; electronic supplementary material, table S9) as compared with in-hive bees.

(d) FPF was more toxic in summer

There was a significant effect of season on the survival of bees exposed to FPF (fit proportional hazards, $p < 0.0001$; electronic supplementary material, table S5). There was also a significant effect of the interaction FPF dose × season ($p = 0.011$). FPF was significantly more toxic in summer, as compared with early spring, at 3000 ng bee^{-1} (Kaplan–$Meier^{DS}$, $p = 0.013$; electronic supplementary material, table S6; figure 1*d*) and 6000 ng bee^{-1} ($p = 0.0003$). At 12 000 ng of FPF, there was no significant effect of season on survival, because bee mortality was too high in both seasons ($p > 0.78$).

The LD_{50} assessments confirmed that FPF toxicity was influenced by season (electronic supplementary material, figure S1). FPF was significantly more toxic in summer, both for in-hive and forager bees (2-fold toxicity increase), as compared with early spring.

There was a significant effect of season on bee abnormal behaviours 1 and 2 h after treatment with FPF (mixed $model_{REML}$, $p < 0.008$; electronic supplementary material, table S8). There was no significant effect of season at 4 h after treatment of FPF ($p > 0.51$). There was a significant effect of the interaction dose × season on bee abnormal behaviours after treatment with FPF (1 h: $p = 0.0004$; electronic supplementary material, table S8; figure S4). FPF significantly increased bee abnormal behaviours in summer, as compared with early spring, at 750 ng bee^{-1} (contrast $test^{DS}$, $p < 0.0001$; electronic supplementary material, table S9; figure S4), 1500 ng bee^{-1} ($p = 0.012$) and 3000 ng bee^{-1} ($p = 0.001$).

The electronic supplementary material contains additional results, including bee weight and DIM toxicity.

4. Discussion

We provide the first demonstration that the combination of two pesticides can synergistically increase the frequency of pollinators with abnormal behaviours (figure 3). We also provide the first evidence of adverse synergistic lethal ($MDR_{Max} = 4$; $RR_{Max} = 11$; $RD_{Max} = 64$) and sublethal ($RR_{Max} = 15$; $RD_{Max} = 63$) effects caused by FPF and an SBI fungicide, PRO (electronic supplementary material, tables S3 and S4; figures 1 and 3). All FPF doses tested significantly impaired bee behaviour as compared with the control treatment (electronic supplementary material, tables S7 and S8; figure 3). FPF can thus impair bee survival and behaviour at field-realistic (worst-case) doses when combined with an SBI fungicide. In addition, the toxic effect of FPF and FPF + PRO on bee survival and behaviour was significantly influenced by worker type and season. Foragers were consistently more susceptible to these pesticides (up to 4-fold; figures 1 and 3; electronic supplementary material, figures S2–S4). This result is troubling because the official guidelines for pesticide risk assessment (RA) only test in-hive bees, thereby underestimating the risk that pesticides pose for foragers. This is also concerning given that foragers are particularly at risk of pesticide exposure since they forage in the field. The lower weight of foragers (−11%), as compared with in-hive bees (electronic supplementary material, table S10), is a possible reason for their increased susceptibility to pesticides.

Our results confirm that abnormal behaviours usually appear shortly (1 h) after exposure to pesticides [40,67,68]. However, official RA guidelines do not require a thorough assessment of abnormal behaviours, and then only 4 h after treatment, when many effects may have declined. Short-term (1–2 h) behavioural alterations can be detrimental for bee health, especially for bees carrying out risky tasks (i.e. foraging) within this time window. We demonstrated that adverse sublethal effects are more frequent in foragers (as compared with in-hive bees) and could impact their foraging efficiency, as well as their survival, if these abnormal behaviours occur while bees are foraging in the field. Future behavioural assessments conducted using standard assays such as locomotion arenas could better establish potentially harmful effects of pesticides on beneficial insects [67,69–71].

Our results show that FPF and the neonicotinoids, both of which target insect nicotinic acetylcholine receptors [9], have relatively similar effects on bee health, sharing side-effects on bee survival and behaviour [67]. The SBI fungicide, PRO, similarly amplifies both FPF (4-fold, $MDR_{summer\ in\text{-}hive} = 3.1$, this study) and neonicotinoid (3-fold [22]) toxicity in bees. With respect to survival, FPF toxicity (based on LD_{50}) is over 559 times lower than the toxicity of the *N*-nitroguanidine neonicotinoids (clothianidin, imidacloprid, thiamethoxam), but more than five times higher than the *N*-cyanoamidine neonicotinoids (acetamiprid, thiacloprid) [33]. To assess the risk for bees, these differences need to be considered based on actual exposure, which depends on application methods (e.g. frequency of treatments and application rate) and active ingredient properties (e.g. toxicity).

The FPF + PRO synergistic effects on bee survival and behaviour are more evident at lower doses, where the effect of FPF is less detrimental. Our assessment suggests that FPF, like the neonicotinoids [40], may lead to a favourable biological response at low exposure levels (i.e. hormesis [72]). In foragers, the low dose of FPF (750 ng bee^{-1}) resulted in reduced mortality than the control treatment (0 ng bee^{-1}; figure 1*c*,*d*). Similarly, our observed synergistic behavioural alterations (figure 3) occurred at low (750–1500 ng bee^{-1}) and high (6000 ng bee^{-1}) doses, but not at intermediate ones (3000 ng bee^{-1}). These non-monotonic effects of pesticides (i.e. hormesis) should be further investigated.

Restrictions on neonicotinoids have been increasing after decades of research demonstrating their adverse effects on beneficial pollinators and persistent environmental contamination [20,58]. New systemic insecticides such as FPF and sulfoxaflor, examples of the novel butenolide and sulfoxamine chemical classes, are the likely successors of the neonicotinoids [73]. While sulfoxaflor has adverse effects on bumblebees [74], FPF is considered 'bee safe' and can be used on flowering crops with actively foraging bees [12,15]. Our study raises concerns about the ecotoxicological profile of FPF and the safety of FPF for bees.

Pesticide toxicity is amplified by multiple interacting stressors [5,20] and thus a holistic approach that tests more of the different conditions that bees naturally experience is beneficial [39,75]. Although sensitivity to pesticides is influenced by season, bee type and concomitant pesticide exposure [44,76], current RA schemes implement only limited tests, requiring only the evaluation of single pesticide effects on in-hive summer bees [77]. Such restricted testing could underestimate or overestimate pesticide effects [38,77] because different worker types (i.e. in-hive bees versus foragers) can be exposed to multiple pesticides over different seasons [6]. FPF (Sivanto) labelling restricts using tank-mixtures with azole fungicides [10]. However, bees can still be exposed to FPF and PRO simultaneously, as discussed above and in the electronic supplementary material.

Future RA should consider sublethal and behavioural effects [78,79]. Studies are necessary to confirm and link laboratory results to field tests, so that specific protection goals, such as avoiding unacceptable decreases in colony population or increases in forager mortality, can be assessed [78]. Monitoring the behaviour of multiple honeybees in a whole colony, though feasible in observation colonies [80], introduces complications [19] and costs that would be likely to constrain general adoption in RA. Testing bees in the laboratory also has the benefit of greater control, ease of testing and thus replication with more colony sources.

Because RA with honeybees is often used as an indicator of potential harm to other bees [50], our results raise broader concerns. However, there can be significant differences, greater and lesser, between the individual sensitivities of different bee species, highlighting the need for future research.

Although risk assessors are beginning to address synergistic effects on survival [79,81] and sublethal effects such as homing behaviour and hypopharyngeal development [51], many important synergistic and behavioural effects that can affect colony fitness are not explored. We propose that RA include more thorough assessments of behaviours and synergies. Behavioural testing should be implemented 1 or 2 h after exposure, since short-term behavioural effects can realistically impair bee survival when ingesting pesticides while foraging [82]. RA could address potential synergies on behaviour and survival by testing limited chemical mixtures of pesticides that have a higher likelihood of interactive effects based on the respective modes of action (e.g. propiconazole with neonicotinoids or FPF) and co-occur in the environment. We provide a simple way to measure synergistic effects on bee behaviour and survival following a standard ecotoxicological test. Our procedure could be implemented fairly easily in pesticide RA procedures, within the LD_{50} toxicity test scheme [60].

Data accessibility. Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.5f87k5v [83].

Authors' contributions. S.T. and J.C.N. designed and supervised the experiments, analysed the data and wrote the manuscript.

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Lethal and sublethal synergistic effects of a new systemic pesticide, flupyradifurone (Sivanto®) on honey bees

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ESM Methods

This study was conducted at University of California San Diego (UCSD), Division of Biological Sciences (La Jolla, CA, USA). We used six healthy honey bee colonies (*Apis mellifera ligustica* Spinola, 1806, 10 frames per colony [1–3]) housed at the UCSD Biology Field Station apiary. In total, we recorded the survival and abnormal behaviours of 1860 bees and the weight of 354 bees. All assessments were conducted by experimenters blind to the treatments. We followed standard toxicological procedures for pesticide tests on bees [4,5].

Honey bee preparation

Foragers are the bees most likely exposed to flupyradifurone (FPF) since they can directly collect pollen and nectar from treated crops. In-hive bees can also be exposed to FPF because they receive the nectar and pollen collected by the foragers and consume potentially contaminated honey and pollen stores. We captured individually returning pollen foragers at hive entrances in vials. We caught in-hive bees located in combs with brood inside the colonies using standard procedures: these bees were likely nurses [6]. The difference between in-hive and forager bees was additionally confirmed by our weight results (see Results). After collection, in-hive and forager bees were separately placed into plastic cages (11 x 11 x 9 cm, 10 bees/cage) and maintained in an incubator at 25 ± 1°C and 50-80% RH for 72 h [5]. To facilitate consumption of the test solution, the bees were starved for 1 h before feeding [5,6].

Pesticide concentrations and doses

FPF (4D IRAC subgroup) is a newly developed systemic insecticide [7] that was first marketed in 2014 in Guatemala and Honduras [8], then in 2015 in USA [9], EU [10], and other countries [11]. Because FPF is a relatively recent pesticide, there is limited environmental contamination data available [12,13]. Nonetheless, it can be used on diverse crops (vegetables, potatoes, pome fruits, grapes, citrus, cotton, soybean, coffee, cocoa, hops, and ornamentals) though multiple application methods (spray, drip irrigation, soil treatments, and seed treatments) [8,14].

Because foragers mainly consume nectar, we calculated the field-realistic exposure of foragers based on the residue of FPF found in nectar (a realistic carbohydrate source) collected

ED_002896_00039492-00011

by a forager, as sampled from its honey stomach [13]. In-hive bees consume nectar and pollen (realistic diet containing carbohydrates and proteins), and we therefore considered nectar and pollen contamination when estimating in-hive bee pesticide intake [15,16].

FPF was found at 4.3 ppm and 4.1 ppm in the nectar in the honey stomachs of bees that were foraging on oilseed rape crops respectively 1 and 3 days after FPF spray treatment (US EPA, 2014). Pollen collected by bees foraging on oilseed rape fields contained 21 ppm of FPF [13]. We simulated a worst-case scenario in which bees were foraging on oilseed rape crops, and therefore used FPF residues in nectar (4.3 ppm) and pollen (21 ppm) of oilseed rape. However, bees can be exposed to FPF at even higher concentrations, and for longer periods. Bees ingested FPF when collecting nectar from cotton (22 ppm) or pollen from apple (39 ppm) or blueberry (68 ppm) crops [13]. FPF was found for about 3 weeks in nectar collected by foragers, and up to nearly 5 months after initial exposure in the nectar and honey stored inside bee colonies [13].

With respect to dosages, we calculated the worst-case field-realistic FPF oral exposure levels for bees using European Food Safety Authority (EFSA) and Environmental Protection Agency (EPA) methods. Using these methods, we estimate that foragers collecting nectar in a field previously treated with FPF can be exposed to 550 ng FPF/bee per foraging flight, and up to 5504 ng FPF/bee per foraging day. These calculations were based on EFSA [16] and used the highest field-realistic FPF concentration found in the honey stomachs of bees that were foraging on oilseed rape crops, 4.3 ppm [13], and the sugar concentration of oilseed rape nectar (10% w/w [17,18]). Bees can be exposed to up to 1564 ng FPF/bee/foraging flight when they forage on nectar from cotton fields. These calculations were based on EFSA [16] and used the highest field-realistic FPF concentration found in the honey stomachs of bees that were foraging on cotton crops, 22 ppm [13], and a low field-realistic sugar concentration of cotton nectar (18%, [19]). Unlike foragers, nurses ingest less nectar and more pollen, leading to a field-realistic exposure of 2402 ng FPF/bee/day [16]. This calculation was based on EFSA guidelines [16] and considered intake of FPF contaminated pollen using the highest field-realistic empirical FPF concentration in oilseed rape pollen (21 ppm [13]). According to other calculations [15], the refined Estimated Environmental Concentration (EEC) of FPF is 970 ng/bee and 1256 ng/bee for nurses and foragers, respectively, when colonies forage in oilseed rape crops [13]. When bees forage nectar in cotton fields, refined EEC for workers reaches 6370 ng FPF/bee [13]. Thus, we used a FPF dose of 375 ng/bee that was lower than the field-realistic scenario in which bees

ED_002896_00039492-00012

ingested FPF contaminated oilseed nectar during a single foraging flight. The tested FPF dose of 750 ng/bee was less than the field-realistic scenario in which bees ingested contaminated oilseed nectar for 1 day or cotton nectar for a foraging flight. Because of the limited FPF residue data availability, these estimations were based on *ad hoc* trials performed for pesticide registration purposes, before product authorisation. Thus, the estimations of FPF field-realistic doses and concentrations should be updated with data collected monitoring real field use in multiple scenarios.

FPF and propiconazole (PRO) are used in a variety of vegetable, fruit, and ornamental plants that are visited by bees. Because FPF is a new pesticide, no monitoring studies have yet tested its co-occurrence as a contaminant with other pesticides. However, FPF and neonicotinoids are approved for use on many of the same crops [8,13,14,20–22], and pollen collected by bees contained both neonicotinoids and SBI (Sterol Biosynthesis Inhibitor) fungicides [23]. Further screening of environmental contamination following large-scale real-world use after widespread commercialization, and testing of lower and field-realistic doses of FPF and PRO, are crucial to appropriately assess real and frequent risks caused by the pesticides [24]. The US EPA [13] assessed the combined effect of FPF and the fungicide, tebuconazole, and found that the addition of tebuconazole (ratio 1:7.5, respectively) decreased FPF LD_{50} of in-hive bees by 6 fold (1200 ng FPF/bee vs. 200 ng FPF+tebuconazole/bee). Although risk assessors are developing models to predict multiple chemical interactions based on chemical characteristics, ultimately reducing the amount of laboratory trials needed to assess risk, the data available is still poor and ultimately require empirical validation [25,26].

The acute oral dose-response relationship (i.e. LD_{50} test) was evaluated using five doses in a geometric series, with a common ratio factor of 2 ($FPF_{LD50\ dose\ range}$: 750-12000 ng/bee) [5]. Because of the high mortality of the FPF+PRO treatments, we tested an additional lower dose of 375 ng FPF/bee (corresponding to 37.5 ppm), instead of the higher 12000 ng FPF/bee, for the combined treatment. In table 1, we compare FPF+PRO treatments that used 375 ng FPF/bee with a higher dose of FPF alone (750 ng/bee), but our estimate of synergism is likely conservative: as expected 750 ng FPF/bee led to stronger effects than 375 ng FPF/bee (data from preliminary test). Because of high summer mortality, we tested an additional lower dose of DIM (25 ng DIM/bee, corresponding to 25 ppm), instead of the higher 800 ng DIM/bee. The synergistic

ED_002896_00039492-00013

effects of FPF+PRO were only tested in the summer, since this season is the most standard one for testing toxicity [5].

We used analytical grade FPF (CAS# 951659-40-8, PESTANAL® analytical standard, Sigma-Aldrich, purity: 99.9%), DIM (CAS# 60-51-5, PESTANAL® analytical standard, Sigma-Aldrich, purity: 99.5%), and PRO (CAS# 60207-90-1, PESTANAL® analytical standard, Sigma-Aldrich, purity: 99.2%) to prepare stock solutions respectively containing 3 mg FPF/g double-distilled H_2O, 1 mg DIM/g double-distilled H_2O, and 100 mg PRO/g acetone [5]. All bees, including control bees, were fed the same amount of solvent (0.7%). The solutions were maintained at 4 °C inside a bottle completely wrapped in aluminum foil to avoid light degradation. The stock solutions were diluted with 1.8 M sucrose solution (corresponding to 50% w/w) to prepare the final solutions that were fed to the bees.

Survival: synergistic and individual effects

Bee mortality was assessed 1 h, 2 h, 4 h, and each 24 h after treatment, up to a maximum of 72 h. A bee was considered dead when was immobile and did not react to any stimulation [4]. The LD_{50} of FPF, DIM, and FPF+PRO were estimated at 48 h after exposure [5]. Because of the very low effect of FPF alone at 375 ng FPF/bee, we only tested this FPF dose in combination with PRO.

Weight assessment

The effects of a given pesticide dose may depend upon bee weight. We therefore measured the weight of 354 pesticide-free bees. Because the amount of food ingested could influence the body weight, the bees were fed the same type of food (50% w/w sucrose solution, *ad libitum*) and frozen at the same time before weighing.

Statistical analysis

We used Fit Proportional Hazards models to separately test the effects of FPF, DIM, or FPF+PRO doses, season (early spring vs. summer), worker type (in-hive vs. forager bees), colony, and all interactions on bee survival (table S5). Because the FPF+PRO treatment was only tested in summer, we used the same model, but without season. Significant effects were further

ED_002896_00039492-00014

analysed with Kaplan-Meier survival analyses (Wilcoxon Chi-square values) following visual data inspection.

Probit analysis [27] was used to estimate the LD_{50} of FPF, DIM, and FPF+PRO across season (early spring vs. summer) and worker type (in-hive vs. forager bees). Because the treatment FPF+PRO was only tested in summer, this treatment was not tested across seasons. Based on LD_{50} values, we defined biologically significant synergy as mixtures with minimum two-fold difference between observed and predicted effect concentrations using the Concentration Addition (CA) reference model [28]. We used the CA model because it is recommended for risk assessment purposes [29], and we used the two-fold difference limit to avoid false positives while focusing on synergistic effects of quantitative importance [28]. The ratio between predicted and observed effects of binary mixtures is defined as the Model Deviation Ratio (MDR) [30]. We used the MDR to define if the interaction of the chemical mixture FPF+PRO caused synergistic ($MDR > 2$), additive ($0.5 \leq MDR \leq 2$), or antagonistic ($MDR < 0.5$) effects [30]. The MDR was calculated using the Toxic Unit (TU) of the individual pesticides (FPF, PRO) and of the binary chemical mixture (FPF+PRO). The TU is defined as the ratio between the concentration of a mixture component and its toxicological acute (LD_{50}) endpoint [16]. Our TU calculations were based on the LD_{50} of FPF (our data, reported in the Results section; $LD_{50,\text{summer in-hive}} = 2995$ ng/bee, $LD_{50,\text{summer foragers}} = 1865$ ng/bee), PRO ($LD_{50} >$ 100000 ng/bee [31]), and FPF+PRO (our data, reported in the Results section; $LD_{50,\text{summer in-hive}} =$ 758 ng/bee, $LD_{50,\text{summer foragers}} = 353$ ng/bee). We calculated the TU of our chemical mixture (FPF+PRO) as the sum of the TUs of each individual chemical in the mixture.

We used a Mixed Model with a REML algorithm to test the effects of FPF or DIM doses, season (early spring vs. summer), worker type (in-hive vs. forager bees), and all interactions on the frequency of bees exhibiting at least one abnormal behaviour 1 h, 2 h, and 4 h after treatment (table S7-S8). Colony was included as a random factor. Because the FPF+PRO treatment was only tested in summer, we used the same model, but without season. We applied the square root-transformation on the frequency of bees exhibiting an abnormal behaviour. We determined the minimum dose that was significantly different from control using the Least-Square Means contrast test and visual data inspection.

We applied a binomial proportion model [32,33] to test for synergistic effects of FPF and PRO on bee survival (figure 1A-B) and behaviour (figure 3A-B). We used the additive effects

ED_002896_00039492-00015

model [34], in which synergism is defined as the combined effect of multiple stressors significantly exceeding the sum of effects elicited by individual stressors. The R scripts (p.adjust function) used are available in the electronic supplementary material (ESM), and focused on testing synergistic, not antagonistic, effects. We used a script modified from Tosi *et al.* [33] and Sgolastra *et al.* [32] that tested for synergistic effects by testing if the difference between the observed and the expected effect (either mortality or presence of abnormal behaviour) of the combined treatment could arise by chance alone or was larger than the simple additive effect of both stressors.

The 0 ng/bee dose treatment was the control for each pesticide. Treatment A consisted of bees exposed only to each specific dose of FPF, for a total of four doses (750, 1500, 3000, 6000 ng/bee). Treatment B consisted of bees only exposed to PRO (7000 ng/bee). Bees exposed to both FPF and PRO (FPF+PRO) were assigned to the combined treatment (AB). We calculated the expected effect proportion of the combined treatment as $P_{ABExp} = P_A + (1-P_A)\, P_B$, where P_A and P_B are the observed effect proportions in the FPF and PRO treatments, respectively. We used Wald confidence intervals to build a hypothesis test for the difference between two proportions. We separately determined the synergistic effects at each assessment time and used the Holm method to correct for multiple comparisons ($\alpha = 0.05$), as recommended by the R script protocol (ESM). We tested the effects across two worker types (in-hive vs. foragers) during summer. We calculated the Risk Ratio (RR) and the Risk Difference (RD) to quantitatively express the size of the interactive effect of the chemical mixture on bee survival (frequency of dead bees, table S3) and behaviour (frequency of abnormally behaving bees, table S4) [35,36]. The RR was determined by dividing the observed effect by the expected effects (i.e. dividing the cumulative incidence in the exposed group by the cumulative incidence in the unexposed group) and therefore cannot be calculated when the expected effect is 0 [35,36]. To estimate the effect size of the pesticide mixture at all time points after treatment, we calculated the RD, the difference between the ratio of observed and expected effects (i.e. subtracting the cumulative incidence in the unexposed group from the cumulative incidence in the exposed group).

A Mixed Model (REML algorithm) was used to test the effects of season (early spring vs. summer), worker type (in-hive vs. forager bees), and all interactions on bee weight (table S10). Colony was included as a random factor.

ED_002896_00039492-00016

Our statistical models were run with R v3.3.2 [37], JMP v10.0 (SAS Statistical Software), and Polo Plus v.2.0 (LeOra Software) software. We used residuals analysis to confirm that our data met parametric assumptions. We report mean ± 1 standard error (s.e.m.), and 95% Confidence Intervals for LD_{50} values [38]. We used an alpha value of 0.05. We applied the Dunn-Sidak method [39] to correct for multiple comparisons when appropriate, and indicated with ^{DS} the corrected statistical tests. We applied stepwise model simplification, building models with all interactions, and then removing them if they were not significant.

ESM Results

Our trials met the official guidelines for toxicity tests [5] because the 24 h LD_{50} of DIM was 114 ng/bee, within the required standard range of 100-350 ng/bee. Moreover, in-hive bees mortality of control treatments was low and within specified limits (≤10%) [5].

FPF doses up to 750 ng/bee caused little mortality (≤10%, not statistically different from control) when we applied the standard toxicological protocol (i.e. using in-hive bees), showing that FPF doses up to 750 ng/bee were sublethal [5].

DIM was more toxic in early spring

There was a significant effect of season on survival of bees exposed to DIM (Fit proportional hazards, $p < 0.0001$, tables S5-S6). DIM was significantly more toxic in early spring, as compared to summer. There were no significant interactions ($p > 0.13$). There was no significant seasonal effect of DIM LD_{50} (figure S2). There was a significant effect of the interaction dose × season or dose × season × worker type on bee abnormal behaviours after treatment with DIM (1-4 h: $p < 0.001$) (table S8).

There was a significant effect of season on bee abnormal behaviours 1 h and 2 h after treatment with DIM (Mixed $Model_{REML}$, $p < 0.0001$, table S8). There was no significant effect of season on bee behaviours at 4 h after treatment of DIM ($p > 0.62$).

Bee weight varied depending on worker type and season

There was a significant effect of worker type ($F_{1,351} = 44.66$, $p < 0.0001$) and season ($F_{1,350} = 5.58$, $p = 0.019$) on bee weight (table S10). In-hive bees were significantly heavier than

ED_002896_00039492-00017

foragers (+11%), and summer bees were significantly heavier than early spring bees (+5%). There was no significant effect of the interaction worker type × season on pesticide-free bee weight ($p > 0.59$). The effect of colony accounted for 1% of model variance.

ED_002896_00039492-00018

ESM Discussion

FPF and DIM showed opposite effects across season

Season consistently influenced pesticide toxicity (figures 2, 4, S2-S4), but its effect varied depending on the active ingredient. Bees were more susceptible to FPF in summer, while bees exposed to DIM were more affected in early spring (ESM). This variability is reflected in the results of prior studies, although we provide the first results showing how this variability can occur even when testing bees from the same apiary during the same seasons. Summer bees are typically more sensitive to pesticides as compared to winter bees [40–43]. However, the neonicotinoid imidacloprid reduced survival of winter bees, as compared to summer bees [41]. In early spring (first few weeks after overwintering), bees were more susceptible to the neonicotinoids, clothianidin and thiamethoxam, than summer bees [44]. The physiological modifications that bees experience across seasons, including variations in midgut structure (which can be a barrier to chemical transfer) and target receptor density, may account for some seasonal variability in pesticide toxicity [42,44]. Interestingly, the transition in bee sensitivity to pesticide seems to occur between early and late spring [44].

ED_002896_00039492-00019

ESM tables

Table S1. Definitions of abnormal behaviours exhibited by bees. We provide a video showing examples of abnormal behaviours in the ESM, and additional details are in table S2.

Name	Definition
Motion coordination deficits	Loss of coordination consisting of falling or stumbling while walking, walking in circles, walking and flying with erratic and irregular movements, and bees that flap their wings while upside down.
Hyperactivity	Excitation manifested as rapid walking, sometimes including short jumps and flight attempts, fast movements of legs and antennae.
Apathy	Hypoactivity consisting of remaining largely motionless or walking very slowly. Such bees also have severely reduced or delayed reactions to stimulation provided by light, movements of other bees, or air currents (e.g. generated by nearby bees).
Curved-down abdomen	The abdomen is unnaturally curved and is flexed ventrally, cramps.
Moribund	The bee appears close to death and exhibits partial paralysis with slight movements of legs and antennae. Will respond slightly to mechanical stimulation.

ED_002896_00039492-00020

Table S2. List of abnormal behaviours observed in the videos recorded during preliminary ecotoxicological trials to highlight and determine the types of common abnormal bee behaviours following pesticide consumption in sucrose solution. The video is available in the ESM (below) and Dryad Digital Repository, and further details are in table S1.

Video ID	Abnormal behaviour type	Description of abnormal behaviour(s)
1	Motion coordination deficits, hyperactivity	One bee shows hyperactivity, loss of coordination, stumbling, and erratic and irregular walking and flight movements.
2	Motion coordination deficits, hyperactivity	One bee is lying on the floor and shows loss of coordination with rapid twitching of legs and wings without flying for prolonged time.
3	Motion coordination deficits	One bee lies on the floor and shows rapid twitching of legs and wings without flying for prolonged time, loss of coordination.
4	Motion coordination deficits, hyperactivity	One bee shows hyperactivity, loss of coordination, stumbles, moves with erratic and irregular movements: atypical circular patterns.
5	Motion coordination deficits, hyperactivity	One bee lies on the floor and shows rapid twitching of legs and antennae, loss of coordination. The bees were recorded soon after exposure (acute oral) to the lower field-realistic dose of FPF (insecticide) and the sublethal dose of PRO (SBI fungicide; this fungicide dose alone caused no abnormal behaviours).
6	Hyperactivity	One bee (bottom, left) shows excitation and rapid movements of legs and antennae.
7	Apathy	Two hypoactive bees on the right side of the video.
8	Apathy	One hypoactive bee on the right side of the cage.
9	Curved-down abdomen, motion coordination deficits	One bee, standing next to the transparent cage door, showing a curved-down abdomen, and exhibiting irregular movements. Behind it, a bee rapidly twitches its legs and is unable to stand. On the left, moribund bees might seem dead but they exhibit partial paralysis and show slight movements of legs and antennae. This video shows a preliminary trial with bees exposed to the lower field-realistic dose of FPF and the sublethal dose of PRO (SBI fungicide) rapidly after acute oral treatment.
10	Moribund	One bee (close to cage front door) showing only slight movements of its legs and antennae, is unable to stand and appears close to death.

ED_002896_00039492-00021

Table S3. Synergistic effects of FPF+PRO on bee survival, depending on FPF dose, worker type, and time after exposure (1-48 h). We show the Risk Ratio (RR, observed/expected), the Risk Difference (RD, observed-expected), and the statistical results (binomial proportion test, Holm corrected, $N = 420$). We report "NA" when the expected or observed mortality was 0. In this R script analysis, we only test for synergism.

Worker type	FPF dose (ng/bee)	PRO dose (ng/bee)	RR after treatment (h)					RD after treatment (h)					*P*-value after treatment (h)				
			1	2	4	24	48	1	2	4	24	48	1	2	4	24	48
In-hive	0	7000	NA	NA	NA	3.0	0.4	0	0	0	7	-15	1.000	1.000	1.000	0.741	1.000
	750		NA	8.0	9.0	5.5	2.0	23	23	27	44	29	**0.005**	**0.007**	**0.005**	**<0.001**	**0.013**
	1500		NA	4.5	3.3	3.3	2.0	23	23	30	37	29	**0.005**	**0.014**	**0.009**	**0.003**	**0.014**
	3000		4.5	3.0	0.9	1.3	1.3	23	20	-3	19	16	**0.036**	0.091	0.605	0.206	0.253
	6000		1.7	2.2	0.7	0.9	0.9	13	23	-20	-10	-7	0.475	0.112	1.000	1.000	1.000
Forager	0	7000	NA	NA	NA	0.2	0.3	0	0	-7	-27	-46	1.000	1.000	1.000	1.000	1.000
	750		NA	NA	NA	4.9	1.7	20	40	57	64	34	**0.006**	**<0.001**	**<0.001**	**<0.001**	**0.006**
	1500		5.0	1.4	1.5	1.4	1.2	27	10	17	19	13	**0.015**	0.387	0.351	0.351	0.387
	3000		11.0	2.6	1.4	1.3	1.1	33	27	20	22	11	**0.001**	**0.037**	0.107	0.107	0.202
	6000		1.0	1.2	0.8	1.0	1.0	0	7	-13	0	0	1.000	1.000	1.000	1.000	1.000

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Table S4. Synergistic effects of FPF+PRO on the frequency of abnormal behaviours, depending on FPF dose, worker type, and time after exposure (1-4 h). We show the Risk Ratio (RR, observed/expected), the Risk Difference (RD, observed-expected), and the statistical results (binomial proportion test, Holm corrected, $N = 420$). We report "NA" when the expected or observed mortality was 0.

Worker type	FPF dose (ng/bee)	PRO dose (ng/bee)	RR after treatment (h)			RD after treatment (h)			*P*-value after treatment (h)		
			1	2	4	1	2	4	1	2	4
In-hive	0	7000	NA	NA	NA	0	0	0	1.000	1.000	1.000
	750		7.3	7.3	10.0	63	63	60	**<0.001**	**<0.001**	**<0.001**
	1500		1.7	2.3	2.3	23	33	27	**0.031**	**0.009**	**0.022**
	3000		0.8	1.0	1.6	-13	-3	23	1.000	1.000	0.094
	6000		1.0	0.7	2.0	-3	-27	27	1.000	1.000	**0.043**
Foragers	0	7000	NA	NA	NA	-7	-7	-7	1.000	1.000	1.000
	750		2.5	15.0	5.5	30	47	30	**0.005**	**<0.001**	**0.002**
	1500		0.7	1.9	2.3	-17	30	27	0.906	**0.022**	**0.022**
	3000		0.7	1.2	1.3	-20	10	7	0.954	0.654	0.654
	6000		1.8	1.1	0.7	30	7	-13	**0.021**	0.604	0.860

ED_002896_00039492-00023

Table S5. Effect of dose, season, worker type, and all interactions on bee survival after exposure to FPF (DF_{Model} = 20), FPF+PRO (DF_{Model} = 8), or DIM (DF_{Model} = 10) (Fit proportional hazard). We report in bold the significant effects. For each factor, we report the statistical values of the last stepwise model simplification that included the factor.

Active ingredient	Factor	DF	L-R χ^2	*P*-value
FPF	Dose	5	297.74	**<0.0001**
	Season	1	29.11	**<0.0001**
	Worker type	1	49.60	**<0.0001**
	Dose × Season	5	14.98	**0.0105**
	Dose × Worker type	5	21.85	**0.0006**
	Season × Worker type	1	17.40	**<0.0001**
	Dose × Season × Worker type	5	4.90	0.4277
	Colony	2	5.86	0.0535
DIM	Dose	6	300.10	**<0.0001**
	Season	1	17.54	**<0.0001**
	Worker type	1	37.89	**<0.0001**
	Dose × Season	4	3.82	0.4306
	Dose × Worker type	6	5.76	0.4511
	Season × Worker type	1	2.25	0.1332
	Dose × Season × Worker type	5	2.69	0.6112
	Colony	2	0.22	0.8946
FPF+PRO	Dose	5	103.29	**<0.0001**
	Worker type	1	12.76	**0.0004**
	Dose × Worker type	5	1.32	0.9328
	Colony	2	3.15	0.2071

ED_002896_00039492-00024

Table S6. Effect of dose, season, and worker type on bee survival, depending on FPF and DIM doses (Kaplan-Meier^{DS}, Wilcoxon). We report in bold the significant effects after Dunn-Sidak correction for multiple comparisons (FPF: dose and season, $k = 4$, adjusted $\alpha = 0.0127$; worker type: $k = 5$, adjusted $\alpha = 0.0102$; DIM: dose, $k = 6$, adjusted $\alpha = 0.0085$; season, $k = 4$, adjusted $\alpha = 0.0127$; worker type: $k = 5$, adjusted $\alpha = 0.0102$). For the dose effect, we compared all doses with control. For the seasonal effect, we did not use 25 and 800 ng DIM/bee in both seasons, since in summer we tested 25 ng DIM/bee instead of 800 ng DIM/bee because using the higher dose of DIM would have resulted in excessively high mortality precluding using this data ("NA"). We report the values of tested comparisons only.

		Dose effect			Season effect			Worker type effect		
Dose (ng/bee)		**χ^2**	**DF**	***P*-value**	**χ^2**	**DF**	***P*-value**	**χ^2**	**DF**	***P*-value**
Control	**0**		NA					21.97	1	**<0.0001**
FPF	**750**									
	1500	3.89	1	**0.0487**	2.96	1	0.0855	14.29	1	**0.0002**
	3000	47.26	1	**<0.0001**	6.22	1	**0.0127**	18.48	1	**<0.0001**
	6000	115.97	1	**<0.0001**	13.23	1	**0.0003**	7.70	1	**0.0055**
	12000	155.89	1	**<0.0001**	0.08	1	0.7802	1.25	1	0.2629
DIM	**25**	8.11	1	**0.0044**		NA				
	50	10.17	1	**0.0014**	6.32	1	**0.012**	11.49	1	**0.0007**
	100	47.82	1	**<0.0001**	1.84	1	0.1744	17.47	1	**<0.0001**
	200	130.64	1	**<0.0001**	9.35	1	**0.0022**	9.38	1	**0.0022**
	400	153.32	1	**<0.0001**	16.99	1	**<0.0001**	15.76	1	**<0.0001**
	800	156.53	1	**<0.0001**		NA		4.80	1	**0.0285**

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Table S7. Effect of FPF or FPF+PRO dose on the frequency of bees exhibiting at least one abnormal behaviour. Based on visual inspection of the data, we made limited tests of the effect of each pesticide dose as compared to control treatments (Mixed Model$_{REML}$, Contrast test^{DS}). We report only tested comparisons.

Active ingredient (name)	Time from treatment (h)	FPF dose (ng/bee)	DF numerator	DF denominator	*F* Ratio	*P*-value
FPF	1	750	1	52	6.96	**0.0109**
		1500	1	52	106.60	**<0.0001**
		3000	1	52	182.58	**<0.0001**
		6000	1	52	193.95	**<0.0001**
		12000	1	52	232.27	**<0.0001**
	2	1500	1	62	24.41	**<0.0001**
		3000	1	62	42.44	**<0.0001**
		6000	1	62	56.77	**<0.0001**
		12000	1	62	67.75	**<0.0001**
	4	1500	1	60	16.07	**0.0002**
		3000	1	60	32.51	**<0.0001**
		6000	1	60	46.74	**<0.0001**
		12000	1	60	18.98	**<0.0001**
FPF+PRO	1	375	1	32	164.81	**<0.0001**
		750	1	32	176.36	**<0.0001**
		1500	1	32	145.40	**<0.0001**
		3000	1	32	176.68	**<0.0001**
		6000	1	32	194.62	**<0.0001**
	2	375	1	32	228.32	**<0.0001**
		750	1	32	276.09	**<0.0001**
		1500	1	32	279.59	**<0.0001**
		3000	1	32	279.07	**<0.0001**
		6000	1	32	241.88	**<0.0001**
	4	375	1	25	148.24	**<0.0001**
		750	1	25	166.23	**<0.0001**
		1500	1	25	157.22	**<0.0001**
		3000	1	25	146.20	**<0.0001**
		6000	1	25	133.70	**<0.0001**

ED_002896_00039492-00026

Table S8. Abnormal behaviours of bees exposed to FPF, FPF+PRO, and DIM, depending on season, worker type, doses, and time after exposure. The effect of FPF+PRO was not tested across season. We tested the effects up to 4 h after treatment because the high mortality elicited by the pesticides at 24-48 h limited the sample size and precluded a rigorous analysis of abnormal behaviours.

Active ingredient	Time after treatment (h)	Colony effect (%)	Factor	DF numerator	DF denominator	*F* Ratio	*P*-value
FPF	1	5	Dose	5	52	82.54	**<0.0001**
			Season	1	52	25.74	**<0.0001**
			Worker type	1	52	4.16	**0.0464**
			Dose × Season	5	52	5.43	**0.0004**
			Dose × Worker type	5	52	4.90	**0.0010**
			Season × Worker type	1	46	1.61	0.2115
			Dose × Season × Worker type	5	46	2.25	0.0654
	2	<1	Dose	5	62	25.60	**<0.0001**
			Season	1	62	7.74	**0.0071**
			Worker type	1	62	0.13	0.7155
			Dose × Season	5	46	0.25	0.9354
			Dose × Worker type	5	46	2.44	**0.0486**
			Season × Worker type	1	46	2.75	0.1041
			Dose × Season × Worker type	5	46	2.40	0.0515
	4	1	Dose	5	60	14.99	**<0.0001**
			Season	1	60	0.43	0.5161
			Worker type	1	60	0.31	0.5798
			Dose × Season	5	44	0.71	0.6221
			Dose × Worker type	5	44	1.15	0.3499
			Season × Worker type	1	44	0.08	0.7759
			Dose × Season × Worker type	5	44	1.48	0.2144

ED_002896_00039492-00027

Active ingredient	Time after treatment (h)	Colony effect (%)	Factor	DF numerator	DF denominator	*F* Ratio	*P*-value
DIM	1	<1	Dose	5	47	19.68	**<0.0001**
			Season	1	47	31.71	**<0.0001**
			Worker type	1	47	27.97	**<0.0001**
			Dose × Season	5	47	5.24	**0.0007**
			Dose × Worker type	5	47	2.84	**0.0254**
			Season × Worker type	1	46	2.28	0.1382
			Dose × Season × Worker type	5	47	2.97	**0.0208**
	2	<1	Dose	5	52	81.00	**<0.0001**
			Season	1	52	44.43	**<0.0001**
			Worker type	1	52	8.65	**0.0049**
			Dose × Season	5	46	1.15	0.3501
			Dose × Worker type	5	52	5.43	**0.0004**
			Season × Worker type	1	46	0.36	0.5515
			Dose × Season × Worker type	5	52	5.23	**0.0006**
	4	2	Dose	5	44	45.40	**<0.0001**
			Season	1	44	0.24	0.6241
			Worker type	1	44	0.08	0.7775
			Dose × Season	5	44	5.06	**0.0009**
			Dose × Worker type	5	44	8.90	**0.0000**
			Season × Worker type	1	44	4.87	**0.0325**
			Dose × Season × Worker type	5	44	5.05	**0.0010**
FPF+PRO	1	<1	Dose	6	32	82.17	**<0.0001**
			Worker type	1	32	1.09	0.3052
			Dose × Worker type	6	26	0.68	0.6662
	2	<1	Dose	6	32	124.83	**<0.0001**
			Worker type	1	32	0.24	0.6289
			Dose × Worker type	6	26	1.35	0.2714
	4	28	Dose	6	25	72.59	**<0.0001**
			Worker type	1	25	4.00	0.0565
			Dose × Worker type	6	25	4.56	**0.0030**

Table S9. The effects of interactions dose × worker type and dose × season on the abnormal behaviours of bees depending on FPF or FPF+PRO exposure. Based upon visual inspection of the data, we conducted limited tests of the effect of worker type (in-hive vs. forager bees) or season (early spring vs. summer) at each specific dose treatment (Mixed Model$_{REML}$, Contrast test^{DS}). The effect of FPF+PRO was not tested across season. We report only tested comparisons.

Effect	Active ingredient (name)	Time from treatment (h)	FPF dose (ng/bee)	DF numerator	DF denominator	*F* Ratio	*P*-value
Dose × Worker type	FPF	1	1500	1	52	17.16	**0.0001**
	FPF	1	3000	1	52	3.35	0.0729
	FPF+PRO	4	375	1	25	9.09	**0.0058**
Dose × Season	FPF	1	750	1	52	26.86	**<0.0001**
	FPF	1	1500	1	52	6.73	**0.0123**
	FPF	1	3000	1	52	13.96	**0.0005**

ED_002896_00039492-00029

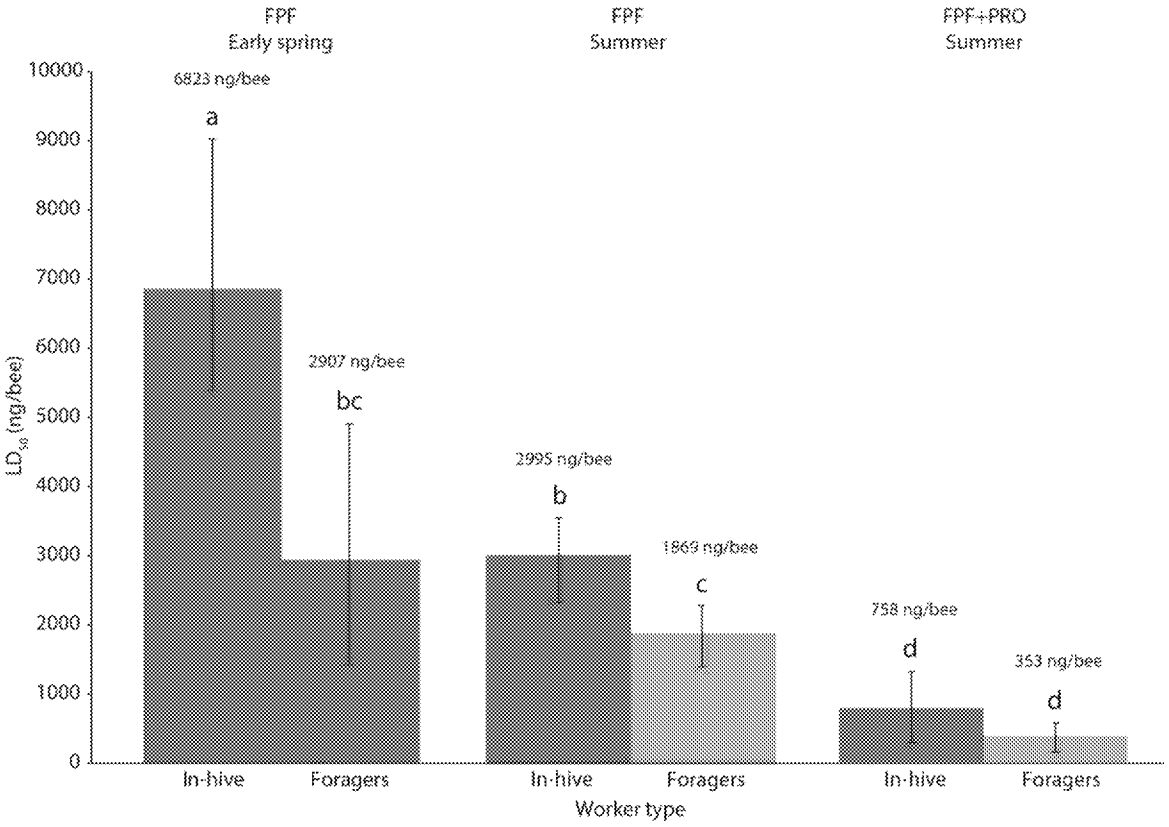
Table S10. Effect of season, worker type, and their interaction on bee weight. For each factor, we report the statistical values of the latest possible stepwise model simplification (Mixed $Model_{REML}$).

Factor	N	R^2	Colony effect (%)	DF numerator	DF denominator	*F* Ratio	*P*-value
Season				1	350	5.58	**0.0187**
Worker type	354	0.15	1	1	351	44.66	**<0.0001**
Season × Worker type				1	350	0.28	0.5974

ED_002896_00039492-00030

ESM figures

Figure S1. The LD_{50} (48 h) of bees exposed to FPF (left and centre) and FPF+PRO (right) across seasons (early spring vs. summer) and worker types (in-hive bees vs. foragers). Above each bar, we show the LD_{50} values. Different letters indicate significant differences. We show the 24 h LD_{50} of summer foragers (light grey bars), because high summer forager mortality at 48 h prevented the accurate estimation of their 48 h LD_{50} (standard LD_{50} estimation time, dark grey bars). Error bars represent 95% confidence intervals ($N_{overall}$ = 1080).



ED_002896_00039492-00031

Figure S2. The LD_{50} (48 h) of bees exposed to DIM across seasons (early spring vs. summer) and worker types (in-hive bees vs. foragers). Above each bar, we show the LD_{50} values. Different letters indicate significant differences. We show the 24 h LD_{50} of summer foragers (light gray bars), because high forager mortality of summer foragers at 48 h prevented the accurate estimation of their 48 h LD_{50}. Error bars represent 95% confidence intervals.

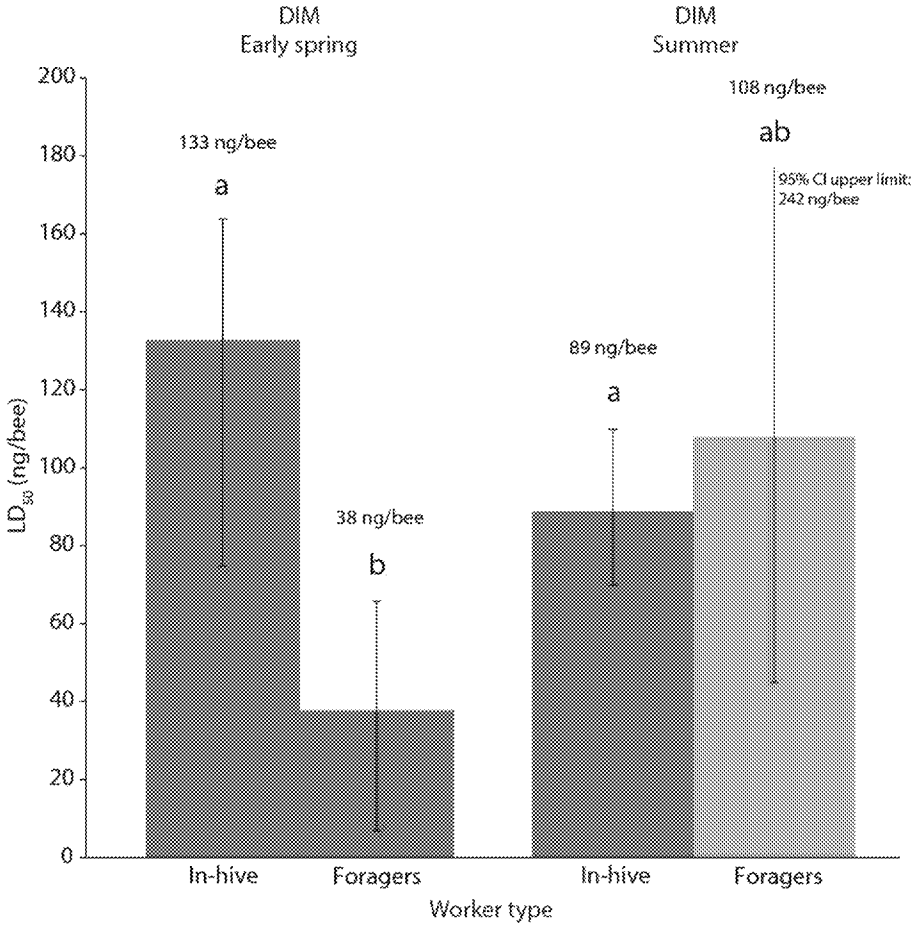
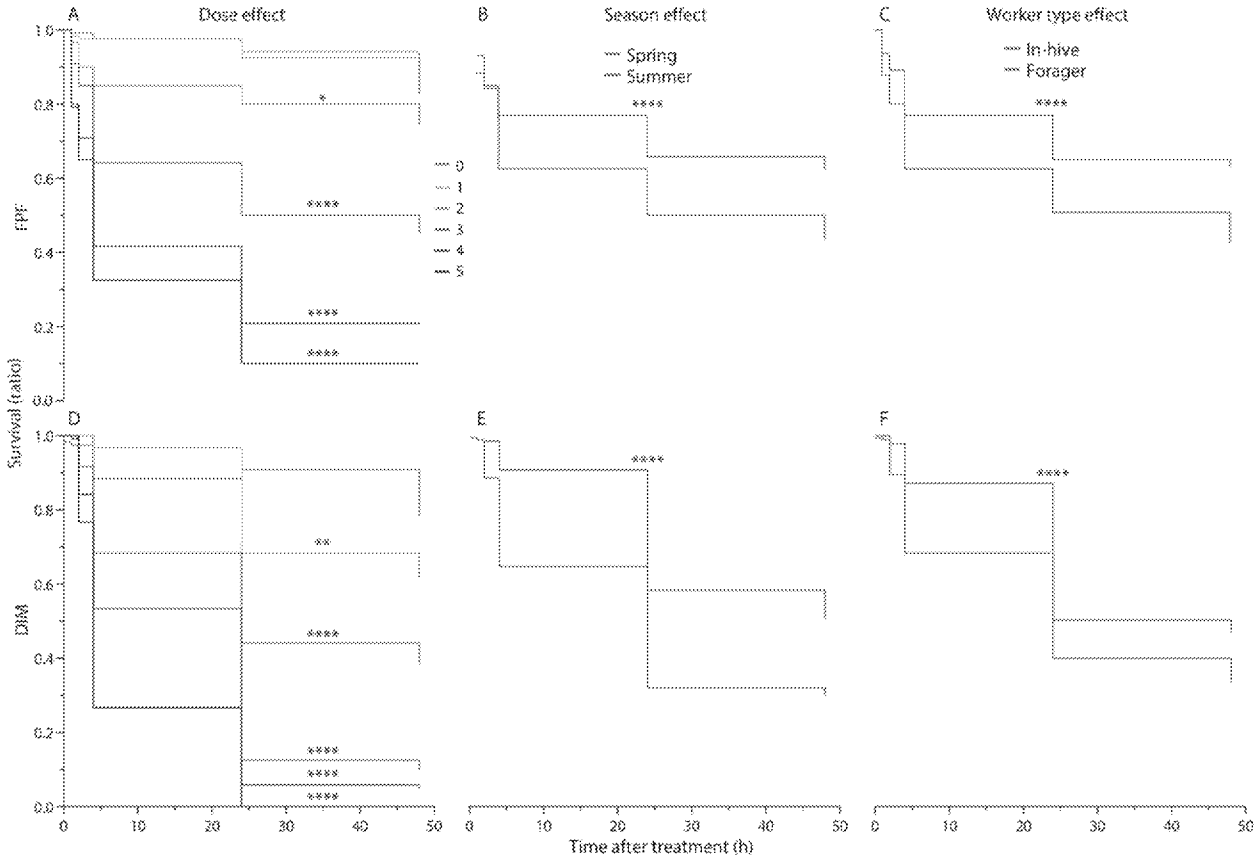
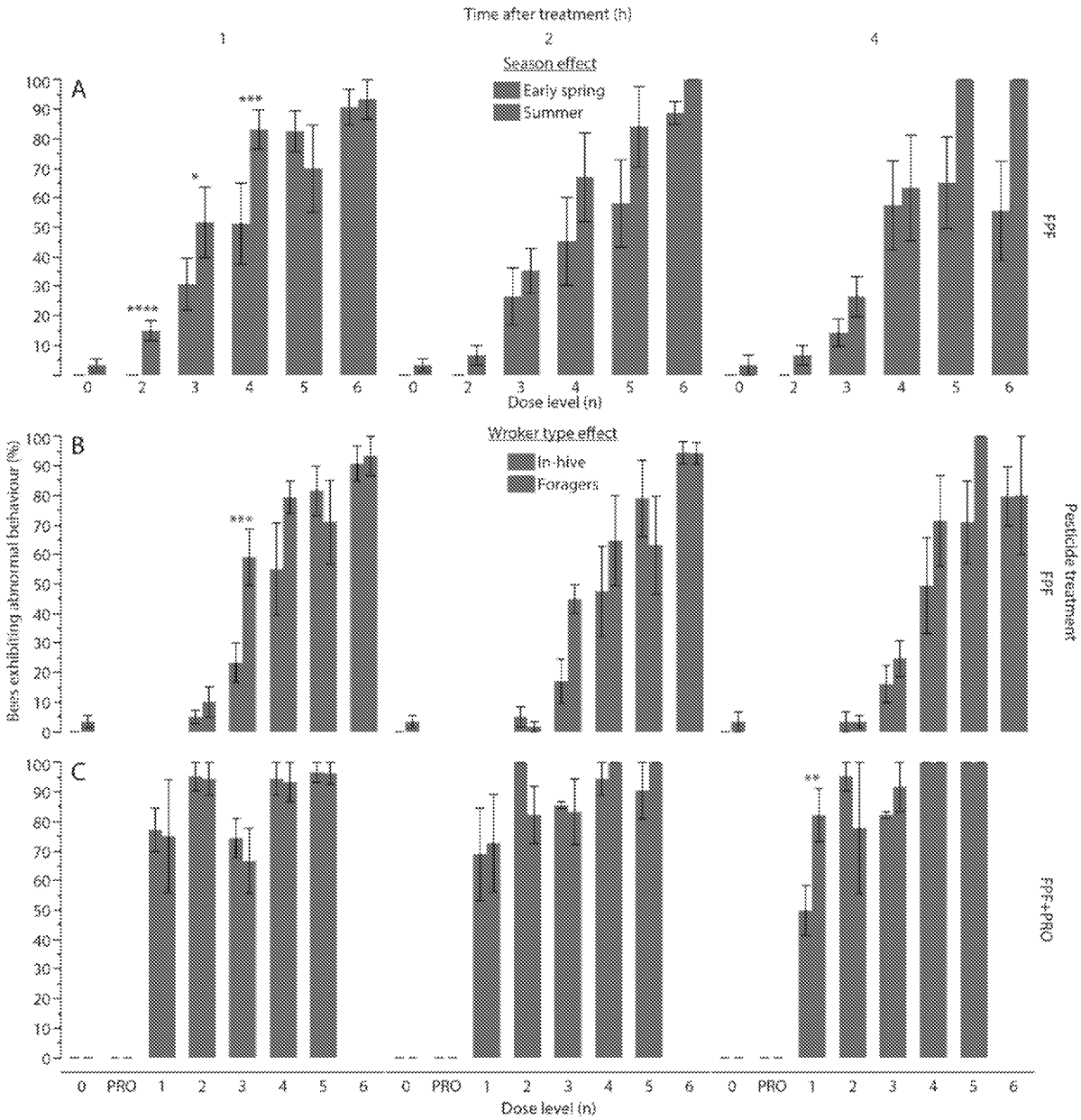


Figure S3. Main effects of (A, D) dose, (B, E) season, and (C, F) worker type on survival of bees exposed to (A, B, C) FPF or (D, E, F) DIM. Asterisks indicate significant differences (Kaplan-Meier[DS], $*p < 0.05$, $**p < 0.01$, $****$ $p < 0.0001$, table S6). In A and D, we made limited pairwise comparisons testing the dose effect (0-5 dose levels, corresponding to a control dose and between 750-12000 ng FPF/bee in A, 50-800 ng DIM/bee in D) comparing each dose to control based upon visual inspection of the data (Dunn-Sidak corrected, table S6).



ED_002896_00039492-00033

Figure S4. Effect of (A) season and (B, C) worker type on the frequency of bees exhibiting abnormal behaviours after exposure to (A, B) FPF or (C) FPF+PRO doses (0-6 dose levels, corresponding to a control dose and between 375-12000 ng FPF/bee). In figure 3C-D of the main text, these results were pooled by worker type, but are here split by worker type to provide further information. Asterisks indicate significant differences (Mixed Model$_{REML}$, Contrast test^{DS}, *p < 0.05, ***p* < 0.01, ****p* < 0.001, *****p* < 0.0001). We compared the effect of season and worker type within each pesticide dose based on visual estimation (Dunn-Sidak corrected). Main effects and further statistical details are reported in tables S8-S9.



ESM videos

Abnormal behaviours observed in videos recorded during preliminary ecotoxicological trials. The videos highlight and better define the types of common abnormal bee behaviours occurring after oral pesticide consumption in sucrose solution. Further details are available in table S1-S2. The video is available in:

- ✓ the Dryad Digital Repository (https://doi.org/10.5061/dryad.5f87k5v)
- ✓ at this YouTube link: YouTube Video of Abnormal Behaviours
- ✓ at this QR code:

ED_002896_00039492-00035

R scripts

R script for testing synergistic effects on survival

```
##############################################################################
# Testing the synergistic effects of two chemicals on survival
# Testing for additivity:
#    Confidence interval for binomial proportion difference under Bliss independence.
#
# INPUTS:
# ndead = vector with 3 elements, containing number of dead individuals under
#        treatment A, B and combined.
# ntot  = vector with 3 elements, containing total number of individuals under
#        the 3 treatments.
# p.signif = significance level (usually 0.05).
# alternative = character string specifying the alternative hypothesis.
#
# OUTPUTS:
# See Tosi et al. 2019
##############################################################################

ci.bliss.additivity <- function(ndead,ntot,p.signif=0.05,alternative="greater") {
  if (alternative=="two.sided") p.signif <- p.signif/2  # Two-tailed test.
  ndead <- unname(ndead)
  ntot <- unname(ntot)
  p <- ndead/ntot
  pa <- p[1]
  pb <- p[2]
  pab.obs <- p[3]
  vara <- p[1]*(1-p[1])/ntot[1]
  varb <- p[2]*(1-p[2])/ntot[2]
  varab.obs <- p[3]*(1-p[3])/ntot[3]
```


ED_002896_00039492-00036

```
  pab.exp <- pa+pb-pa*pb
  varab.exp <- vara+varb+pb^2*vara+pa^2*varb    # Derived with the Delta method.
  p.dif <- pab.obs-pab.exp
  sd.all <- sqrt(varab.obs+varab.exp)
  z <- qnorm(1-p.signif)
  out <- list(pA=pa,pB=pb,pAB.obs=pab.obs,pAB.exp=pab.exp,p.Dif=p.dif,
          VarA=vara,VarB=varb,VarAB.obs=varab.obs,VarAB.exp=varab.exp,Var.All=sd.all^2,
          CI=switch(alternative,
                two.sided=c(lower=p.dif-z*sd.all,upper=p.dif+z*sd.all),
                less=c(upper=p.dif+z*sd.all),
                greater=c(lower=p.dif-z*sd.all)))
  return(out)
}

# Calculates the exact p-value by inverting the hypothesis test.
invert.hypothesis.bliss <- function(n.mort,n.total) {
  fbliss <- function(signif)
ci.bliss.additivity(n.mort,n.total,signif,alternative="greater")$CI["lower"]
  loglik <- function(signif) abs(fbliss(signif))
  return(optimize(loglik,interval=c(0,1),maximum=F,tol=1e-32)$minimum)
}

# Mortality data. Column 1 (e.g. datamort[[1]][,1]) contains the total number of individuals,
labelled "N".
datamort <- list()
datamort[[1]] <- cbind(c(30,30,30),c(0,0,0),c(0,0,0),c(2,0,0),c(7,4,2),c(13,10,5)) # forager, 0
datamort[[2]] <- cbind(c(30,30,30),c(0,0,6),c(0,0,12),c(0,0,17),c(1,4,24),c(7,10,25)) # forager,
750
datamort[[3]] <- cbind(c(30,30,30),c(2,0,10),c(7,0,10),c(10,0,15),c(13,4,21),c(15,10,24)) #
forager, 1500
```


ED_002896_00039492-00037

```
datamort[[4]] <- cbind(c(30,30,30),c(1,0,11),c(5,0,13),c(16,0,22),c(20,4,28),c(25,10,30)) #
forager, 3000
datamort[[5]] <- cbind(c(30,30,30),c(8,0,8),c(11,0,13),c(25,0,21),c(30,4,30),c(30,10,30)) #
forager, 6000
datamort[[6]] <- cbind(c(30,30,30),c(0,0,0),c(0,0,0),c(0,0,0),c(0,1,3),c(3,5,3)) # in-hive, 0
datamort[[7]] <- cbind(c(30,30,30),c(0,0,7),c(1,0,8),c(1,0,9),c(2,1,16),c(4,5,17)) # in-hive, 750
datamort[[8]] <- cbind(c(30,30,30),c(0,0,7),c(2,0,9),c(4,0,13),c(4,1,16),c(4,5,17)) # in-hive, 1500
datamort[[9]] <- cbind(c(30,30,30),c(2,0,9),c(3,0,9),c(12,0,11),c(17,1,23),c(17,5,24)) # in-hive,
3000
datamort[[10]] <- cbind(c(30,30,30),c(6,0,10),c(6,0,13),c(22,0,16),c(29,1,26),c(29,5,27)) # in-
hive, 6000

for (i in 1:10) rownames(datamort[[i]]) <- c("TREAT.A","TREAT.B","TREAT.AB") #
TREAT.A = FPF; TREAT.B = PRO; TREAT.AB = FPF+PRO
for (i in 1:10) colnames(datamort[[i]]) <- c("N","1h","2h","4h","24h","48h")

cat("-----------------------------------------------------------------\n")

# Testing Bliss additivity. All we need to do is to define "n.total" and "n.mort", and then feed
invert.hypothesis.bliss() with those two numbers.
# Index i runs from 1 to the number of synergies tested (=1).
# For a generic dataset with 1 endpoint and where nt=total number of individuals and nd=number
of dead individuals, we would do: p <- invert.hypothesis.bliss(nt,nd)

for (i in 1:10) {
  a <- datamort[[i]]
  b <- a[,-1]
  p.value <- NULL

# For each endpoint j we test the Bliss hypothesis.
  for (j in 1:5) {
```

```
  n.total <- a[c(1,2,3),1]    # Total number of individuals
  n.mort <- a[c(1,2,3),j+1]   # Number of dead individuals.
  p <- invert.hypothesis.bliss(n.mort,n.total)  # p-value from inverting the hypothesis test.
  p.value <- c(p.value,p)
 }
# Control for multiple comparison, Holm methodology. For cases where there is only 1 endpoint
this is obviously not needed.
 p.correct <- p.adjust(p.value,method="holm")

# Formatted output.
 name.data <- c("forager, 0","forager, 750","forager, 1500","forager, 3000","forager, 6000","in-
hive, 0","in-hive, 750","in-hive, 1500","in-hive, 3000","in-hive, 6000")
 cat(paste(name.data[i],"\n",sep=""))
 names(p.correct) <- c("1h","2h","4h","24h","48h")
 print(datamort[[i]])
 cat("\n")
 cat(paste(name.data[i],". Observed and expected binomial proportions.\n",sep=""))
 pab <- a[,-1]/a[,1]
 pab <- rbind(pab,pab[1,]+pab[2,]-pab[1,]*pab[2,])
 rownames(pab) <- c("TREAT.A","TREAT.B","TREAT.AB","Expected")
 print(pab)
 cat("\n")
 cat(paste(name.data[i],". Control of type I errors (Holm method) in binomial proportion
test.\n",sep=""))
 print(p.correct)

 cat("-----------------------------------------------------------------\n")
}
```

R script for testing synergistic effects on abnormal behaviours

```
##################################################################################
```


ED_002896_00039492-00039

```
# Testing the synergistic effects of two chemicals on abnormal behaviours
# Testing for additivity:
#     Confidence interval for binomial proportion difference under Bliss independence.
#
# INPUTS:
# nabnbe = vector with 3 elements, containing number of individuals exhibiting abnormal
#         behaviour under treatment A, B and combined.
# ntot  = vector with 3 elements, containing total number of individuals under
#         the 3 treatments.
# p.signif = significance level (usually 0.05).
# alternative = character string specifying the alternative hypothesis.
#
# OUTPUTS:
# See Tosi et al. 2019
##############################################################################

ci.bliss.additivity <- function(nabnbe,ntot,p.signif=0.05,alternative="greater") {
  if (alternative=="two.sided") p.signif <- p.signif/2  # Two-tailed test.
  nabnbe <- unname(nabnbe)
  ntot <- unname(ntot)
  p <- nabnbe/ntot
  pa <- p[1]
  pb <- p[2]
  pab.obs <- p[3]
  vara <- p[1]*(1-p[1])/ntot[1]
  varb <- p[2]*(1-p[2])/ntot[2]
  varab.obs <- p[3]*(1-p[3])/ntot[3]
  pab.exp <- pa+pb-pa*pb
  varab.exp <- vara+varb+pb^2*vara+pa^2*varb    # Derived with the Delta method.
  p.dif <- pab.obs-pab.exp
  sd.all <- sqrt(varab.obs+varab.exp)
```

```
  z <- qnorm(1-p.signif)
  out <- list(pA=pa,pB=pb,pAB.obs=pab.obs,pAB.exp=pab.exp,p.Dif=p.dif,
          VarA=vara,VarB=varb,VarAB.obs=varab.obs,VarAB.exp=varab.exp,Var.All=sd.all^2,
          CI=switch(alternative,
                two.sided=c(lower=p.dif-z*sd.all,upper=p.dif+z*sd.all),
                less=c(upper=p.dif+z*sd.all),
                greater=c(lower=p.dif-z*sd.all)))
  return(out)
}

# Calculates the exact p-value by inverting the hypothesis test.
invert.hypothesis.bliss <- function(n.abnbe,n.total) {
  fbliss <- function(signif)
ci.bliss.additivity(n.abnbe,n.total,signif,alternative="greater")$CI["lower"]
  loglik <- function(signif) abs(fbliss(signif))
  return(optimize(loglik,interval=c(0,1),maximum=F,tol=1e-32)$minimum)
}

# Abnormal behaviour data (individuals exhibiting the behaviour).
# Column 1 (e.g. dataabnbe[[1]][,1]) contains the total number of individuals, labelled "N".
dataabnbe <- list()

dataabnbe[[1]] <- cbind(c(30,30,30),c(0,0,0),c(0,0,0),c(0,0,0)) # in-hive, 0
dataabnbe[[2]] <- cbind(c(30,30,30),c(3,0,22),c(3,0,22),c(2,0,20)) # in-hive, 750
dataabnbe[[3]] <- cbind(c(30,30,30),c(10,0,17),c(8,0,18),c(6,0,14)) # in-hive, 1500
dataabnbe[[4]] <- cbind(c(30,30,30),c(24,0,20),c(21,0,20),c(12,0,19)) # in-hive, 3000
dataabnbe[[5]] <- cbind(c(30,30,30),c(21,0,20),c(23,0,15),c(8,0,16)) # in-hive, 6000
dataabnbe[[6]] <- cbind(c(30,30,30),c(2,0,0),c(2,0,0),c(2,0,0)) # foragers, 0
dataabnbe[[7]] <- cbind(c(30,30,30),c(6,0,15),c(1,0,15),c(2,0,11)) # foragers, 750
dataabnbe[[8]] <- cbind(c(30,30,30),c(19,0,14),c(10,0,19),c(6,0,14)) # foragers, 1500
dataabnbe[[9]] <- cbind(c(30,30,30),c(23,0,17),c(14,0,17),c(6,0,8)) # foragers, 3000
```


ED_002896_00039492-00041

```
dataabnbe[[10]] <- cbind(c(30,30,30),c(12,0,21),c(15,0,17),c(13,0,9)) # foragers, 6000

for (i in 1:10) rownames(dataabnbe[[i]]) <- c("TREAT.A","TREAT.B","TREAT.AB") #
TREAT.A = FPF; TREAT.B = PRO; TREAT.AB = FPF+PRO
for (i in 1:10) colnames(dataabnbe[[i]]) <- c("N","1h","2h","4h")

cat("-----------------------------------------------------------------\n")

# Testing Bliss additivity. All we need to do is to define "n.total" and "n.abnbe",
#                          and then feed invert.hypothesis.bliss() with those two numbers.
# Index i runs from 1 to the number of synergies tested.
# For a generic dataset with 1 endpoint and where nt=total number of individuals
#                          and nab=number of individuals exhibiting abnormal behaviour,
#                                  we would do: p <- invert.hypothesis.bliss(nt,nab)

for (i in 1:10) {
  a <- dataabnbe[[i]]
  b <- a[,-1]
  p.value <- NULL

# For each endpoint j we test the Bliss hypothesis. J is the # of time assessments
  for (j in 1:3) {
    n.total <- a[c(1,2,3),1]    # Total number of individuals
    n.abnbe <- a[c(1,2,3),j+1]   # Number of bees exhibiting abnormal behaviour.
    p <- invert.hypothesis.bliss(n.abnbe,n.total)  # p-value from inverting the hypothesis test.
    p.value <- c(p.value,p)
  }
# Control for multiple comparison, Holm methodology. For cases where there is only 1 endpoint
this is obviously not needed.
  p.correct <- p.adjust(p.value,method="holm")
```

```
# Formatted output.
  name.data <- c("in-hive, 0","in-hive, 750","in-hive, 1500","in-hive, 3000","in-hive,
6000","foragers, 0","foragers, 750","foragers, 1500","foragers, 3000","foragers, 6000")
  cat(paste(name.data[i],"\n",sep=""))
  names(p.correct) <- c("1h","2h","4h")
  print(dataabnbe[[i]])
  cat("\n")
  cat(paste(name.data[i],". Observed and expected binomial proportions.\n",sep=""))
  pab <- a[,-1]/a[,1]
  pab <- rbind(pab,pab[1,]+pab[2,]-pab[1,]*pab[2,])
  rownames(pab) <- c("TREAT.A","TREAT.B","TREAT.AB","Expected")
  print(pab)
  cat("\n")
  cat(paste(name.data[i],". Control of type I errors (Holm method) in binomial proportion
test.\n",sep=""))
  print(p.correct)

  cat("-----------------------------------------------------------------\n")
}
```

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Appointment

To: Suarez, Mark [Suarez.Mark@epa.gov]; Lennartz, Steven [Lennartz.Steven@epa.gov]; Peck, Charles [Peck.Charles@epa.gov]; Perry, Tracy [Perry.Tracy@epa.gov]; Sims, Diann [Sims.Diann@epa.gov]; Paisley-Jones, Claire [Paisley-Jones.Claire@epa.gov]; Becker, Jonathan [Becker.Jonathan@epa.gov]; Kyle, Lee [Kyle.Lee@epa.gov]; Connolly, Jennifer [Connolly.Jennifer@epa.gov]; Panger, Melissa [Panger.Melissa@epa.gov]; Anderson, Brian [Anderson.Brian@epa.gov]; Rossmeisl, Colleen [Rossmeisl.Colleen@epa.gov]; Anderson, Brian [Anderson.Brian@epa.gov]; Panger, Melissa [Panger.Melissa@epa.gov]; Connolly, Jennifer [Connolly.Jennifer@epa.gov]; Kyle, Lee [Kyle.Lee@epa.gov]; Becker, Jonathan [Becker.Jonathan@epa.gov]; Paisley-Jones, Claire [Paisley-Jones.Claire@epa.gov]; Sims, Diann [Sims.Diann@epa.gov]; Perry, Tracy [Perry.Tracy@epa.gov]; Garber, Kristina [Garber.Kristina@epa.gov]; Peck, Charles [Peck.Charles@epa.gov]; Lennartz, Steven [Lennartz.Steven@epa.gov]; Suarez, Mark [Suarez.Mark@epa.gov]

Location: DCRoomPYS7771D/Potomac-Yard-One

Start: 12/4/2018 2:15:00 PM
End: 12/4/2018 3:00:00 PM
Show Time As: Tentative

Recurrence: (none)

Agenda for meeting today:

- Moving start time to 9:15 am today
- Discuss slide deck and thoughts for briefing next week. I have received comments on several slide decks and Steve has created new maps, so it may be easier to discuss then send around. We also received new slide edits from NMFS last night. Based on discussions with EPA/Services management, a proposed agenda for the briefing was created last week (see below). However, we have not yet received feedback on this agenda or comments on slides from FWS.
- Other items?

- Introduction: 5 min (Brian and Patrice)
  - Purpose: We are going to present progress on the action area development related to agricultural uses of malathion.
  - Deliberative Process / Ex. 5
  - Each agency will give their perspective on the pros and cons of the methods at the end of the presentation.

- Presentation – (EPA and FWS). 30 min
- Perspectives on pros and cons of the methodology and recommendations
  - EPA: 10 minutes
  - FWS: 10 minutes
  - NMFS: 10 minutes
  - USDA: 10 minutes
- Discussion: 30 minutes

- Wrap up and next steps: 15 minutes

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Location: DCRoomPYS7100/Potomac-Yard-One

Start: 2/12/2019 7:00:00 PM
End: 2/12/2019 8:00:00 PM
Show Time As: Busy

Recurrence: (none)
every Tuesday from 2:00 PM to 3:00 PM

For EPA folks, you are getting this email twice (sorry). I sent late yesterday but it got kicked back to me for everyone outside of EPA due to attachment #2 being too large. I have removed that attachment and will work on getting that to outside folks shortly (will probably put on SharePoint and send a link).
Thanks!
Colleen

****
Hi all –

Proposed agenda for tomorrow's meeting:

Discuss two attached documents:

1) Summary of proposed methodology for the AA based on our discussions to date, including areas where decisions still need to be made. This is based largely on the meeting minutes I sent around last week and other previous meetings.
2) Census crop presence/absence table by County (if folks have time to review, EPA can provide any clarifications on how to use table).

We would also like to discuss the need for redacted mosquito control usage data – we had previously discussed obtaining that data, but there may be some concerns from the registrant in being able to release the data. It would be good to discuss what form we may want this data in, or if it is needed.

Please let us know if folks will be available to meet at 2 pm and if there are additional agenda items.

Thanks!
Colleen

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Sent: 5/21/2019 7:28:24 PM
To: Franzosa, Jill [Franzosa.Jill@epa.gov]; Tietge, Joe [Tietge.Joe@epa.gov]; Baghdikian, Christina [Baghdikian.Christina@epa.gov]; Purucker, Tom [Purucker.Tom@epa.gov]; Blackwell, Brett [Blackwell.Brett@epa.gov]; Elonen, Colleen [Elonen.Colleen@epa.gov]; Etterson, Matthew [Etterson.Matthew@epa.gov]; LaLone, Carlie [lalone.carlie@epa.gov]; Lavelle, Candice [lavelle.candice@epa.gov]; Nichols, John [Nichols.John@epa.gov]; Raimondo, Sandy [Raimondo.Sandy@epa.gov]; Kolanczyk, Richard [Kolanczyk.Rick@epa.gov]; Nacci, Diane [Nacci.Diane@epa.gov]; Stedeford, Todd [Stedeford.Todd@epa.gov]; Garber, Kristina [Garber.Kristina@epa.gov]; Raffaele, Kathleen [raffaele.kathleen@epa.gov]; Latier, Andrea [Latier.Andrea@epa.gov]; Biales, Adam [Biales.Adam@epa.gov]
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Subject: CSS ETAM RACT - ORD + Partners Meeting
Attachments: ETAM Research Area Coordination Team_ORD and Partners slides_20190604.pptx; ETAM Research Area Template_DRAFT_Output and Product Description_20190528_OPP+Regional Feedback.docx; ETAM Research Area Template_DRAFT_Output and Product Description_20190528_kg.docx
Location: Skype Meeting; +1 (202) 991-0477, 5027339#; RTP-B311K

Start: 6/4/2019 6:00:00 PM
End: 6/4/2019 7:00:00 PM
Show Time As: Busy

Recurrence: Weekly
every Tuesday from 2:00 PM to 3:00 PM

Rescheduled CSS ETAM ORD + Partners Meeting from 5/24 to 6/4

Agenda

- Current Timeline and Goals for Meeting – Jill 5 min
- Research Area Template Feedback (see attached Word doc) – Output Lead (45 min)
  - ETAM RA Overview – Jill – 6 min
  - ETAM2 – Matt - 6 min
  - ETAM3– Tom – 6 min
  - ETAM4 – Colleen – 6 min
  - ETAM5-7 – Rick and Candice – 6 min
  - ETAM8– Sandy – 6 min
  - ETAM9-10 – Brett & John – 6 min
- Partner Engagement Template Feedback (see attached Word doc) – all - 5-10 min
- Next Steps/ Action Items

Join Skype Meeting

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Conference ID: Conference Line/Code / Ex. 6
Forgot your dial-in PIN? | Help

Appointment

From: Raimondo, Sandy [Raimondo.Sandy@epa.gov]
Sent: 7/5/2018 1:14:06 PM
To: Raimondo, Sandy [Raimondo.Sandy@epa.gov]; TenBrook, Patti [TenBrook.Patti@epa.gov]; Miller, Amy [Miller.Amy@epa.gov]; Blake, Ellen [Blake.Ellen@epa.gov]; Denton, Debra [Denton.Debra@epa.gov]; Small, Matthew [Small.Matthew@epa.gov]; Etterson, Matthew [Etterson.Matthew@epa.gov]; Garber, Kristina [Garber.Kristina@epa.gov]; Justice, JamesR [Justice.JamesR@epa.gov]; Kanarek, Andrew [Kanarek.Andrew@epa.gov]; Martin, Roy [Martin.Roy@epa.gov]; Oliver, Leah [Oliver.Leah@epa.gov]; Purucker, Tom [Purucker.Tom@epa.gov]; Sharpe, Leah [sharpe.leah@epa.gov]; Thursby, Glen [Thursby.Glen@epa.gov]; Waits, Eric [Waits.Eric@epa.gov]; Yuan, Yongping [Yuan.Yongping@epa.gov]; Nash, Maliha [Nash.Maliha@epa.gov]

Subject: Final Agenda: ORD-R9 California endangered species case study
Attachments: EPA Region 9 workshop final agenda 8-8-18.docx; CA ESA Case Study 8-3-18_Region9.pptx
Location: EPA Region 9 - Havasupai Room

Start: 8/8/2018 3:30:00 PM
End: 8/8/2018 8:00:00 PM
Show Time As: Tentative

All,

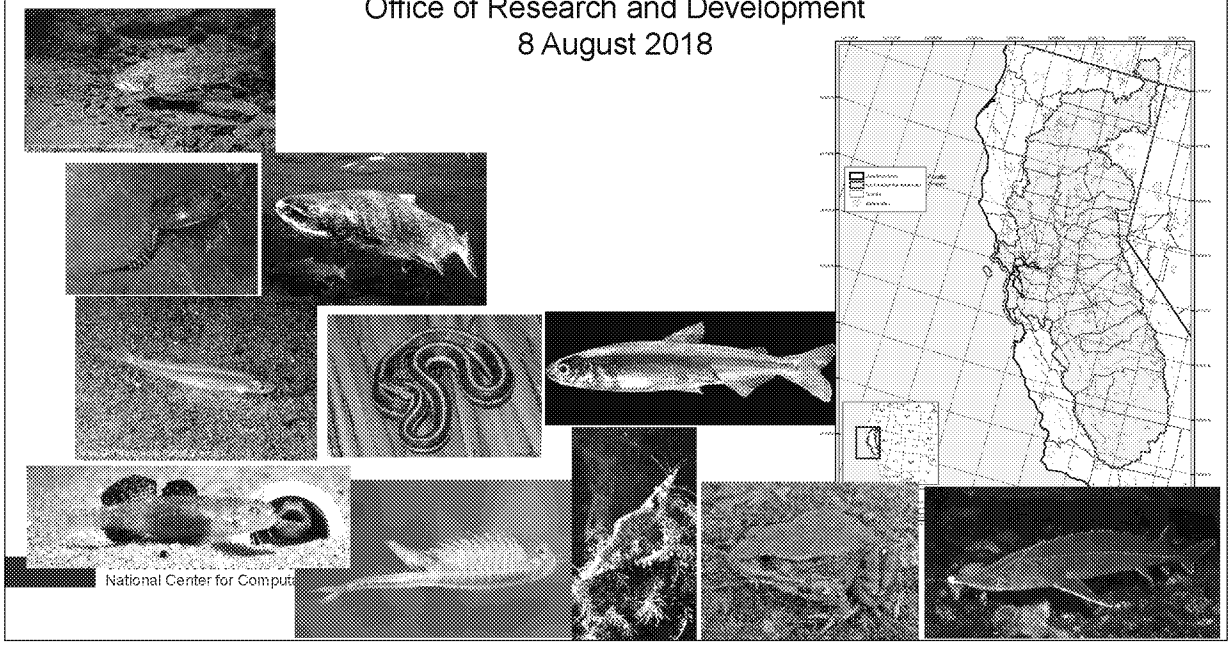
Attached is the final agenda for the meeting on Wednesday August 8 with EPA Region 9 and the ORD California endangered species project team. Slides are attached for those calling in. We will provide remote access information if available, otherwise, remote participation can occur via the conference line.

Looking forward to our discussions,
Sandy

United States Environmental Protection Agency

Chemical Safety for Sustainability
California Endangered Species Case Study

Sandy Raimondo
Office of Research and Development
8 August 2018



United States Environmental Protection Agency

The EPA Team

ORD/NHEERL

- Gulf Ecology Division (GED)
  - Sandy Raimondo (task lead)
  - Leah Oliver
  - Leah Sharpe
- Mid-Continent Ecology Division (MED)
  - Matthew Etterson
  - Nathan Pollesch
- Atlantic Ecology Division (AED)
  - Glen Thursby

Region 9

- Debra Denton

ORD/NERL

- System Exposure Division (SED)
  - Yongping Yuan
  - Tom Purucker
  - Sumathy Sinnathamby
  - Maliha Nash
  - Roy Martin
  - Eric Waits
- OPP/EFED
  - Kris Garber
  - Andrew Kanarek

Stakeholders & Collaborators

EPA United States Environmental Protection Agency

Water Boards

USDA

California Fish & Wildlife

NOAA — National Oceanic and Atmospheric Administration, U.S. Department of Commerce

Department of Water Resources, State of California

CalEPA California Environmental Protection Agency

USGS

Pacific States Marine Fisheries Commission

SWAMP Surface Water Ambient Monitoring Program

DWR · USBR · USFWS · SWRCB · DFW · USEPA · USACE · NMFS · USGS

UCDAVIS

Cramer Fish Sciences

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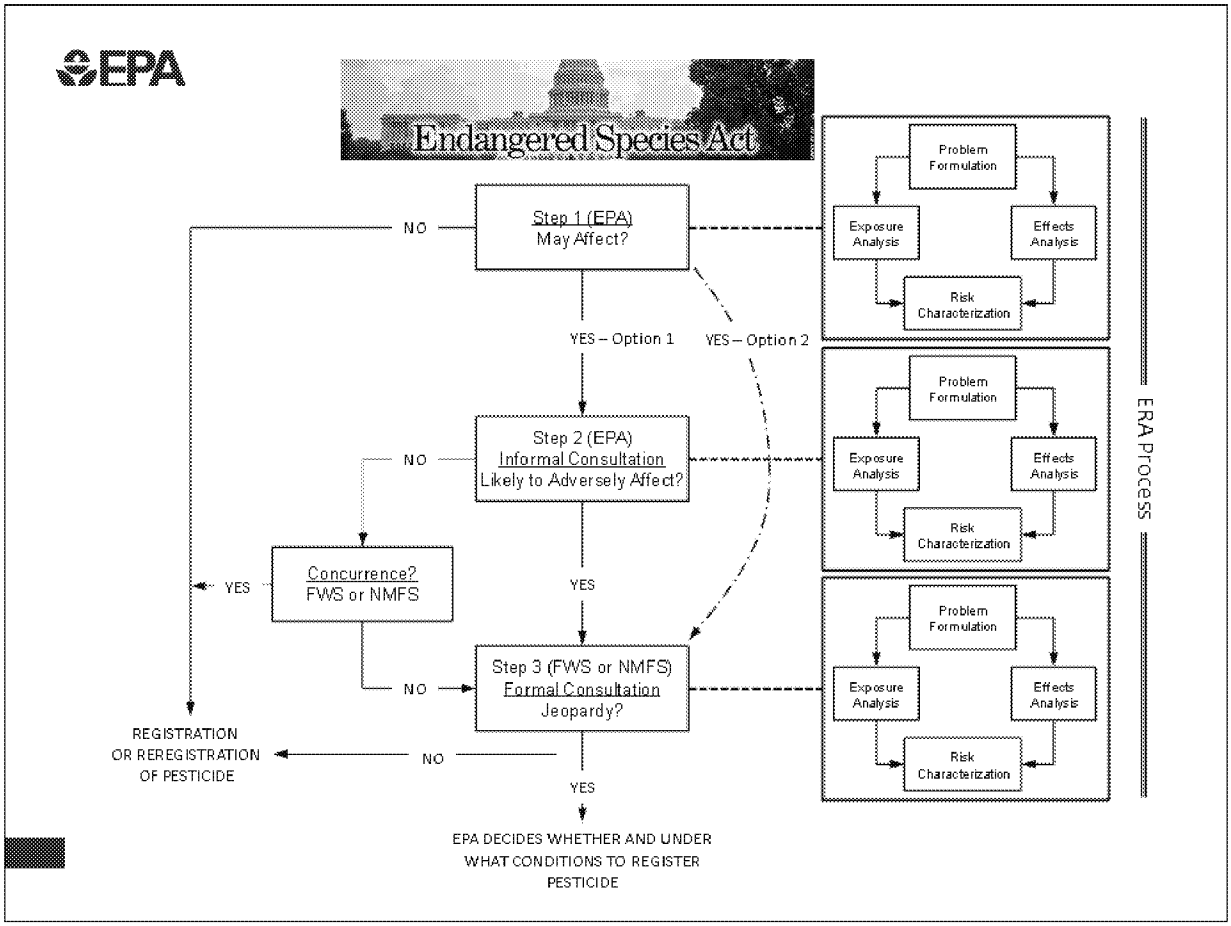
Task Drivers and Goals

- EPA research priorities highlighted in the National Academy of Science's report (2013) "Assessing Risks to Endangered and Threatened Species from Pesticides"
- Develop, evaluate, and demonstrate approaches that improve endangered species risk assessments and reduce knowledge-based uncertainties
- Approaches that transition from the current EPA process toward probabilistic risk assessment for threatened and endangered (T&E) species
- Develop, evaluate, and provide guidance on approaches that make use of minimal data, increasing the number and types of exposure scenarios, and implementing models that project population-level impacts to listed species.


EPA
Endangered Species Act
Step 1 (EPA)
May Affect?
NO
YES – Option 1
YES – Option 2
Step 2 (EPA)
Informal Consultation
Likely to Adversely Affect?
NO
YES
Concurrence?
FWS or NMFS
YES
NO
Step 3 (FWS or NMFS)
Formal Consultation
Jeopardy?
NO
YES
REGISTRATION
OR REREGISTRATION
OF PESTICIDE
EPA DECIDES WHETHER AND UNDER
WHAT CONDITIONS TO REGISTER
PESTICIDE
Problem Formulation
Exposure Analysis
Effects Analysis
Risk Characterization
Problem Formulation
Exposure Analysis
Effects Analysis
Risk Characterization
Problem Formulation
Exposure Analysis
Effects Analysis
Risk Characterization
ERA Process

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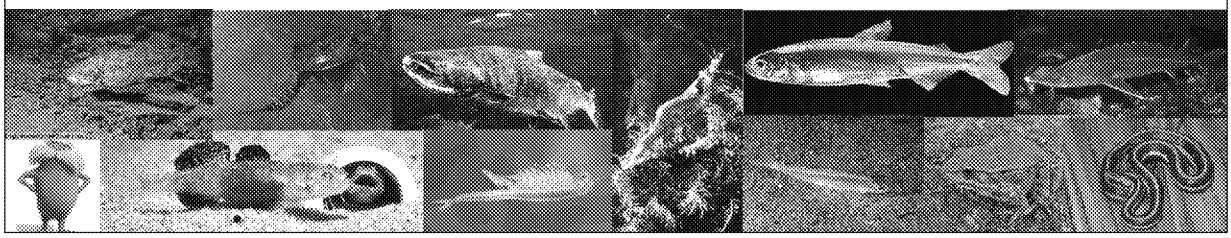
Anticipated Project Outcomes

- Reduced uncertainties associated with predicting pesticide impacts to endangered species
- Provide improved and defensible approaches available for Section 7 consultation
- Develop framework for integrating exposure and effects into probabilistic assessments
- Minimize impacts to listed species from pesticide exposure that provides greater protection of other species



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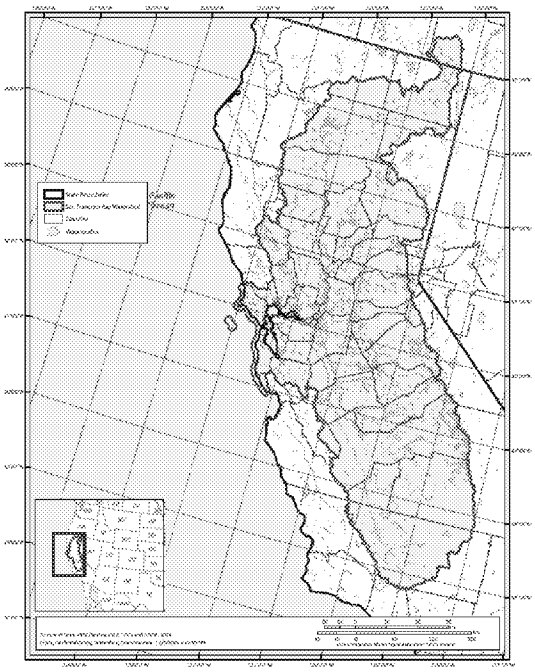
California Central Valley

- Diversity
  - Listed species
  - Pesticide use
  - Habitat types
- Relatively "abundant" data
  - Pesticide use
  - Environmental monitoring
  - Species data
- Region & State partnership
- Historical processes for ESA well documented



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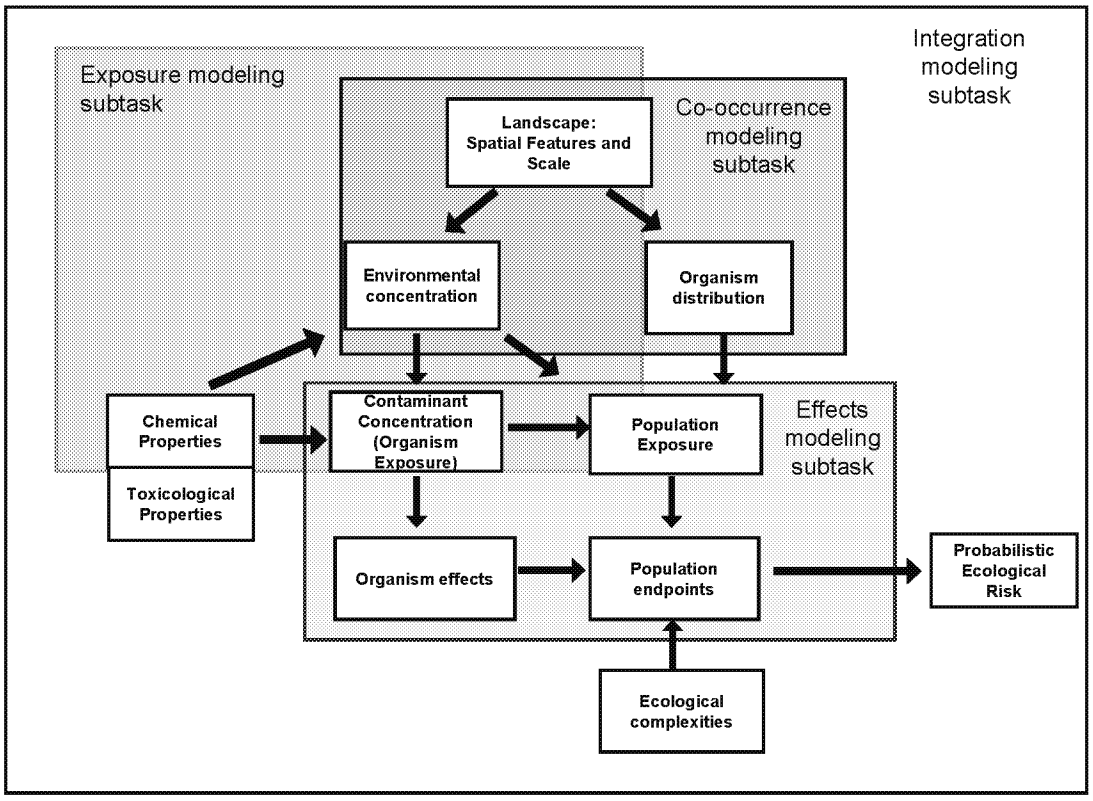
2015 Project Scoping Meeting

- Understand processes required of the Agency, Services, and States to comply with the Endangered Species Act (ESA)
- Overview species and pesticide usage within the study area
- Engagement with representatives of the Regional and State Offices
  - EPA Office of Pesticide Programs
  - EPA Office of Water
  - EPA Region 9
  - US Fish and Wildlife
  - CA State Water Resource Board (Rich Breuer)
  - CA Department of Pesticide Registration (Yuzhou Luo, Rich Marovich)
  - CA Department of Fish and Wildlife (Stephen Louie)
- Task framework established

Task Conceptual Model and Structure



California Central Valley Species

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Tier 1

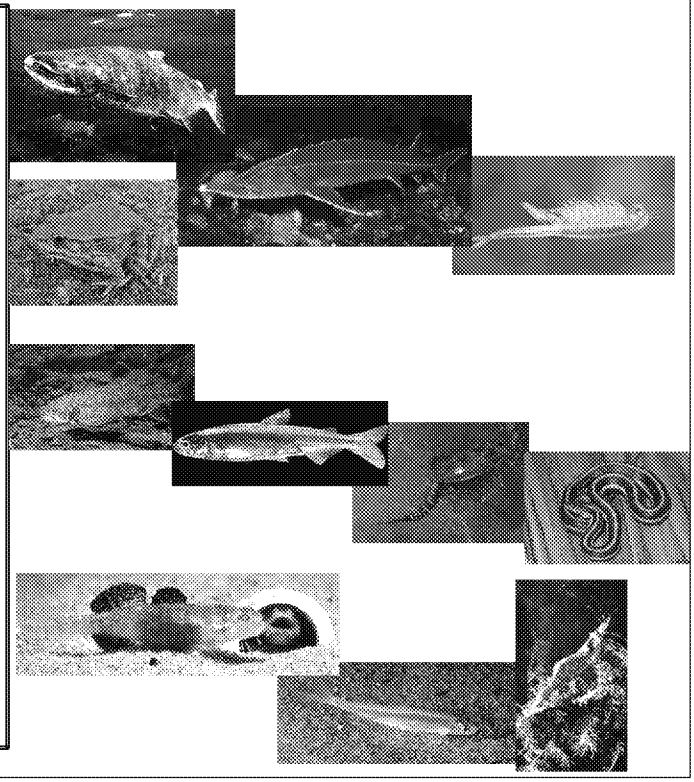
- Chinook Salmon (*Oncorhynchus tshawytscha*)
  - Sacramento River winter-run
  - Central Valley spring-run
- Southern North American Green Sturgeon (*Acipenser medirostris*)
- Fairy shrimp (*Branchinecta lynchi*)
- California Red-legged Frog (*Rana draytonii*)

Tier 2

- Central Valley steelhead (*Onchoryncus mykiss*)
- Delta Smelt (*Hypomesus transpacificus*)
- Tadpole shrimp (*Lepidurus packardi*)
- Garter snake (*Thamnophis gigas*)

Tier 3

- Tidewater goby (*Eucyclogobius newberryi*)
- San Francisco Longfin Smelt (*Spirinchus thaleichthys*)
- California Freshwater Shrimp (*Syncaris pacifica*)



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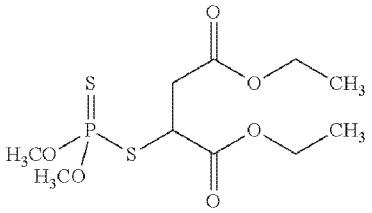
Priority Chemicals

- 40 chemicals, priority tiered
- OPP/Region 9 priorities
- Diverse chemical class
- Varying available data



Malathion

Chemical	CAS	Use pattern	Chemical Class	Tier
Bifenthrin	82657043	Insecticide	pyrethroid	1
Carbaryl	63252	Insecticide	carbamate	1
Chlorpyrifos	2921882	Insecticide	organophosphate	1
Cypermethrin	52315078	Insecticide	pyrethroid	1
Diazinon	333415	Insecticide	organophosphate	1
Fipronil	120068373	Insecticide	phenylpyrazole	1
Imidacloprid	138261413	Insecticide	neonicotinoid	1
Malathion	121755	Insecticide	organophosphate	1
Methomyl	16752775	Insecticide	carbamate	1
Permethrin	52645531	Insecticide	pyrethroid	1
Thiobencarb	28249776	Herbicide	carbamate	1
Deltamethrin	52918635	Insecticide	pyrethroid	2
Captan	133062	Fungicide	thiophthalimide	2
Chlorothalonil	1897456	Fungicide	chloronitrile	2

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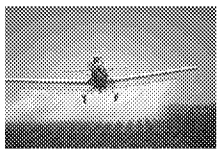
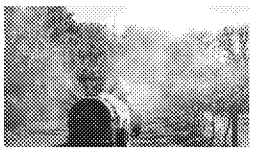
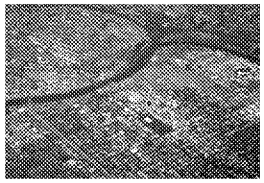
Exposure Modeling Subtask





Pesticide Use
- Row crops
- Orchards
- Urban
- Rice

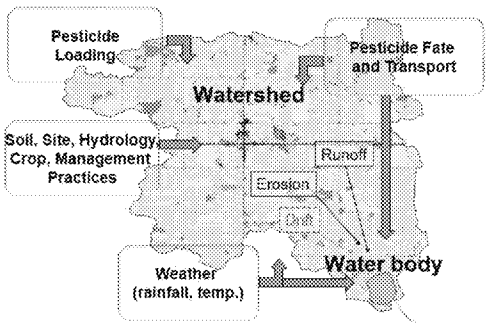


Habitat type
- Streams
- Delta
- Vernal pools

Exposure Modeling Subtask

- Spatial Aquatic Model (SAM; agriculture)
  - Watershed based model for surface water
  - Improve approach for higher-tiered assessments
  - Where and when exceedance will occur
  - Daily estimates

- Pesticide in Water Calculator (PWC)
  - Urban, vernal pool applications
  - Watershed delineation using Soil and Water Assessment Tool (SWAT)
  - Crop parameters
  - Weather data
  - Physicochemical properties



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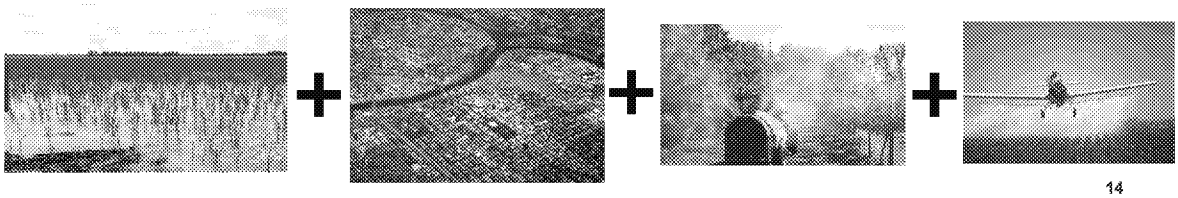
Exposure Modeling Subtask

- Rice
  - Collaborations: Martin Locke, Ron Bingner, USDA; Minghua Zhang, Ruoyu Wang, UC-Davis
  - RiceRQ; Pesticide in Flooded Application Model (PFAM) and other candidate models
- Comparison to monitoring data
- Cumulative exposure
  - additive exposure from multiple applications



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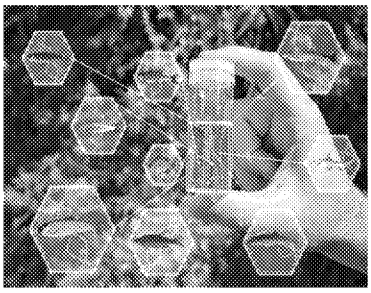
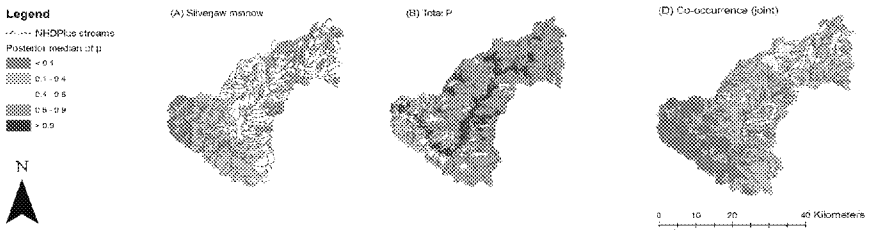
Co-Occurrence Subtask



- Environmental DNA (eDNA)
  - Species occurrence
  - Evaluate the use of eDNA to validate and improve risk models for specific endangered species
  - Comparison to predicted occupancy and existing monitoring data.

- Co-occurrence modeling
  - Data-driven, joint distribution modeling to predict and map coincidence of contaminants and sensitive species
  - Integrate with exposure models
  - High-resolution perspective (spatio-temporal dynamics / uncertainty)
    - Daily estimates of probability of co-occurrence across catchments



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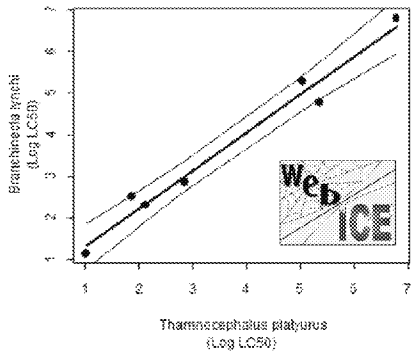
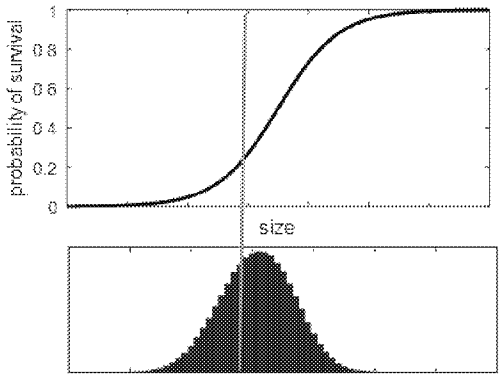
Effects Modeling Subtask

- Web-based Interspecies Correlation Estimation
  - Sensitivity of untested species
- Fish translator
  - Model that integrates the results of FIFRA (Part 158) studies to estimate population-relevant endpoints for fish exposed to pesticides
  - Apply existing FIFRA – based studies (ELS & LC50) in population-level risk assessment
- Surrogacy and minimal data
  - Scenario specific



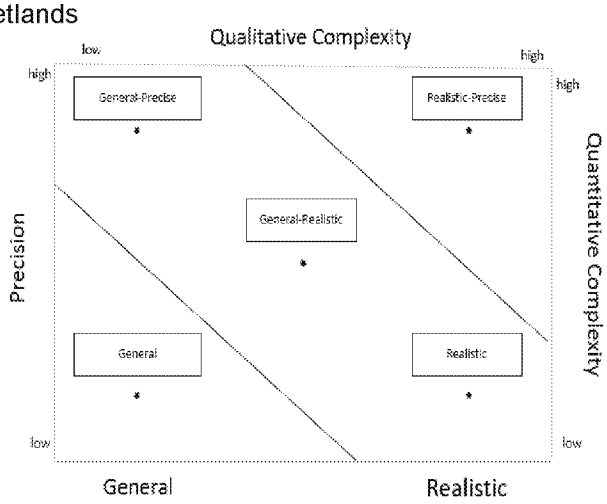


Integration Subtask

- Population models
  - Crustacean population model for time-varying exposures
  - Salmon population model in collaboration with NOAA
  - *A Framework for Linking Population Model Development with Ecological Risk Assessment Objectives.*

- Critical habitat – Geographically Isolated Wetlands
  - Vernal pools

- Weight of Evidence
  - Decision Science



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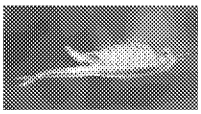
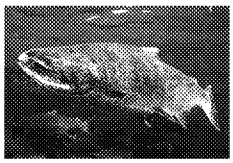
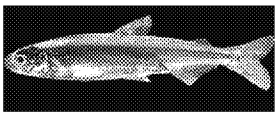
Pulling Pieces Together: Probabilistic Assessments

Probabilistic species risk assessment for one or more endangered California aquatic species

- Fairy shrimp (critical habitat focus)
  - PWC
  - eDNA
  - Lab, field, Web-ICE
  - Population model
- Salmon
  - Cumulative exposure from all modeling sources
  - Population model vs. “traditional” analysis
- Delta smelt
  - Cumulative exposure from all modeling sources
  - Co-occurrence model
  - eDNA
  - Fish translator

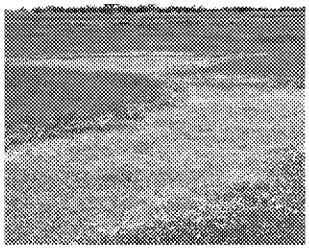






Tier 1 chemicals: Carbaryl, methomyl, bifenthrin, thiobencarb

Fairy Shrimp & Vernal Pools



Exposure estimates
- PWC
- RiceRQ
- Field samples for chemical detection (water, soil)

Co-occurrence
- eDNA

Effects estimates
- Toxicity testing
- Web-ICE estimates

Integration
- VP fairy shrimp (meta)population model
- Framework for critical habitat assessment using community-level protection goals and weight of evidence approaches

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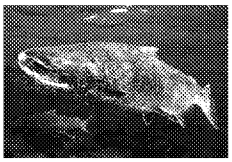
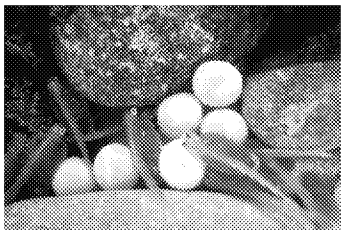
Salmon



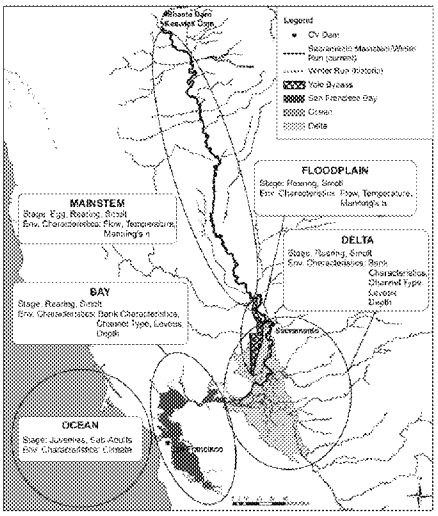


Exposure estimates
- SAM
- RiceRQ
- PWC for Urban applications
- Monitoring data

Co-occurrence
- NA

Effects estimates
- Literature

Integration
- Population model with NOAA collaboration
- Demonstration of models of varying complexity
- Comparison to traditional ERA



Office of Research and Development
National Center for Computational Toxicology

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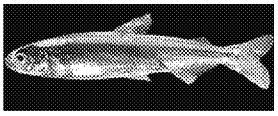
Delta Smelt

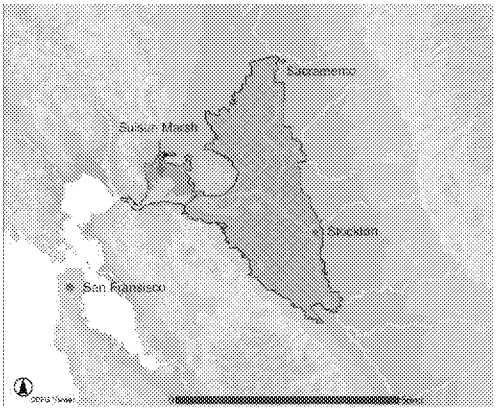
Exposure estimates
- Cumulative exposure from all sources
- Monitoring data

Co-occurrence
- Probabilistic modeling
- eDNA

Effects estimates
- Fish Translator

Integration
- Do we need population models?





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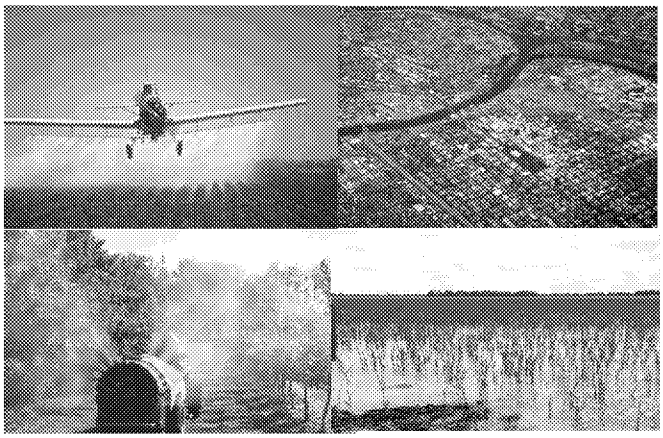
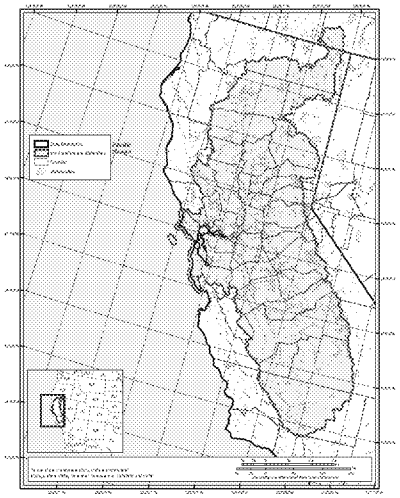
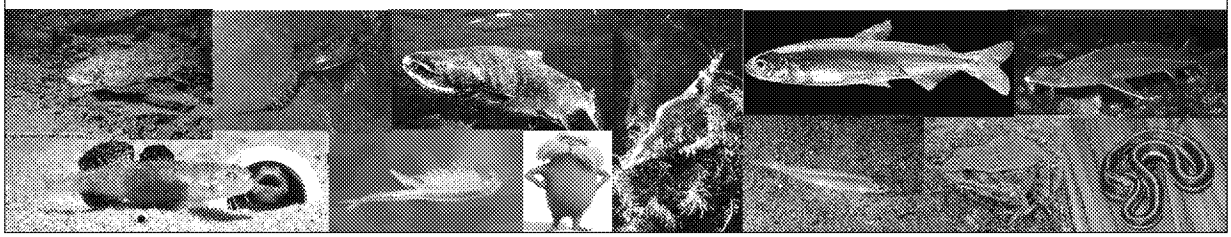
Questions?







Appointment

From: Garber, Kristina [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=C4FBFBF6569041F4BD559765E027AA31-KRISTINA GARBER]
Sent: 4/16/2018 4:20:35 PM
To: Garber, Kristina [Garber.Kristina@epa.gov]; Kanarek, Andrew [Kanarek.Andrew@epa.gov]; Kyle, Lee [Kyle.Lee@epa.gov]; Odenkirchen, Edward [Odenkirchen.Edward@epa.gov]
CC: Sankula, Sujatha [Sankula.Sujatha@epa.gov]
BCC: DCRoomPYS10731/Potomac-Yard-One [DCRoomPYS10731@epa.gov]

Subject: discuss pop modeling slides
Attachments: popmod_briefing_4.18.18_kg_ark.pptx
Location: DCRoomPYS10731/Potomac-Yard-One

Start: 4/17/2018 3:00:00 PM
End: 4/17/2018 4:00:00 PM
Show Time As: Busy

I'd like to talk through the population modeling slides for the briefing with Marietta. Draft slides are attached.

Applications of Population Modeling in Ecological Risk Assessment of Pesticides

April 18, 2018

Objective of this briefing

- Provide EFED management update on population model development
- Discuss Crop Life America RISE panel on population modeling
- Get EFED management input on next steps for population model development efforts

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Appointment

From: Anderson, Brian [Anderson.Brian@epa.gov]
Sent: 2/14/2018 3:36:43 PM
To: Anderson, Brian [Anderson.Brian@epa.gov]; Panger, Melissa [Panger.Melissa@epa.gov]; Garber, Kristina [Garber.Kristina@epa.gov]; Rossmeisl, Colleen [Rossmeisl.Colleen@epa.gov]; Connolly, Jennifer [Connolly.Jennifer@epa.gov]; Lennartz, Steven [Lennartz.Steven@epa.gov]; Eckel, William [Eckel.William@epa.gov]; Sims, Diann [Sims.Diann@epa.gov]; Becker, Jonathan [Becker.Jonathan@epa.gov]; Paisley-Jones, Claire [Paisley-Jones.Claire@epa.gov]; Miller, Wynne [Miller.Wynne@epa.gov]; Myers, Clayton [Myers.Clayton@epa.gov]; Echeverria, Marietta [Echeverria.Marietta@epa.gov]; Guilaran, Yu-Ting [Guilaran.Yu-Ting@epa.gov]
CC: Suarez, Mark [Suarez.Mark@epa.gov]; Perry, Tracy [Perry.Tracy@epa.gov]; Sherman, Kelly [Sherman.Kelly@epa.gov]; Schroeder, Carolyn [Schroeder.Carolyn@epa.gov]; Peck, Charles [Peck.Charles@epa.gov]

Subject: use and usage - pre-meet with DDs to go over slides and plan
Attachments: Usage_step2_2-14-18(BEAD scientists).pptx; Why Usage Data Are Important (3).pptx
Location: DCRoomPYS9100/Potomac-Yard-One

Start: 2/14/2018 4:00:00 PM
End: 2/14/2018 5:00:00 PM
Show Time As: Busy

Please forward to anyone I missed

Proposed method for incorporating usage data into revised ESA method

2/14/18 DRAFT

Presentation to BEAD scientists

Internal deliberative, do not cite or distribute

1

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EFED ESA team

- Amy Blankinship
- Jen Connolly
- Elizabeth Donovan
- Bill Eckel
- Kris Garber
- Ethan Harwood
- Steve Lennartz
- Melissa Panger
- Chuck Peck
- Colleen Rossmeisl
- Christina Wendel
- Katrina White

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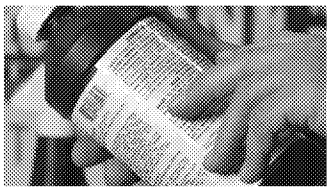
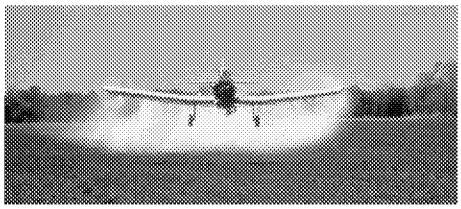
Why Usage Data Are Important

Terms

- **USE** – applications as described on the labels



- **USAGE** – how applications are actually made when a pesticide is being used



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Appointment

From: Rossmeisl, Colleen [Rossmeisl.Colleen@epa.gov]
Sent: 11/20/2018 2:14:44 AM
To: Rossmeisl, Colleen [Rossmeisl.Colleen@epa.gov]; Panger, Melissa [Panger.Melissa@epa.gov]; Patrice Ashfield [patrice_ashfield@fws.gov]; Anderson, Brian [Anderson.Brian@epa.gov]; Nancy Golden [nancy_golden@fws.gov]; Sims, Diann [Sims.Diann@epa.gov]; Becker, Jonathan [Becker.Jonathan@epa.gov]; Myers, Clayton - ARS [clayton.myers@ars.usda.gov]; Paisley-Jones, Claire [Paisley-Jones.Claire@epa.gov]; David Baldwin [david.baldwin@noaa.gov]; Hill2, Elizabeth - ARS [elizabeth.hill2@ars.usda.gov]; Connolly, Jennifer [Connolly.Jennifer@epa.gov]; Lennartz, Steven [Lennartz.Steven@epa.gov]; Peck, Charles [Peck.Charles@epa.gov]; Suarez, Mark [Suarez.Mark@epa.gov]; Ryan DeWitt [ryan.dewitt@noaa.gov]; Kathryn Bissell [kathryn_bissell@fws.gov]; jennifer_thompson@fws.gov; Garber, Kristina [Garber.Kristina@epa.gov]; Tony Hawkes [tony.hawkes@noaa.gov]; Andrew Raabe [andrew_raabe@fws.gov]; Sara Pollack [sara_pollack@fws.gov]; Leona Laniawe [leona_laniawe@fws.gov]; Keith Paul [keith_paul@fws.gov]; Burk, Rosemary [rosemary_burk@fws.gov]; Hill2, Elizabeth - OCE [Elizabeth.Hill2@oce.usda.gov]; Thomas Hooper - NOAA Federal [thomas.hooper@noaa.gov]

Subject: ESA Interagency Usage Data Methodology Workgroup
Attachments: Canceled: ESA Interagency Usage Data Methodology Workgroup ; Untitled Attachment; Untitled Attachment; Untitled Attachment; Canceled: ESA Interagency Usage Data Methodology Workgroup
Location: DCRoomPYS7100/Potomac-Yard-One

Start: 11/20/2018 7:00:00 PM
End: 11/20/2018 8:00:00 PM
Show Time As: Busy

Recurrence: Weekly
Occurs every Tuesday from 2:00 PM to 3:00 PM effective 11/20/2018 until 2/26/2019.

Agenda to be forwarded each week.
Thanks!
Colleen

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Update on incorporation of pesticide usage data into the Action Area

12/10/18

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Appointment

To: Panger, Melissa [Panger.Melissa@epa.gov]; Anderson, Brian [Anderson.Brian@epa.gov]; Nancy Golden [nancy_golden@fws.gov]; Sims, Diann [Sims.Diann@epa.gov]; Becker, Jonathan [Becker.Jonathan@epa.gov]; Paisley-Jones, Claire [Paisley-Jones.Claire@epa.gov]; Connolly, Jennifer [Connolly.Jennifer@epa.gov]; Lennartz, Steven [Lennartz.Steven@epa.gov]; Peck, Charles [Peck.Charles@epa.gov]; Suarez, Mark [Suarez.Mark@epa.gov]; Ryan DeWitt [ryan.dewitt@noaa.gov]; jennifer_thompson@fws.gov; Keith Paul [keith_paul@fws.gov]; Patrice Ashfield [patrice_ashfield@fws.gov]; Rossmeisl, Colleen [Rossmeisl.Colleen@epa.gov]; Patrice Ashfield [patrice_ashfield@fws.gov]; Panger, Melissa [Panger.Melissa@epa.gov]; Anderson, Brian [Anderson.Brian@epa.gov]; Nancy Golden [nancy_golden@fws.gov]; Sims, Diann [Sims.Diann@epa.gov]; Becker, Jonathan [Becker.Jonathan@epa.gov]; Myers, Clayton - ARS [clayton.myers@ars.usda.gov]; Paisley-Jones, Claire [Paisley-Jones.Claire@epa.gov]; David Baldwin [david.baldwin@noaa.gov]; Hill2, Elizabeth - ARS [elizabeth.hill2@ars.usda.gov]; Connolly, Jennifer [Connolly.Jennifer@epa.gov]; Lennartz, Steven [Lennartz.Steven@epa.gov]; Peck, Charles [Peck.Charles@epa.gov]; Suarez, Mark [Suarez.Mark@epa.gov]; Ryan DeWitt [ryan.dewitt@noaa.gov]; Thomas Hooper - NOAA Federal [thomas.hooper@noaa.gov]; Kathryn Bissell [kathryn_bissell@fws.gov]; jennifer_thompson@fws.gov; Garber, Kristina [Garber.Kristina@epa.gov]; Tony Hawkes [tony.hawkes@noaa.gov]; Andrew Raabe [andrew_raabe@fws.gov]; Sara Pollack [sara_pollack@fws.gov]; Leona Laniawe [leona_laniawe@fws.gov]; Keith Paul [keith_paul@fws.gov]; Burk, Rosemary [rosemary_burk@fws.gov]; Hill2, Elizabeth - OCE [Elizabeth.Hill2@oce.usda.gov]

Location: DCRoomPYS7100/Potomac-Yard-One

Start: 12/4/2018 7:00:00 PM
End: 12/4/2018 8:00:00 PM
Show Time As: Busy

Recurrence: (none)
every Tuesday from 2:00 PM to 3:00 PM

Hi all –

As Brian indicated in his email, a new draft agenda was discussed by the managers last week for the upcoming briefing. That agenda is below and we can talk about it this afternoon – time allotments may need to shift depending on where certain material will be covered.

Thank you for all the comments on the presentation! There are a lot of different comments and we have created some new maps as well (as have the other agencies)... I am trying to address as much as I can and pull them together in one slide deck, roughly following the outline below. For the meeting today, we would like to at least finalize (or nearly finalize) the slides where we have some interagency agreement on methods.

Thanks –
Colleen

<u>Proposed agenda for December 10 briefing</u>:

- Introduction: 5 min (Brian and Patrice)
  - Purpose: We are going to present progress on the action area development related to agricultural uses of malathion.
  -
  -
  -
  -

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    - Each agency will give their perspective on the pros and cons of the methods at the end of the presentation.

- Presentation – (EPA and FWS). 30 min
- Perspectives on pros and cons of the methodology and recommendations
    - EPA: 10 minutes
    - FWS: 10 minutes
    - NMFS: 10 minutes
    - USDA: 10 minutes
- Discussion: 30 minutes
- Wrap up and next steps: 15 minutes

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Message

From: Etterson, Matthew [Etterson.Matthew@epa.gov]
Sent: 4/12/2019 3:32:19 PM
To: Garber, Kristina [Garber.Kristina@epa.gov]
Subject: RE: common effects datasets

THANK YOU!

-----Original Message-----
From: Garber, Kristina
Sent: Friday, April 12, 2019 10:32 AM
To: Etterson, Matthew <Etterson.Matthew@epa.gov>
Subject: RE: common effects datasets

The data should be in tables in the ssd attachments at the following links:

https://www.epa.gov/endangered-species/biological-evaluation-chapters-chlorpyrifos-esa-assessment#appendices 2

https://www.epa.gov/endangered-species/biological-evaluation-chapters-diazinon-esa-assessment#append2

https://www.epa.gov/endangered-species/biological-evaluation-chapters-malathion-esa-assessment#append2

-----Original Message-----
From: Etterson, Matthew
Sent: Friday, April 12, 2019 11:27 AM
To: Garber, Kristina <Garber.Kristina@epa.gov>
Subject: RE: common effects datasets

Yes, I wondered about that. Good idea. Are they on the docket?

-----Original Message-----
From: Garber, Kristina
Sent: Friday, April 12, 2019 8:47 AM
To: Etterson, Matthew <Etterson.Matthew@epa.gov>
Subject: Re: common effects datasets

Ok. Another option would be to use the datasets from the ESA assessments for chlorpyrifos, malathion and diazinon. They are publicly available

> On Apr 11, 2019, at 7:51 PM, Etterson, Matthew <Etterson.Matthew@epa.gov> wrote:
>
> No don't worry about it. I have versions of the files, but was wondering whether there are public versions sitting out there somewhere. It's not a big deal. Jiust looking for some data sets that people can use for learning the SSDToolbox. I have downloaded some datesets from Ecotox that'll work fine. They aren't quite as comprehensive as the datasets that we compiled from multiple sources, but for demonstration purposes I think they're good enough.
>
> Thanks!
> Matt
> _____
> From: Garber, Kristina
> Sent: Thursday, April 11, 2019 4:55 PM
> To: Etterson, Matthew
> Subject: Re: common effects datasets
>
> We pulled the data from WEBICE, ECOTOX and existing criteria docs. I don’t think that we posted them. I think that Russ Erickson had a version with more study info. I can look through my files to see if I have the data. Would you like me to do so?
>
> On Apr 10, 2019, at 5:26 PM, Etterson, Matthew <Etterson.Matthew@epa.gov<mailto:Etterson.Matthew@epa.gov>> wrote:
>
> Hi Kris,
> I was just poking around the docket for the common effects projects looking to see whether the acute datasets that we used for the SSD analyses were posted there anywhere. Are those datasets considered in the public domain? Are there any versions of those that you know about that have study attribution next to the acute values?
> Thanks,
> Matt

Message

From: Etterson, Matthew [Etterson.Matthew@epa.gov]
Sent: 4/18/2019 5:26:03 PM
To: Garber, Kristina [Garber.Kristina@epa.gov]
Subject: RE: common effects datasets

fish

-----Original Message-----
From: Garber, Kristina
Sent: Thursday, April 18, 2019 12:25 PM
To: Etterson, Matthew <Etterson.Matthew@epa.gov>
Subject: Re: common effects datasets

Are you interested in the fish or the invert data?

> On Apr 18, 2019, at 1:22 PM, Etterson, Matthew <Etterson.Matthew@epa.gov> wrote:
>
> Ok, thanks Kris. Fair enough. I'll stand by and see whether you find it online. I can get by without it, but it's an interesting dataset.
> Matt
>
> -----Original Message-----
> From: Garber, Kristina
> Sent: Thursday, April 18, 2019 12:21 PM
> To: Etterson, Matthew <Etterson.Matthew@epa.gov>
> Subject: Re: common effects datasets
>
> Hi Matt,
>
> I remember now that some of the appendices did not include the tables with the raw data. Let me check another location online and see if I can find the data for the SSD. If it’s not available publicly, I suggest that you do not distribute the data.
>
> Kris
>
>> On Apr 18, 2019, at 12:19 PM, Etterson, Matthew <Etterson.Matthew@epa.gov> wrote:
>>
>> Thanks Kris,
>> So, here's a follow-up question. The Diazinon SSD analysis for the BEs did not include the dataset. Turns out I did that analysis for Scott Glaberman, so I have the file sitting on my computer. Do you think it's OK for me to use and distribute with the SSD Toolbox? It has some interesting properties that would be useful. But if you think I should avoid it, that's OK.
>> Thanks,
>> Matt
>>
>> -----Original Message-----
>> From: Garber, Kristina
>> Sent: Friday, April 12, 2019 10:32 AM
>> To: Etterson, Matthew <Etterson.Matthew@epa.gov>
>> Subject: RE: common effects datasets
>>
>> The data should be in tables in the ssd attachments at the following links:
>>
>> https://www.epa.gov/endangered-species/biological-evaluation-chapters-chlorpyrifos-esa-assessment#appendices 2
>>
>> https://www.epa.gov/endangered-species/biological-evaluation-chapters-diazinon-esa-assessment#append2
>>
>> https://www.epa.gov/endangered-species/biological-evaluation-chapters-malathion-esa-assessment#append2
>>
>> -----Original Message-----
>> From: Etterson, Matthew
>> Sent: Friday, April 12, 2019 11:27 AM
>> To: Garber, Kristina <Garber.Kristina@epa.gov>
>> Subject: RE: common effects datasets
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>>
>> -----Original Message-----
>> From: Garber, Kristina
>> Sent: Friday, April 12, 2019 8:47 AM

>> To: Etterson, Matthew <Etterson.Matthew@epa.gov>
>> Subject: Re: common effects datasets
>>
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>>> No don't worry about it. I have versions of the files, but was wondering whether there are public versions sitting out there somewhere. It's not a big deal. Jiust looking for some data sets that people can use for learning the SSDToolbox. I have downloaded some datesets from Ecotox that'll work fine. They aren't quite as comprehensive as the datasets that we compiled from multiple sources, but for demonstration purposes I think they're good enough.
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>>> Thanks!
>>> Matt
>>> _____
>>> From: Garber, Kristina
>>> Sent: Thursday, April 11, 2019 4:55 PM
>>> To: Etterson, Matthew
>>> Subject: Re: common effects datasets
>>>
>>> We pulled the data from WEBICE, ECOTOX and existing criteria docs. I don’t think that we posted them. I think that Russ Erickson had a version with more study info. I can look through my files to see if I have the data. Would you like me to do so?
>>>
>>> On Apr 10, 2019, at 5:26 PM, Etterson, Matthew <Etterson.Matthew@epa.gov<mailto:Etterson.Matthew@epa.gov>> wrote:
>>>
>>> Hi Kris,
>>> I was just poking around the docket for the common effects projects looking to see whether the acute datasets that we used for the SSD analyses were posted there anywhere. Are those datasets considered in the public domain? Are there any versions of those that you know about that have study attribution next to the acute values?
>>> Thanks,
>>> Matt

BASF
We create chemistry

U.S. Environmental Protection Agency
Document Processing Desk
Office of Pesticide Programs
Room S4900, One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202-4501

August 15, 2019

Subject: Comments on EPA Proposed Revised Method for National Level Endangered Species Risk Assessment Process for Biological Evaluations of Pesticides
Comments Submitted at www.regulations.gov,
Docket ID EPA-HQ-OPP-2019-0185

BASF appreciates the opportunity to submit comments on the draft proposed methodology for pesticide assessments conducted pursuant to the Endangered Species Act (ESA) developed by the United States Environmental Protection Agency (EPA). BASF commends EPA for their efforts to improve the scientific defensibility of the methodology utilized in the three pilot Biological Evaluations (BEs) for chlorpyrifos, diazinon, and malathion. The draft proposed methodology is a positive step toward developing a process that can distinguish between species that are likely or not likely to be adversely impacted by certain aspects of modern-day agriculture.

Although the draft proposed methodology represents an improvement compared to the pilot BEs, it still adopts some of the same technical deficiencies, provides too much discretion to the reviewer in the weight-of-evidence determinations, and is often vague and fails to provide sufficient details to allow for proper understanding. Providing informed comments on certain aspects of the draft proposed methodology was difficult (if not impossible) simply because the methodology could not be followed without actual seeing it played out in an actual assessment.

For this reason, BASF asks that EPA would consider a path forward that involves: A) holding an in-person workshop where participants could work through the process using actual data to see how the assessment would play out in the context of an actual risk assessment; and B) allowing for another round of public comment once stakeholders had a thorough understanding of all aspects of the methodology so that more informed comments could be provided.

Thank you for the opportunity to provide feedback on this critical topic.

Yours sincerely,

Karen Cain

Karen Cain,
Senior Country Regulatory Manager

Attachment: BASF Reg. Doc. No. 2019/2039897

BASF Corporation
26 Davis Drive
PO Box 13528
Research Triangle Park, NC 27709-3528

BASF COMMENTS ON EPA's PROPOSED REVISED METHOD FOR ESA PESTICIDE ASSESSMENTS

BASF Reg. Doc. No. 2019/2039897

This comment document is organized into two sections: general comments and detailed comments on specific aspects of the draft proposed methodology. The detailed section of the comments is organized in the same logical process flow as outlined in the methodology.

General Comments

Further stakeholder engagement is necessary

BASF appreciates the effort the United States Environmental Protection Agency (EPA) put forth to provide stakeholders the opportunity to provide comments on the methodology during the "Public Meeting on Revised Method for ESA Pesticide Assessments" (further referred to as the 'public meeting') held on June 10, 2019. After thoroughly reviewing the proposed methodology, BASF believes that additional stakeholder engagement by EPA is necessary to allow for true understanding of the methodology. While the proposed methodology represents a significant improvement of the methods employed in the pilot BEs, there are many areas where the proposals are either too vague to allow for proper understanding or describe a series of calculations for which it is very difficult (if not impossible) to follow to their logical conclusion (*i.e.*, how they would result in meaningful refinements to the risk assessment) without looking at an actual assessment. This is particularly true for the calculation and application of state-level percent crop treated (PCT) factors, composite factor, refining the off-site transport zone, summation of state-level spatial overlaps, and the nationwide probabilistic modeling process. Ideally, this engagement would be in the form of another in-person meeting or workshop where EPA could walk stakeholders through some of the more complex aspects of the process using actual data. Once the methodology is fully understood, stakeholders should be afforded the opportunity to provide more-informed comments on the draft proposed methodology. This would help to establish transparency in the process and to demonstrate confidence in how the proposed methodology would actually work in a nationwide endangered species assessment.

Use of GOPHER for ESA

As a member company of the Federal Endangered Species Task Force (FESTF), BASF has been involved with the development of GOPHER, a web-based platform that houses all of the datasets (both tabular and spatial) required to conduct complex nationwide endangered species assessments. BASF has firsthand knowledge of the key advantages that GOPHER affords for ESA, specifically:

- Data quality: All of the spatial data in GOPHER have been converted to a common projection to ensure alignment of spatial data to avoid erroneous overlaps.
- Efficiency:
    - GOPHER currently includes many functionalities that rely on pre-processed spatial layers, including screening-level county co-occurrence maps and queries, and nearest proximity calculations for each use/species combination in the continental United States, which required a very significant amount of computational power and time to create.
    - Species information from the Services has been compiled and uploaded to GOPHER, including information on species traits, extinction/extirpation, dormancy and other

temporal behavior patterns, critical habitats and their primary constituent elements (PCEs), and dietary preferences, among other information.

- Reproducibility: All of the data in GOPHER are updated at regular time intervals that are appropriate for each individual dataset (*e.g.*, once per year for Cropland Data Layer, three times per year for FWS range files) and archived once superseded by a newer version.

The detailed comments to the proposed ESA methodology submitted by FESTF contain a thorough list of examples of how GOPHER can be utilized to improve the quality, efficiency, transparency, and reproducibility of EPA's ESA assessments.

Label mitigations

The draft proposed methodology only refers to the product label in terms of the labeled uses and the maximum application rate for each use. While there are often many labels for a given active ingredient, they often include common language regarding application instructions that can he used in Step 2 to help refine the analysis, including:

- Soil type restrictions: could be used to refine the use footprint in Step 2 and PWC modeling (*e.g.*, don't include scenarios with restricted soil types in the probabilistic modeling);
- Geographic restrictions (*e.g.*, don't apply in certain counties/states): could be used to refine the use footprint in Step 2
- Spray drift mitigations (*e.g.*, environmental conditions, boom height, nozzle, boom pressure); could be used to refine Step 2 offsite transport zone and PWC modeling;
- No-spray buffers: could be used to refine PWC modeling; and
- Vegetative filter strips: could be used to refine PWC modeling.

Qualitative assessments

In the draft proposed methodology, EPA proposes the use of qualitative methods several times due to lack of quantitative data or accepted models to simulate processes. These include qualitative evaluation of:

- Step 1 – potential use sites; page 10: Use footprints for uses without reliable spatial use information;
- Step 1 – offsite transport zone; page 12: Downstream impacts to aquatic species in medium- and high-flowing bins located in areas that have been removed from consideration during Steps 1 and 2 based solely on usage data;
- Step 2b – precision of the species range data (GIS layers; page 26-7): proximity (*i.e.*, whether a species is within 2600 ft) of certain habitat types utilized by a species to potential use sites (for species with range is wholly or partially at the county/state level that is not found in 'many' habitat types and not exclusively on federal lands) given lack of spatial data; and
- Step 2b – confidence in the exposure assessment; page 28-9): confidence in the exposure assessment (*i.e.*, how well the conceptual model of the relevant exposure models(s) matches up with the specific species being assessed).

While BASF generally agrees with the use of qualitative assessments as proposed in the draft methodology, more information is needed to guide how these qualitative assessments would proceed, specifically: how the complex processes involved with downstream dilution will be assessed (step 1 – offsite transport zone) and how the supplemental species range will be created given lack of necessary quantitative data (Step 2b – precision of the species range data).

Additionally, EPA should consider the use of qualitative processes for minor crop uses rather than relying on quantitative information (Cropland Data Layer, USDA Census of Agriculture) to group them into use data layers, which greatly distorts the extent of their spatial footprints.

Concentrated flow

During the "Public Meeting on Revised Method for ESA Pesticide Assessments" held on June 10, 2019 there was discussion about the topic of concentrated flow or channelized flow (CF). In the meeting, EPA described CF as a "flowing waterbody". It was not clear from the discussion how this flowing waterbody will be addressed, if at all, in ESA.

It seems that CF flow could be relevant in two contexts within ESA: 1) the transition from uniformly-distributed sheet flow to CF flow is assumed to occur after 30 m (100 ft), which is the justification for setting the terrestrial plant exposure zone (T-PEZ) equal to a width of 30 m in the Plant Assessment Tool (PAT) model; and 2) CF is a potential route of exposure to aquatic species outside of the field (*i.e.*, in the offsite transport zone). Given that the 30 m threshold for the transition from sheet flow to CF as described in #1 is a standard hydrologic assumption, we do not currently have questions about the use of 30 m for the delineation of the width of T-PEZ. Our questions regarding CF are associated with #2.

We request that EPA provide additional information about how CF will be evaluated as an exposure route (Note: The proposed methodology makes no mention of runoff or CF as it relates to the offsite transport zone), specifically:

- How will CF be simulated and which bin will it fit into?
- What is the off-field distance that CF could reasonably be expected to transport chemicals, and how would the concentration of chemicals in CF be affected by attenuation processes such as dilution, sorption, infiltration, and degradation?
- How does CF affect commonly used mitigations such as vegetated filter strips (VFS)?

Very long VFSs (*e.g.*, 100 ft or more in the direction of flow) could be susceptible to CF based on the threshold noted above, but it is safe to assume that shorter VFSs would be less susceptible to CF and would still be effective at removing pesticides transported in both dissolved and bound phases. Therefore, it is important to retain VFS in the runoff mitigation toolbox and to prescribe reasonable VFS lengths (*i.e.*, < 100 ft) that would be less susceptible to CF than VFSs with lengths greater than 100 ft.

It should also be acknowledged that there are programs that encourage farmers to address CF. Because of the destructive nature of CF to farmland, farmers are motivated to avoid, or remediate, extensive CF on their fields. There are national programs in place that require farmers on highly erodible land (HEL) to be in conservation compliance in order to receive some farm program benefits. Compliance includes the requirement to address ephemeral gullies that are associated with CF[1]. As another example, a pilot project in five states is providing additional financial assistance to farmers to address ephemeral gullies on HEL[2]. The best management practices supported by programs such as these would reduce off-field exposure due to CF and should be accounted for in the risk assessment process.

[1] See: https://www.nrcs.usda.gov/wps/portal/nrcs/detail/wa/programs/farmbill/cc/?cid=nrcseprd1331645
[2] See: https://www.nrcs.usda.gov/wps/portal/nrcs/detail/ne/newsroom/releases/?cid=NRCSEPRD1462837

Detailed Comments

Step 1: May Affect (MA) / No Effect (NE) Determinations

Step 1C: Overlap analysis: 1% overlap of species range and action area

Potential use sites: Grouping of crops into use data (page 9)
The process of identifying potential use sites relies on the United States Department of Agriculture's (USDA) Cropland Data Layer (CDL). EPA aggregates these classes into the following 13 use data layers (UDLs): corn, cotton, rice, soybeans, wheat, vegetables and ground fruit, other grains, other row crops, other crops, pasture/hay, citrus, vineyards and other orchards. The stated purpose of this grouping is "to improve the accuracy of the data and to help ensure that agricultural fields that are mis-identified with respect to the crop being grown are captured in the aggregated spatial layer."

BASF does not agree that grouping certain crops together into UDLs improves the accuracy of the data. The only scenario that would seem to improve the overall capture of all crops within a UDL would be the case where pixels that should be classified for a given crop in the UDL are systematically mis-identified as belonging solely to other crops within the same UDL. EPA has not provided any data indicating that this case occurs with any frequency for the prescribed "low-confidence crop" UDLs, if it occurs at all. Furthermore, grouping of low-acreage crops can lead to vast overestimation of crop area by accounting for all of the additional acreage associated with every other crop included in the UDL. For example, consider a product label that allows for use on a single crop within a "low-accuracy UDL". This grouping procedure will systematically overestimate the crop footprint by not accounting:

- For all of the remaining crops in the UDL that are not on the product label;
- For use restrictions for crops that are on the product label;
- That not all fields are treated with pesticide in any given year;
- That not all fields that are treated with pesticide are treated with a given active ingredient;
- That not all fields treated with a given active ingredient are treated on the same day; and
- The maximum application rate/number of applications is not always used.

Vastly distorting the crop footprint to include areas not relevant for the crop and then assuming that all fields are treated with a single AI at the maximum application rate on the same day is not a valid approach even for a screening assessment, and in fact, many of the comments on the pilot BEs reflected as such.

BASF respectfully requests that EPA revisit this approach, as this has the potential to significantly overestimate the action area for minor crops and consider other sources of information to inform accuracy such as the CDL state/crop accuracy metadata and USDA Census of Agriculture (CoA) coefficient of variation (CV) estimates.

Potential use sites: 'Region growing' to align CDL and CoA usage data (page 9)
The revised methodology continues to adopt the 'region growing' concept employed in the pilot BEs to identify potential use sites for each UDL. This process involves increasing the spatial footprint of CDL use sites until area of the use sites will "meet or exceed" the cropped area reported in the CoA. No details are provided in the proposed methodology on how this process will be carried out. For instance, will the specific methodology outlined in the pilot BEs be used in which use footprints are increased one 30-meter grid cell at a time, using the CDL cultivated layer as a mask, until the CoA acreage is met?

If so, the same concerns apply to the proposed methodology as for the pilot BEs, namely that using the CDL cultivated data layer as a mask will include areas that were misclassified as being cultivated in the use site footprint. The cultivated layer includes any pixel that has been classified as being cultivated in 2 of 5 years (or in the most recent year). Therefore, any erroneous classification of an individual grid cell has the potential (or certainty if in the most-recent year's CDL dataset) to be included in the cultivated data layer. This has the potential to increase the use site footprint to areas that are not cultivated, especially for minor crops with lower-reported CDL accuracies and a high number of crops being aggregated into a single UDL.

In addition, it is unclear under what circumstances would necessitate region growing to be used to increase the spatial use footprints so that the total use area would *exceed* the CoA estimate. EPA should provide specific reasons why such an adjustment is necessary (*i.e.*, rather than adjusting the spatial use footprint to *meet* the CoA acreage) and under what specific circumstances it would be carried out.

Pesticide usage data: SUUM Memo (pages 10-11)
The compilation of pesticide usage data to refine the UDLs includes the development of the National and State Summary Use and Usage Memo (SUUM memo) by EPA, which compiles usage data from publicly-available (California Pesticide Use Reporting, USDA) and proprietary sources. The proposed methodology is the first time the public has been informed of the existence/use of these SUUM memos and, therefore, it is very important that more information is provided on how the data are collected, filtered, organized, and analyzed for use in the ESA process. In addition, the final SUUM memo for each use needs to be made publicly available to the fullest-extent possible (*i.e.*, with respect to proprietary usage data). If providing proprietary data would violate a license agreement, the data should be aggregated (with documentation) to the minimalist extent necessary to protect proprietary data and then be made publicly available.

Pesticide usage data: Refining agricultural UDLs (pages 10-11)
The CoA and SUUM memo are used to refine the agricultural UDL footprints after the 'region growing' process has been applied but prior to aggregation with non-agricultural UDLs to form the initial action area. While BASF appreciates EPA's attempt to include a process for refinement of the agricultural use areas in Step 1, the potential for refinement seems limited, particularly for UDLs containing a large number of individual agricultural uses (i.e., "low-accuracy crops").

For example, the proposed methodology will:

- Remove a county from the agricultural UDL only if all labeled agricultural uses within a UDL are not grown in the county according to CoA.
- Remove a state from the UDL only if, based on survey data, no usage occurred (based on the SUUM) for all labeled uses within a given agricultural UDL in the entire state.

The first bullet does nothing to refine the use footprint for minor uses that have been grouped together with other crops, particularly if one or more of the other crops has a large use footprint. In these cases, the agricultural UDL for these minor crops will still be vastly overestimated.

Regarding the second bullet, it is unclear why actual pesticide usage data can only be used to clear areas from the agricultural UDL at the state level. Publicly-available usage data from USDA and CAPUR, for example, are available at the county- and Public Land Survey System (PLSS)-level (typically 1-mile grid cells), respectively. Page 10 indicates that these data sources are included in the SUUM memo; therefore, this information should also be used to clear individual counties from the agricultural UDL if there is no usage.

Furthermore, under the proposed methodology, only areas with survey data indicating no usage can be used to refine the initial UDL crop footprint. This ignores the fact that many areas are not surveyed because the crops are not grown in particular location and/or region. BASF encourages EPA to make use of the CoA survey metadata, which includes descriptions of why survey locations were or were not selected. Also, any use restrictions on the existing product label should be taken into account, which can prescribe certain regions, counties, or soil types for which application is not allowed and thus could be used to refine the agricultural UDL footprint.

Step 1D: Overlap analysis: species range and federal lands

Use of <1% Overlap for NE determinations

BASF appreciates EPA's proposal to take the precision of the spatial datasets into account when determining whether spatial overlap of species range and/or critical habitat has occurred. Notwithstanding previous comments on the conservatism of the initial action area, BASF believes the 1% cutoff is a reasonable cutoff threshold. However, BASF agrees with EPA that larger cutoff thresholds (than 1%) should be considered when, for example, county/state boundaries are used to define the extent of species range as there is inherently higher inaccuracy. Use of county/state boundaries is a common occurrence in the range maps and a process should be developed to develop appropriate cutoff thresholds in these cases.

Species range overlaps completely (≥99%) with federal lands

The proposed methodology indicates existing BEs will be considered for species with range overlap ≥99% with federal lands to potentially screen out federal areas that do not allow for pesticide application and also to avoid duplication of work effort (with existing BEs). Considering that cutoff thresholds may be higher than 1% for species ranges with low accuracy (*e.g.*, county boundaries for species range as discussed in the above comment), EPA may consider a lower overlap threshold (than 99%) with federal lands in these cases. For example, if the overlap threshold for NE determination for a particular UDL/species was determined to be 3%, a 97% overlap threshold would be used to evaluate exclusive use on federal lands.

Step 2: Likely to Adversely Affect (LAA) / Not Likely to Adversely Affect (NLAA) Assessment

Step 2A: Refinement of species range and action area

Refinement of off-site transport zone due to spray drift (page 21)

While BASF believes that using AgDrift, as described on page 11 regarding the initial development of the off-site transport zone, is acceptable for developing a worst-case initial drift buffer, the use of AgDrift is not appropriate for refining drift buffers given its extreme conservatism for predicting downwind drift, particularly at distances far from the edge-of-field. The AgDrift empirical drift curves are 90th percentile fits to the Spray Drift Task Force (SDTF) data generated in the 1992/1993 assuming 20 spray passes or swaths. Given the age of the data, these studies do not include any data for drift reducing nozzles that are commonly used in modern-day agriculture (and are mandated by some regulatory labels) to reduce offsite drift. Further, the assumption that 20 swaths are required to capture drift using modern DRT is not supported by field data. When comparing an AgDrift-predicted drift deposition to the lowest NOEC for the most sensitive taxon, it is not difficult to envision many cases where little to no reduction in the drift buffer (*i.e.*, offsite transport zone) will be achieved, given that AgDrift estimates are especially conservative at large downwind distances from the treated field.

BASF recognizes that EFED does not currently have a tool to quantitatively assess drift from modern drift-reducing nozzles; however, other options exist that EPA could utilize, such as REGDISP. In addition to the SDTF data, REGDISP includes Agriculture and Agri-Food Canada (AAFC) field study data generated in 2000, 2004, and 2011, which tested drift-reducing Air Induction (AI) and Turbo TeeJet Induction (TTI) nozzles. The empirical fits to the AAFC data are still conservative but brings some realism to the drift estimates that will allow for refinement of often impractical drift estimates from AgDrift.

Further, many federal labels have specific language to reduce offsite transport vis spray drift, such as specification of nozzle type/droplet size, nozzle/boom pressure, boom height, and wind speed. This label language must be taken into account when refining the offsite transport zone due to spray drift.

Aggregate PCT calculation (page 20)

EPA states the following with respect to utilizing usage data to develop statewide percent crop treated (PCT) values, "as noted previously in Step 1, some uses are not surveyed at all and some uses are only surveyed in some states. In such cases, a surrogate assumption may be used." It is very important that EPA first check the CoA metadata to see if the area was not surveyed because the crop is not grown in state area, otherwise, surrogate data could introduce additional error into the assessment.

Using PCT to refine spray drift offsite transport zone (page 21)

The proposed methodology relies on the PCT factor for each state (based on usage data) to reduce the number of treated acres in the state through the following calculation:

$$\textit{Area treated within the state} = PCT_{STATE} \times \textit{total UCL acres within the state}$$

The process for refining drift distances for each UDL/state combination relies on a "composite factor"; which is applied to the drift area. However, no information on how the "composite factor" is calculated or how it will be applied is provided. EPA needs to provide an example of how the composite factor would be developed and applied for a case in which several UDLs (including single-crop UDLs such as corn and a UDL containing multiple crops) exist in the state, each with different maximum application rates.

Further, as written, it appears the only potential refinement of the spray drift offsite transport zone is to base the drift buffer on the use with the highest number of treated acres in the state rather than defaulting to the use with the maximum application rate (as in step 1). Therefore, UDLs with one use are afforded no opportunity for refinement at this step. For UDLs with multiple uses, if after the PCT adjustment the use with the highest treated acres is the also the use with the maximum application rate, there is also no possible refinement.

As a general comment at this point in the proposed process, there are too many proposed calculations to comprehend what impact they would have, in total, in an actual risk assessment and if the proposed methodology would result in conclusions that are grounded in any reality. For this reason, it is critical that EPA further engage stakeholders in the form of an in-person meeting/workshop followed by an opportunity to provide additional comments.

Concentration of statewide treated acres within the species range (page 20-21).

On page 21 EPA states (emphasis added): *Note that part of the weight of evidence (discussed below) will employ alternative assumptions of how treated acres may be distributed relative to the range of the species in cases where the species range is small relative to the range of the state. For example, rather than assuming that all treated acres within the state are concentrated within a species range, it*

could be assumed that they are uniformly distributed throughout the state. BASF notes that in the Public Meeting on Revised Method for ESA Pesticide Assessments" held on June 10, 2019 EPA mentioned that, for use/species with LAA determinations, that they will consider a similar approach as described above (*i.e.*, look at uniform distribution of treated acres in the state) as a refinement.

NLAA determination based <1 Individual potentially exposed (page 21)
On page 21, EPA explains a process to derive the percent of the species range that may be exposed to the pesticide of interest. Briefly, the percent of species range that overlaps with the use footprint is multiplied by the "best available population size estimate to determine the number of individuals potentially exposed. If <1 individual is exposed, a NLAA determination is made." It is important to note that this process relies on the underlying assumption that the species is uniformly distributed within its range, which is not noted in the document. Given a spatial overlap of 1% as the smallest value that can be treated as representing actual overlap (based on the accuracy of the spatial data layers), using this equation it becomes quickly apparent that any endangered species population ≤100 individuals will be may effect (ME) at this step (*i.e.*, 1% × 100 individuals = 1 individual) regardless of the percent overlap. Furthermore, this process seems to contradict the protection goals that should be afforded very small populations as opposed to large ones. Consider the following example of two populations of varying size each with 1% overlap with the use footprint:

	Population 1	**Population 2**
% range overlap with pesticide use footprint	1	1
# individuals in population	50	1,000
# individuals potentially exposed	0.5	10
Determination	NLAA	LAA

In this example, a NLAA determination would be made for the population consisting of 50 individuals while a LAA determination would be made for the population consisting of 1,000 individuals.

Step 2B/C: Weight of evidence determinations

Weight-of evidence: Timing of applications relative to dormancy state

The proposed methodology introduces the criterion of 'most likely dormant' that the assessor uses to determine whether a NLAA determination can be made for particular use/species based on species dormancy during the window of application. More information is needed on what level of confidence is being proposed to indicate that dormancy is 'most likely'. For example, if the FWS/NMFS documentation clearly indicates species dormancy from November through April and the applications are only made in January through February, would that satisfy this requirement? As written, it seems to leave a lot of discretion to the assessor.

Weight-of evidence: Precision of species range data

BASF requests more clarity on what is meant by "many habitat types" in the context of species presence within ranges wholly defriend by geopolitical boundaries (page 26). Does many simply mean more than one or does it refer to a total habitat area greater than some fraction of the species range? This is important because a species may inhabit more than one habitat type that, the total area of which is still small when compared to the areas of the species range polygon.

Weight-of evidence: Confidence in the exposure determination

BASF appreciates the inclusion of this check to ensure exposures are realistic or even possible in certain cases and thinks the two examples provided reflect real world situations that are likely to be encountered when conducting nationwide endangered species risk assessments. Another example

would be a species that has range/critical habitat that overlaps a particular use; however, its habitat is incompatible with agricultural land, such as a species that inhabits only high elevations where agriculture does not occur. In this scenario, there would be low confidence in the exposure conceptual model and it may even want to be investigated whether the exposure pathway is complete. (Note: GOPHER has the capability to perform simple queries on habitat information for each species such as wetland habitat, or elevation of habitat, based on FWS/NMFS documentation, and could be used to facilitate this process).

Finally, this checkpoint is well placed in the workflow as it conducted after making NLAA determinations based on weight-of-evidence in the preceding steps but prior to conducting complex exposure modeling. BASF notes that it may be more appropriate to conduct the most-conservative screening analysis described in the *Effects Determination* section at this point in the process since it may be possible to eliminate the need for evaluating whether the exposure conceptual model is too conservative if a NLAA determination can be made.

Probabilistic analysis

BASF commends EPA for moving from the deterministic process utilized in the pilot BEs, which assumed the worst-case EEC for each use/bin combination and assigned this to a vast area of the country defined by the HUC-2, to a probabilistic approach that is used to develop a distribution of EECs to account for variability in sensitive parameters, including the application date and runoff curve number (RCN), both of which have significant heterogeneity, especially at the vast HUC-2 scale. While this is a significant step in the right direction, there are still areas of the process that could be further improved. Finally, this is yet another step in the process for which a trial evaluation or public workshop is needed so that the public/industry has the opportunity to see how this process works using actual data.

Aquatic probabilistic exposure assessment (pages 33-35)

In the June 10, 2019 public meeting, EFED mentioned that environmental exposure concentrations (EECs) will be developed for each relevant use/aquatic bin combination at the HUC-2 level. However, no mention of HUC-2 spatial aggregation of EECs is mentioned in the method document. This process needs to be described in the method document along with its anticipated impact on the exposure assessment.

Also, the document does not state what percentile of aquatic EECs should be used to create the distribution of aquatic EECs for the effects determination. For example, in FIFRA 1-in-10 return frequency was used, corresponding to the 90th percentile EEC is used; however, in the previous pilot BEs the 1-in-15 year return frequency (93.3rd percentile EEC) was used.

Aquatic bins as receiving waterbodies (pages 33-35)

As in the pilot BEs, the proposed process relies on the aquatic bins to represent all receiving waterbodies used in the aquatic exposure assessment. BASF continues to believe that the aquatic bin approach is flawed, particularly for moderate and high-flowing aquatic systems that will have large watersheds, and questions the appropriateness of the bins for reflecting habitats. For example, the static, low volume aquatic bin has an area of 1 m^2 and is 10 cm deep, essentially equating to a small puddle that could exist almost anywhere both spatially (including in the middle of an agricultural field) and temporally (after a large rainfall).

Monte Carlo simulation of application date and runoff curve number (page 34)

BASF believes the process that EPA has outlined, creating distributions of application date and runoff curve number (RCN) factors that relate to the resulting EECs to the worst-case initial simulation,

overall is a sound approach. BASF has the following clarifying comments. Regarding application date, the proposed methodology states that "...simulations are run using alternate application dates that would fall within a reasonable application window..." Based on this description, it is unclear if these dates are selected at random or if every date in the application window is simulated. Given the current application window batch analysis capability of the Pesticide in Water Calculator (PWC), it seems that simulating every day of the entire application window is appropriate to create the true distribution of application date factors.

Regarding the creation of the soil factor, the proposed methodology states that "similarly, an analysis is conducted using a different hydrologic soil group [than the worst-case]." The RCN is dependent on a variety of factors, including HSG, cover type, hydrologic condition and antecedent runoff conditions. Therefore, it is unclear how a distribution of soil factors will be created solely from moving from runoff-prone to less runoff-prone HSG soils (*i.e.*, D→ C → B → A). Also, the distribution of soil factors should reflect the distribution of soil types that are present in the action area for a given use. The current proposed methodology does not mention any spatial considerations of soil heterogeneity. Ultimately, more information is needed on how the RCNs are selected to create the distribution of the soil factors.

Effects determinations (page 35)

A screening approach is proposed in this section where the most-conservative assumptions are all initially assumed to see if less than 1 mortality of 1 individual exceeding sub-lethal effects is anticipated. If effects are anticipated using these assumptions, then the least-conservative assumptions are simulated to see if making a NLAA determination is possible. These steps are somewhat confusingly referred to as probabilistic analyses. Based on the description in the text, they appear to be screening-level deterministic approaches that bound whether a) an NLAA determination can be made initially for a given use/species; or b) an NLAA determination is even possible for a given use/species. The only scenario where probabilistic analyses are warranted under the proposed methodology is if NLAA cannot be initially determined but is possible. Further, the language in this section states that the confidence level for these two steps must be at the 95th percentile; however, since there is no probabilistic distribution of EECs or species sensitivities at these screening steps, it is unclear to what percentile the document is referring.

Finally, it seems that a more appropriate place for the most- and least-conservative screening analyses may be just after the weight-of-evidence steps but prior to calculating application/soil factors and creating a distribution of species locations, as these are only needed for the probabilistic analysis (if necessary).

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SINGLE AND COMBINED EFFECTS OF THE AGRICULTURAL INSECTICIDES CHLORPYRIFOS, IMIDACLOPRID AND DIMETHOATE ON FRESHWATER INSECT LARVAE

by

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BSc University of New Brunswick, 2006

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ABSTRACT

This study investigated lethal and sublethal effects of chlorpyrifos, imidacloprid and dimethoate on mayfly and chironomid larvae, individually and as mixtures. The work was relevant because pesticides are applied to crops in combination and are entering aquatic systems and exerting effects jointly. In acute bioassays, chlorpyrifos and imidacloprid were more toxic than dimethoate for both organisms. Despite their common mode of action (MOA), the chlorpyrifos-dimethoate mixture was synergistic, while the chlorpyrifos-imidacloprid and dimethoate-imidacloprid mixtures resulted in opposing synergism and antagonism, respectively. MOA may be an ineffective mixture effects predictor. During the chronic bioassay, chironomids recovered from single toxicant pulses for all endpoints. Potential stimulatory effects on survival were observed with chlorpyrifos and imidacloprid exposure, and on overall emergence with dimethoate and the mixture, which may indicate a hormetic effect. Pesticide mixtures do not necessarily behave strictly by current models and can be affected by exposure regimes; further investigation is warranted.

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PREFACE

This thesis has been prepared in article format. Chapter 1 contains a general introduction and Chapter 4 contains a discussion of general conclusions. Chapters 2 and 3 contain the following articles:

CHAPTER 2: Single versus combined lethal effects of three agricultural insecticides on freshwater insect larvae

(This chapter will be prepared and submitted for publication with the main authors being H.M.K. LeBlanc and J.M. Culp)

and

CHAPTER 3: Sublethal effects of a pulsed exposure to a mixture of three agricultural insecticides on the midge *Chironomus tentans*

Given that these two articles are inherently related, there will be some overlap in the introduction sections of the chapters, as well as with the general introduction in Chapter 1.

This thesis has been composed in its entirety by the candidate, and no part of this work has been submitted for any other degree.

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TABLE OF CONTENTS

ABSTRACT ii
PREFACE iii
ACKNOWLEDGEMENTS iv
TABLE OF CONTENTS v
LIST OF TABLES ix
LIST OF FIGURES xi
CHAPTER 1: Introduction ..... 1 - 20

1.1 Background 1
1.1.1 Mixtures 1
1.1.2 Sublethal Effects 3
1.2 Aims and Objectives 4
1.2.1 Study Mixture 4
1.2.1.1 Modes of Action 5
1.2.1.2 Existing Knowledge of Lethal Effects 6
1.2.2 Study Organisms 8
1.2.3 Mixture Modelling 9
1.2.4 Objectives 11
1.2.4.1 Lethal effects 11
1.2.4.2 Modelling 11
1.2.4.3 Sublethal effects 11
1.3 Overall Significance of Work 12
1.4 Literature Cited 13
1.5 Tables 17

ED_002896_00040210-00006

1.6 Figures 20

CHAPTER 2: Single versus combined lethal effects of three agricultural insecticides on freshwater insect larvae ..... 21 - 63

2.0 Abstract 21

2.1 Introduction 21

2.2 Materials and Methods 25

2.2.1 Exposure Solutions and Dilutions 25

2.2.2 Mayfly Experiment 26

2.2.2.1 Test species and collection 26

2.2.2.2 Exposure Procedures 26

2.2.3 Chironomid Experiment 27

2.2.3.1 Exposure organisms 27

2.2.3.2 Exposure procedures 28

2.2.4 Chemical Analysis 29

2.2.5 Data Analysis 29

2.2.6 Modelling 30

2.3 Results 31

2.3.1 Mayfly Experiment 31

2.3.2 Chironomid Experiment 32

2.3.2.1 Single insecticide exposure 32

2.3.2.2 Binary and ternary insecticide exposure 33

2.3.2.3 Modelling 34

2.4 Discussion 36

ED_002896_00040210-00007

2.5 Conclusions 42

2.6 Acknowledgements 43

2.7 Literature Cited 44

2.8 Tables 48

2.9 Figures 54

CHAPTER 3: Sublethal effects of a pulsed exposure to a mixture of three agricultural insecticides on the midge *Chironomus tentans* ..... 64 - 89

3.0 Abstract 64

3.1 Introduction 64

3.2 Materials and Methods 66

3.2.1 Exposure Solutions and Dilutions 66

3.2.2 Exposure Organisms 67

3.2.3 Exposure Procedures 68

3.2.4 Sample Processing 70

3.2.5 Chemical Analysis 70

3.2.6 Data Analysis 71

3.3 Results 72

3.4 Discussion 74

3.5 Conclusions 79

3.6 Acknowledgements 80

3.7 Literature Cited 81

3.8 Tables 84

3.9 Figures 86

CHAPTER 4: Conclusion ..... 90 - 100

4.1 Introduction 90

ED_002896_00040210-00008

4.2 Findings and Implications 91

4.3 Recommendations and Conclusions 94

4.4 Literature Cited 97

CURRICULUM VITAE

ED_002896_00040210-00009

LIST OF TABLES

CHAPTER 1: Introduction ..... **1 – 20**

Table 1.1 - Summary of formulation parameters of study insecticides 17

Table 1.2 - Summary of guidelines and usage data for study insecticides..... 18

Table 1.3 - Objectives and hypotheses by chapter 19

CHAPTER 2: Single versus combined lethal effects of three agricultural insecticides on freshwater insect larvae ..... **21 – 64**

Table 2.1 – Exposure concentrations used in 96-h acute bioassays of mayflies..... 48

Table 2.2 – Exposure concentrations used in 96-h acute bioassays of chironomids..... 49

Table 2.3 – Summarized interpretation of additional MIXTOX model parameters for the reference models..... 50

Table 2.4 – Provisional acute toxicity bioassay endpoints for 96-h static exposures of Heptageniidae mayflies. 50

Table 2.5 - Acute toxicity bioassay endpoints calculated using probit method for 96-h exposures of *C. tentans* to individual insecticides..... 51

Table 2.6 – Acute toxicity bioassay endpoints for 96-h exposures of *C. tentans* to binary and ternary insecticide mixtures 51

Table 2.7 – Summary of the MIXTOX analysis of the effect of 96-h binary mixture exposure on *C. tentans* compared to the concentration addition (CA) model..... 52

Table 2.8– Summary of the MIXTOX analysis of the effect of 96-h binary mixture exposure on *C. tentans* compared to the independent action (IA) model 53

CHAPTER 3: Sublethal effects of a pulsed exposure to a mixture of three agricultural insecticides on the midge *Chironomus tentans* ..... **65 – 90**

Table 3.1 - Exposure concentrations used in chronic 10-d and 28-d bioassays of *C. tentans*. 84

Table 3.2 - Summary of investigated effects..... 84

ED_002896_00040210-00010

Table 3.3 - Sex ratio and time to first emergence results 85

ED_002896_00040210-00011

LIST OF FIGURES

CHAPTER 1: Introduction ..... **1 – 20**

Figure 1.1 - Chemical structures of study insecticides 20

CHAPTER 2: Single versus combined lethal effects of three agricultural insecticides on freshwater insect larvae ..... **21 – 64**

Figure 2.1 – Mayfly study exposure set-up 54

Figure 2.2 – Diagram of *C. tentans* exposure vessel set-up 54

Figure 2.3 – Mean % survival of Heptageniidae mayflies with exposure to chlorpyrifos over 96 h 55

Figure 2.4 – Mean % survival of Heptageniidae mayflies with exposure to imidacloprid over 96 h 56

Figure 2.5 – Mean % survival of Heptageniidae mayflies with exposure to dimethoate over 96 h 57

Figure 2.6 - Mean % survival of *C. tentans* larvae per treatment level after 96-h static exposure to chlorpyrifos, imidacloprid and dimethoate 58

Figure 2.7 - Mean % survival of *C. tentans* larvae per treatment level after 96-h static exposure to binary mixtures of chlorpyrifos-imidacloprid, dimethoate-chlorpyrifos and imidacloprid-dimethoate 59

Figure 2.8 - Mean % survival of *C. tentans* larvae per treatment level after 96-h static exposure to a ternary mixture of chlorpyrifos, imidacloprid and dimethoate 60

Figure 2.9 – MIXTOX description of the effect of chlorpyrifos - imidacloprid mixture on mean % survival of *C. tentans* 61

Figure 2.10 - MIXTOX description of the effect of imidacloprid - dimethoate mixture on mean % survival of *C. tentans* 62

Figure 2.11 - MIXTOX description of the effect of dimethoate - chlorpyrifos mixture on mean % survival of *C. tentans* 63

CHAPTER 3: Sublethal effects of a pulsed exposure to a mixture of three agricultural insecticides on the midge *Chironomus tentans* ..... **65 – 90**

Figure 3.1 – Diagram of *Chironomus tentans* exposure vessel set-up 86

ED_002896_00040210-00012

Figure 3.2 – Mean % survival of *C. tentans* per treatment level at 10 d and 28 d of chronic exposure to 96-h pulse of chlorpyrifos, imidacloprid, dimethoate and ternary mixture of chlorpyrifos, imidacloprid and dimethoate, followed by recovery period 87

Figure 3.3 - Mean AFDM per *C. tentans* larvae (mg) per treatment level at day 10 of chronic bioassay 88

Figure 3.4 - Cumulative % emergence over time of *C. tentans* throughout 96-h pulse of chlorpyrifos, imidacloprid, dimethoate and ternary mixture of chlorpyrifos, imidacloprid and dimethoate, followed by a recovery period..... 89

ED_002896_00040210-00013

CHAPTER 1: Introduction

1.1 Background

Aquatic ecosystems in agricultural catchments are often exposed to mixtures of pesticides as opposed to single chemical exposures (De Zwart and Posthuma 2005). These mixture exposures frequently occur at relatively low contaminant concentrations that often produce sublethal effects (Teuschler *et al.* 2002). Thus, it is important to consider mixtures of contaminants at low concentrations when investigating pesticide effects on aquatic systems.

1.1.1 Mixtures

There are numerous sources for pesticides to enter aquatic systems, and the nature of these sources increases the likelihood of the presence of pesticide mixtures. In a survey of US streams, Gilliom *et al.* (2007) found that in 90 % of stream water samples taken, two or more pesticides were detected, in 70 % of samples, five or more pesticides were detected and in 20 % of samples, 10 or more pesticides were detected. Pesticides typically enter aquatic systems via the air (e.g., from spray drift) or through runoff (e.g., from irrigation or precipitation) from agricultural sites. Differing pest management requirements for neighbouring crops, intermingling of crops that have different pesticide regimes and co-application of multiple chemicals for the same crop (Lydy *et al.* 2004; Gilliom *et al.* 2007) can all lead to pesticides entering aquatic environments as mixtures as opposed to single products.

Due to these various environmental and application factors present in real-world systems, it is important to investigate mixture toxicities. Mixture studies are, however,

ED_002896_00040210-00014

complex and results that can be used for regulation of mixtures are not always evident. In real world situations, chemical mixtures are usually composed of unknown mixtures with dissimilar structures. In experimental studies, as the number of contaminants being investigated increases, the number of treatment combinations quickly escalates, along with equipment, space and personnel requirements.

The detrimental effects of agricultural pesticides entering aquatic systems are likely underestimated. This underestimation occurs because the guidelines in place for use of agricultural pesticides are based only on the toxicity of the individual chemicals, despite their presence in mixture form in aquatic systems (Donald *et al.* 1999). In Canada and the US, regulatory agencies do not formally assess pesticide mixtures (Lydy *et al.* 2004), largely due to the fact that there are simply too many mixture possibilities in agricultural settings to investigate each one. However, chemicals do not necessarily behave the same in mixture form as individual chemicals. Furthermore, the effects of a mixture on organisms and communities are likely different and more complex than the effects of individual chemicals. Relyea (2009) determined that a mixture of 10 herbicides and insecticides at low concentrations (less than 16 μg/L) had dramatic effects on amphibians, eliminating 99 % of leopard frogs in the study, but causing no mortality in gray tree frogs also present in the study. The same pesticides had a wide range of direct and indirect effects on amphibians. In a study by Walter *et al.* (2002), researchers observed that a mixture of 11 aquatic contaminants combined at their no observed effect concentrations was clearly more toxic when the chemicals were combined than any of individual contaminants. Additionally, several studies have observed synergistic effects of pesticide mixtures to aquatic organisms (e.g., Pape-Lindstrom and Lydy 1997;

ED_002896_00040210-00015

Anderson and Zhu 2004; Lydy and Austin 2004; Belden and Lydy 2006). In order to reduce the risk that regulations governing pesticide use are underestimating their effects on aquatic systems, it is therefore necessary to establish methods and models to address the issue of pesticide mixtures (Teuschler *et al.* 2002; Lydy *et al.* 2004).

1.1.2 Sublethal Effects

In addition to typically being exposed to pesticides at relatively low concentrations, aquatic organisms are often exposed to pulses of pesticides that rapidly dissipate throughout the aquatic system during run-off events (Zhao and Newman 2006). Thus, it is important to consider the consequences of these non-continuous, low dose exposures to pesticides, which are more likely to cause sublethal effects rather than lethal effects.

Growth, development, feeding rate and emergence are all ecotoxicological endpoints of freshwater insects that may be affected by sublethal exposures to pesticides. These factors affect individual fitness which can lead to negative population level effects. For example, feeding inhibition reduces energy intake potentially leading to growth inhibition. Adult mayflies generally do not consume food, instead focusing on reproduction, therefore energy consumption ends when emergence occurs. Fecundity, or the reproductive rate, of adult mayflies is consequently a function of nymph size at emergence (Scrimgeour and Culp 1994). Alexander *et al.* (2008) found that two species of mayflies developed less and emerged smaller when subjected to sublethal concentrations of imidacloprid. Sex ratio may also be affected by sublethal pesticide exposure. A study investigating the effects of three pesticides (atrazine, DDT and

ED_002896_00040210-00016

chlorpyrifos) on *Chironomus tentans* found that sex ratios were biased towards males after sublethal exposure to these chemicals (Rakotondravelo *et al.* 2006). Alternatively, Alexander *et al.* (2008) found that sublethal effects were only found in male mayflies.

It is critically important to understand the effects of pesticide mixture toxicity at both lethal as well as sublethal concentrations which are environmentally-relevant. In addition to measuring lethal toxicity, toxicity tests need to be designed to measure the effects of multiple chemical exposure, alternative routes of exposure and sublethal effects so as not to underestimate contaminant toxicity when determining water quality guidelines. In order to adequately protect the environment from the toxicity associated with mixtures of chemical pollutants, we need improved knowledge of how chemicals in mixtures exert toxic effects, especially for sensitive non-target species such as midges, mayflies and other aquatic invertebrates.

1.2 Aims and Objectives

My thesis investigates the single and combined effects of three insecticides on non-target freshwater insect larvae (chironomid midges and mayflies) at both lethal and sublethal levels.

1.2.1 Study Mixture

The focus of this study will be a pesticide mixture composed of three insecticides: chlorpyrifos (*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate), imidacloprid (1-[(6-chloro-3-pyridinyl)methyl]-*N*-nitro-2-imidazolidinimine) and dimethoate (*O,O*-dimethyl *S*-[2-(methylamino)-2-oxoethyl] phosphorodithioate) (Figure

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1.1, Table 1.1 and Table 1.2). These chemicals were chosen for study based on consultation with staff from Health Canada's Pest Management Regulatory Agency. The chemicals are currently used in potato production in New Brunswick, Canada (GNB 2009). Chlorpyrifos is used primarily to control beetles, dimethoate is used to control aphids and imidacloprid is used against Colorado potato beetle, flea beetle and aphids (GNB 2009).

1.2.1.1 Modes of Action

Insecticides differ widely in the mechanism by which they exert their toxicity on organisms known as the mode of action (MOA), which is the biochemical method by which a chemical reacts in a living organism. MOA encompasses the initial interaction of a pesticide with a receptor site in the affected organism along with the mechanisms by which subsequent lethal and sublethal effects are produced. When investigating chemical mixtures, it is important to keep the MOAs of the study chemicals in mind, as the effects of the mixture could be related to the way in which the chemicals are interacting with each other, as well as with the study organism.

Chlorpyrifos and dimethoate are both systemic organophosphate insecticides. They exert toxic action as acetylcholinesterase (AChE) inhibitors. In both vertebrates and invertebrates, acetylcholine (ACh) is an excitatory neurotransmitter that is necessary for transmission of nerve impulses across synapses. When ACh is released from the pre-synaptic membrane of a neuron, it travels across the synapse to interact with binding sites on the post-synaptic membrane. Once it is no longer required, ACh is hydrolyzed by the enzyme AChE, into acetic acid and choline. When an organophosphate such as

ED_002896_00040210-00018

chlorpyrifos or dimethoate is present, the insecticide molecules bind to AChE and phosphorylate the enzyme, making it unable to hydrolyze ACh (Fukuto 1990; Tomizawa and Casida 2003). Consequently, ACh builds up in the synapse, over-stimulating the neurological activity in the affected organism, resulting in twitching of involuntary muscles leading to paralysis and death (Cox 1994).

Imidacloprid is a systemic neonicotinoid insecticide. Neonicotinoid insecticides are one of the newest classes of pesticides (Tomizawa and Casida 2003). These chemicals mimic the nicotine molecule, which has long been used as an insecticide. Imidacloprid and other neonicotinoids target nicotinic acetylcholine receptors (nAChR), which are agonist-gated ion channels that allow rapid neurotransmission. Nicotine and ACh serve as the agonists for nAChRs (Tomizawa and Casida 2001). Because there are fundamental differences in the nAChRs of vertebrates and invertebrates, neonicotinoids bind more readily to invertebrate nAChRs than to vertebrate nAChRs, therefore these chemicals are not considered toxic to mammals (Liu and Casida 1993; Tomizawa and Casida 2003). For this reason, this class of insecticides is widely used. Neonicotinoids bind irreversibly to nAChRs, thereby prohibiting ACh from binding and opening the ion channels. This irreversible binding by imidacloprid causes nervous system disruption, especially in the central nervous system, and ultimately death (Cox 2001).

1.2.1.2 Existing Knowledge of Lethal Effects

Several studies have been conducted to investigate the effects of chlorpyrifos, dimethoate and imidacloprid individually. Chlorpyrifos is highly volatile and rapidly lost from experimental systems, but still acutely toxic to aquatic organisms. Pablo *et al.*

ED_002896_00040210-00019

(2008) determined the LC_{50} (the concentration of a chemical that kills 50 % of test organisms in a given time period) value for both a cladoceran and a mayfly nymph to be less than 1 μg/L at 6 h. *Chironomus tentans* larvae were sensitive to chlorpyrifos with a 48 h LC_{50} value of 0.3 μg/L (Moore *et al.* 1998). Imidacloprid is persistent in aquatic systems and highly toxic to aquatic organisms and honey bees (Cox 2001). Recent studies have found the 96-h LC_{50} value of imidacloprid in *C. tentans* to be 5.4-5.5 μg/L (Phillips and Bode 2004; Stoughton *et al.* 2008) and in late mayfly instars to be 0.65 μg/L (Alexander *et al.* 2007). *Lumbriculus variegates* had a 96-h EC_{50} of 6.2 μg/L, where immobility was the effective response (Alexander *et al.* 2007). Additionally, feeding inhibition occurred in mayfly larvae exposed to short pulses (24 h) of imidacloprid at concentrations greater than 1 μg/L. Alexander *et al.* (2008) also found that male mayflies exposed to low concentrations (0.1 ppb) of imidacloprid developed less rapidly and emerged at a smaller size relative to control organisms. Dimethoate is very stable, highly water soluble and has a very low potential to bioaccumulate (Anderson *et al.* 2006). It is less toxic than chlorpyrifos to aquatic invertebrates, however both lethal and sublethal effects were observed. 48-h EC_{50} or LC_{50} values for *Daphnia magna*, *C. tentans* and *Chironomus riparius* were 1.1 mg/L, 0.249 mg/L and 0.481 mg/L respectively (Anderson and Zhu 2004; Anderson *et al.* 2006; Domingues *et al.* 2007). Anderson *et al.* (2006) found that when *D. magna* were exposed to multiple pulses of dimethoate, with a recovery period in between each pulse, mortality during the 48-h post-exposure period was significantly increased compared to a single pulse of dimethoate, indicating that recovery ability was inhibited. Additionally, emergence delay of *C. riparius* larvae was observed with exposure to dimethoate (Domingues *et al.* 2007).

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1.2.2 Study Organisms

Chironomids (Order Diptera, Family Chironomidae) are a family of non-biting midges that are globally distributed and one of the most abundant insect groups in freshwater locations (Pinder 1983; EC 1997). As collector-gatherers, chironomids feed on algae, diatoms, and detritus. There are four stages in the chironomid life cycle: egg, larvae, pupa and adult (imago). Mating of adult midges is initiated by males and occurs in flight. Approximately 24 to 48 h after mating, female *C. tentans* lay gelatinous C-shaped masses containing approximately 2300 eggs directly into the water. Larvae emerge from hatched eggs within 2 to 4 days at 19 to 23 °C, and then construct tubes from surface sediment approximately 2 to 3 days after hatching. This stage consists of four instars, during which the organism changes from white to dark red in colour. Metamorphosis occurs within the open-ended larval tube. After moulting, the pupa emerges from the larval tube, and swims on the sediment surface for approximately 3 days before rising to the water surface and emerging as a winged imago. Adult midges do not feed; therefore they typically survive for 3 to 5 days upon emergence, during which time copulation occurs (EC 1997). In total, the lifecycle of *C. tentans* takes on average 23 to 30 d at 23°C under laboratory conditions (Benoit *et al.* 1997).

Mayflies (Order Ephemeroptera) are ubiquitous benthic invertebrates in streams and rivers around the world. There are four stages in the mayfly lifecycle: egg, nymph, subimago, and adult (imago). Mating typically occurs in swarms of male and female adults. Females typically deposit eggs onto the water surface, or by releasing eggs while flying low over the water surface. Eggs often hatch within weeks to months of being laid, upon which nymph stage emerges. This nymph stage undergoes several moults

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(reports for different species range from 12 to 45 or more). The length of the nymphal stage typically lasts from 3 to 6 months. The third stage, the winged but most often sexually immature subimago stage, is unique to Ephemeroptera. Larvae typically crawl onto stream-side vegetation and rocks or the water surface to moult into the subimago, which then flies to nearby vegetation to moult again into adults. The subimago stage can last from minutes to hours to days, depending on the species. After moulting into adults, most mayflies die within 2 hours to 3 days (Waltz and Burian 2008).

Chironomids and mayflies are often prey for fish and other stream predators, transferring energy along trophic pathways and thus are integral parts of aquatic food webs (Allen 1995). These invertebrates also transfer contaminants such as pesticides along trophic pathways.

1.2.3 Mixture Modelling

It is often difficult to predict and determine how a group of pesticides interact in a mixture form, especially in mixtures of chemicals with differing MOAs. Traditionally, there are two main models used to describe pesticide mixture toxicity: concentration addition (CA) and independent action (IA). The CA model is generally used to predict and evaluate the toxicity of a mixture of chemicals with the same MOA (Bliss 1939). It is expected that if two chemicals have the same MOA on an organism, then their effects should be additive. The CA model involves summing the concentrations of the components of the mixture and then adjusting the values for different potencies (De Zwart and Posthuma 2005). Alternatively, the IA model is used to predict and evaluate the toxicity of chemicals with differing MOAs (Bliss 1939). Such chemicals are believed

ED_002896_00040210-00022

to be acting on the organism by different pathways, therefore the toxicity that they exert are expected to be independent of one another. The toxicity of such a mixture is therefore based on probability statistics.

CA and IA models are good predictors of mixture toxicity for mixtures of only a few chemicals, at large concentrations (De Zwart and Posthuma 2005). Such mixture constraints are not environmentally-relevant and mixtures are frequently not composed of chemicals with solely similar or different MOAs. Traditionally, for mixtures of toxicants with multiple MOAs, concentration addition was used as a worst-case scenario for determining toxicity, even though the model is based on the assumption that the mixture being modelled has only one MOA (De Zwart and Posthuma 2005). Cedergreen *et al.* (2008) investigated the use of CA and IA in describing the toxicity of several binary mixtures of compounds with different MOAs, using 158 data sets. They determined that half of their mixtures could not be correctly described by either of the two models.

Given that mixtures do not generally meet the strict assumptions for the CA and IA models, nor do they necessarily behave as these models would predict, Jonker *et al.* (2005) developed a descriptive MIXTOX model, which aims to describe the antagonistic and synergistic effects that are often observed with mixtures of toxicants. The MIXTOX model begins with the two reference models and sequentially extends them using a series of deviations to better describe mixture effects, comparing the fit of the deviations with the reference models using Chi-squared tests.

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1.2.4 Objectives

The objectives of my research were to determine both lethal (i.e., LC_{50} values) and sublethal effects (i.e., growth, emergence and reproduction) of the study mixture through laboratory experiments (Table 1.3). Additionally, I determined whether either the CA or IA reference models, alone or coupled with MIXTOX model deviations, would best represent the toxicity of the pesticide mixture in order to provide a more comprehensive view of the additive and synergistic effects of the study mixtures.

1.2.4.1 Lethal effects

The first objective of my thesis was to determine 96-h LC_{50} values for both mayfly and *C. tentans* larvae when exposed to chlorpyrifos, imidacloprid and imidacloprid both individually, as well as in binary and ternary combinations. Organisms were exposed to a range of chemical concentrations via static, non-renewal test systems.

1.2.4.2 Modelling

Using the lethal toxicity data that I gathered from my laboratory work, my second objective was to determine whether the CA and IA reference models alone were good predictors of the toxicity of the mixtures, or whether their fit to the mixtures would be improved by including MIXTOX deviations.

1.2.4.3 Sublethal effects

The third objective of my thesis was to investigate the sublethal effects of exposure to a mixture of chlorpyrifos, dimethoate and imidacloprid on *C. tentans*. I conducted a chronic test over 28 days, with second-instar chironomid larva, where

ED_002896_00040210-00024

organisms were exposed to a pesticide pulse for 4 days and then transferred to uncontaminated recovery vessels for the remainder of the experiment. Endpoints were measured throughout the experiment to determine effects on growth (i.e. larval dry weight), emergence (i.e. time to first emergence, total and cumulative emergence) and reproduction (i.e. sex ratio). Each of these effects, should they occur with exposure to the tertiary mixture, could potentially reduce the genetic diversity and abundance of the chironomid population and even lead to extinction of the species.

1.3 Overall Significance of Work

Given that aquatic systems in agricultural and urban catchments are often exposed to mixtures of pesticides as opposed to single chemical exposures, it is important to determine what the effects of those chemicals entering the system will be. Studying the toxic effects of a set of agricultural insecticides on benthic organisms will allow further studies to occur with respect to the sublethal effects of these chemicals on these non-target organisms. It is important to understand the effects of these locally used agricultural pesticides on benthic organisms, which are an integral part of aquatic food webs. The effects that are evident in the current study could be indications of effects on a larger, more far reaching scale.

ED_002896_00040210-00025

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1.5 Tables

Table 1.1 - Summary of formulation parameters of study insecticides

Active Ingredient	Formulation Used	Manufacturer	Active Ingredient Concentration
Chlorpyrifos	Lorsban® 4E	Dow AgroSciences	480 g/L
Imidacloprid	Admire® 240F Flowable Systemic Insecticide	Bayer CropScience	240 g/L
Dimethoate	Lagon® 480E	United Agri Products Canada	480 g/L

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Table 1.2 - Summary of guidelines and usage data for study insecticides

	Chlorpyrifos	Imidacloprid	Dimethoate
Guideline for protection of freshwater aquatic life (CCME 2007a)	0.0035 µg/L	0.23 µg/L	6.2 µg/L
Reported usage of active ingredient in Canada (Brimble *et al.* 2005)	252,649.1 kg	19,572.9 kg	26,956.0 kg
Reported usage of active ingredient in New Brunswick in 2003 (Brimble *et al.* 2005)	2,749.3 kg	7,821.8 kg	1,476.5 kg
Reported presence in aquatic systems	– 0.06 µg/L in urban stream (Phillips and Bode 2004) – 180 ng/L in Salinas River, California, USA (Anderson *et al.* 2006) – 0.3 µg/L in urban stream (Hoffman *et al.* 2000, as cited in Rakotondravelo *et al.* 2006)	– >0.1 µg/L in urban stream (Phillips and Bode 2004) – 0.3 µg/L maximum concentration in New Brunswick surface waters (EC 2006; Murphy *et al.* 2006, as cited in CCME 2007b)	– 0.27 µg/L in agricultural study ditches (Wan *et al.* 1994) – 0.4 µg/L maximum concentration in New Brunswick surface waters (EC 2006)

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Table 1.3 - Objectives and hypotheses by chapter

Chapter	Objectives	Hypotheses
2 Single versus combined lethal effects of three agricultural insecticides on freshwater insect larvae	– Determine the lethal effects of 3 insecticides individually on *C. tentans* and Heptageniid mayflies	– Dimethoate will be less toxic than imidacloprid and chlorpyrifos
	– Determine the lethal effects of all combinations of the 3 insecticides on *C. tentans*	– Ternary mixture will be more toxic than individual insecticides. The similar MOA of chlorpyrifos and dimethoate will result in additive toxicity
	– Evaluate which reference model, concentration addition and independent action, with or without applied MIXTOX deviations, best represents the toxicity of the binary mixtures	– Chlorpyrifos-dimethoate mixture will be strictly additive given common MOA; independent action model will best describe both organophosphate-imidacloprid mixtures
3 Sublethal effects of a pulsed exposure to a mixture of three agricultural insecticides on the midge *Chironomus tentans*	– Determine the chronic, sublethal effects of ternary insecticide mixture on *C. tentans*	– Growth and emergence will be reduced with exposure to the ternary mixture, as opposed to individual chemicals. Also, a shift in sex ratio towards more emerged adult males
		– Less recovery from pulse of ternary mixture as opposed to individual chemicals

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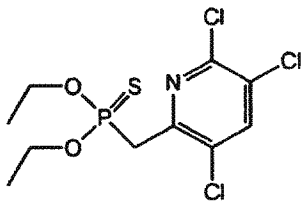
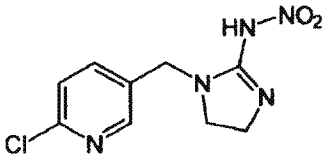
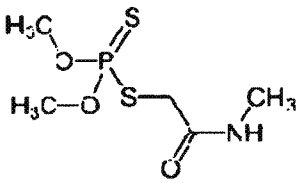
1.6 Figures



a) chlorpyrifos



b) imidacloprid



c) dimethoate

Figure 1.1 - Chemical structures of study insecticides (Available from: en.wikipedia.org)

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CHAPTER 2: Single versus combined lethal effects of three agricultural insecticides on freshwater insect larvae

2.0 Abstract

Pesticides are currently regulated individually but enter aquatic systems as mixtures of toxicants. The lethal effects of three agricultural insecticides – chlorpyrifos, imidacloprid and dimethoate – on Heptageniid mayfly and *Chironomus tentans* larvae were examined. 96-h static bioassays were performed using single, binary and ternary toxicant mixtures. Effects of the binary mixtures were investigated using the MIXTOX model, to determine if the mixtures behaved additively, synergistically or antagonistically. Dimethoate was much less toxic than chlorpyrifos or imidacloprid to both organisms. Chlorpyrifos and dimethoate were not additive in mixture despite their common mode of action (MOA) and resulted in opposing effects (synergism vs. antagonism) when combined with imidacloprid. These results suggest that current mixture models which are based on MOA alone may not be adequate in describing mixture effects. Other factors such as water solubility, secondary MOA and mechanisms of toxicity need to be considered. Further investigation into multi-chemical mixture effects is required.

2.1 Introduction

The effects of pesticides on non-target organisms in aquatic ecosystems traditionally have been regulated as individual, non-interacting compounds (Lydy *et al.*

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2004). However, field exposure invariably involves a mixture of chemicals that can produce a variety of effects, including additive, synergistic or antagonistic effects compared to the individual chemicals (Jonker *et al.* 2005). Currently, it is unclear how to accurately incorporate mixture effects into regulatory work, a task made more difficult by the fact that the composition of a mixture is often poorly quantified. Due to an absence of knowledge, pesticides are regulated individually rather than as mixtures, potentially underestimating their effects (Donald *et al.* 1999), leading to insufficient protection of exposed organisms.

Although the regulation of pesticides has mostly disregarded the effects of mixtures, there is a long history of mixture toxicity research. The models of concentration addition (CA) and independent action (IA) are well established as reference models for describing mixture effects of toxicants (Bliss 1939). The CA model describes strictly additive effects and is based on an assumption that the compounds in a mixture have similar modes of action (MOA). Effects are predicted by treating mixture components as dilutions of one another. In contrast, the IA model describes mixture effects by adding up the effect probabilities of each component of the mixture (Bliss 1939). IA is typically used to describe mixtures whose components have differing MOAs. Both the CA and IA reference models assume that there are no interactions between mixture components; however, this is rarely the case in mixtures that occur in aquatic environments (Syberg *et al.* 2008). Furthermore, it must be assumed that the modes of action of the mixture components can be adequately determined (Borgert *et al.* 2004).

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It is unlikely that environmental mixtures follow the assumptions of the straight CA or IA reference models (Lydy *et al.* 2004). For this reason, a model such as the MIXTOX model (Jonker *et al.* 2005) is useful because it attempts to describe antagonistic and synergistic effects. The MIXTOX model incorporates the reference models, and sequentially extends these model equations using a series of deviations to better describe binary mixtures effects. Firstly, the synergism/antagonism (S/A) deviation is applied to determine whether straight synergism or antagonism better describes the mixture effects than the reference model. Next, significant effects at different chemical ratios (dose-ratio (DR) deviation) are checked, followed by assessing for the effects of dose level (DL) deviation. The fit of the deviations compared to the reference model are tested using Chi-squared tests.

The current study aimed to investigate the acute effects of three agricultural insecticides – chlorpyrifos (*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate), imidacloprid (1-[(6-chloro-3-pyridinyl)methyl]-*N*-nitro-2-imidazolidinimine) and dimethoate (*O,O*-dimethyl *S*-[2-(methylamino)-2-oxoethyl] phosphorodithioate) – on non-target aquatic invertebrates. The chemicals are all currently used in potato production in New Brunswick, Canada (Dunn 2004; Murphy *et al.* 2006; GNB 2009). Chlorpyrifos and dimethoate are both organophosphorus insecticides and have a common MOA as acetylcholinesterase inhibitors. Imidacloprid is a neonicotinoid insecticide which exerts its toxic action by blocking nicotinic acetylcholine receptors. All three insecticides disrupt the nervous system of insects, ultimately causing death (Cox 1994; Cox 2001).

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The ability to accurately describe and predict mixture toxicity is vitally important, given the high likelihood of chemicals being present in the environment as mixtures. Therefore, my objectives were to: (1) determine the LC_{50} values for the three study insecticides; (2) evaluate the lethal effects of the binary and ternary combinations of those same insecticides; and (3) investigate which model, CA or IA, with or without applied MIXTOX deviations, best represented the toxicity of the mixtures. I hypothesized that dimethoate would be less toxic than chlorpyrifos or imidacloprid, based on established literature values (USEPA ECOTOX database: http://cfpub.epa.gov/ecotox/). Additionally, I reasoned that the binary mixture of chlorpyrifos-dimethoate would be strictly additive, given the common MOAs, while the IA model would best represent the effects of the chlorpyrifos-imidacloprid and imidacloprid-dimethoate mixtures. Finally, the ternary mixture was predicted to have greater than additive toxicity given that other studies have shown that mixtures of contaminants at the no observable effects concentration are clearly more toxic in combination than individually (Walter *et al.* 2002). Mayflies collected from a local river were used to determine LC_{50} values for the individual chemicals, while the midge *Chironomus tentans* cultured in the lab was used to determine LC_{50} values for the individual chemicals and mixture combinations. Mayflies and *C. tentans* larvae were used as test organisms because they are common in stream and river ecosystems, where they play ecologically important roles as grazers and collector consumers, respectively.

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2.2 Materials and Methods

2.2.1 Exposure Solutions and Dilutions

Test solutions were prepared using agricultural formulations of the three test insecticides (Lorsban 4E® (active ingredient (a.i.): chlorpyrifos; Dow AgroSciences Canada, Calgary, AB, Canada), Admire 240F® (a.i.: imidacloprid; Bayer CropScience, Calgary, AB, Canada), and Lagon 480® (a.i.: dimethoate; United Agri Products Canada, Dorchester, ON, Canada)). Stock solutions of each chemical were prepared using the appropriate amount of the chemicals into dechlorinated groundwater (Aquaculture Facility, University of New Brunswick, Fredericton, NB, Canada) which had been acclimated to test conditions and aerated for a minimum of 24 h in a 25 L Nalgene® carboy. Stock solutions were stored at 4 °C in amber glass bottles.

Exposures were conducted for each of the three individual insecticides as well as for all binary and ternary combinations of the same chemicals. Exposure concentrations were prepared by pipetting calculated concentrations of the stock solution into known volumes of test water to achieve desired concentrations. Exposure mixtures were prepared by adding aliquots of nominally equivalent toxic units (TU) of the individual chemicals to the same container of test water. One TU was equivalent to the 96-h LC_{50} of each chemical determined in the individual exposures.

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2.2.2 Mayfly Experiment

2.2.2.1 Test species and collection

Late-instar Heptageniidae mayfly nymphs were collected in the Nashwaak River, a tributary of the greater Saint John River, near Nashwaak Village, NB, Canada. The animals were gently washed from rocks and transferred into a container of aerated river water and transported back to the laboratory at the University of New Brunswick, Fredericton, NB, Canada within 8 hours. In the lab, nymphs were acclimated in an environmental growth chamber (Percival Scientific, Inc., Perry, IA, USA) to 20 ± 1°C over a 24-h period with a 16:8 light: dark cycle and a 50 % water exchange to dechlorinated groundwater. Organisms were not fed during the acclimation or testing periods.

2.2.2.2 Exposure Procedures

Groups of five to nine mayfly larvae (numbers depended on the number of animals available before each test) were randomly allocated to each exposure vessel (glass beaker, 12-cm diameter, 300 mL) containing a stir bar, four 1-inch square ceramic tiles, and 200 mL of test solution (Figure 2.1). Five concentrations were used per chemical combination, with four to five replicates per concentration (Table 2.1). Each exposure vessel was placed on a stir plate, in order to increase water flow over gills, and eliminate the need for aeration (Lowell *et al.* 1995). A piece of expanded polystyrene foam (13 mm thick) was placed between each vessel and stir plate to insulate the exposure vessel from temperature changes associated with the running stir plate. Test

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vessels were placed in an environmental chamber maintained at 20 °C with a photoperiod of 16:8 hours light to dark.

Test organisms were observed daily for 4 d. The number of mortalities was recorded, and exuviae removed after 24, 48, 72 and 96 h.

2.2.3 Chironomid Experiment

2.2.3.1 Exposure organisms

Culturing and exposure procedures were a combination of those outlined by Environment Canada (1997) and Stoughton *et al.* (2008). *C. tentans* egg ropes were obtained from laboratory cultures at the Toxicology Centre, University of Saskatchewan, Saskatoon, SK, Canada. Eggs were hatched in an environmental growth chamber at the University of New Brunswick, Fredericton, NB, Canada and subsequently transferred to 28 L culture tanks containing a layer of washed silica sand (212 – 425 µm) approximately 1 cm deep and 12 L of dechlorinated groundwater. Culture tanks were maintained in a growth chamber at 23 °C with a 16:8 light: dark schedule. Cultures were fed 20 mL of 100 g/L Nutrafin® fish food slurry three times per week.

96-h tests were conducted with approximately 10-d old larvae. Fourteen days prior to the start of a test, adults were collected and mated overnight to produce egg ropes. Egg ropes were held in a chamber until hatching occurred, and were then transferred to a new culture tank under the same conditions as the main culture tank, with the exception that larvae were fed 5 mL of 100 g/L Nutrafin® fish food every second day.

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2.2.3.2 Exposure procedures

Groups of seven to ten larvae were randomly allocated to each exposure vessel (Kimax® 300 mL tall form beaker without spout) containing a 0.5 cm deep layer of washed silica sand (212 – 425 μm) and 200 mL test solution (Figure 2.2). Five concentrations per chemical combination were used, with five replicates per concentration (Table 2.2). Additionally, five control replicates were used, which contained dechlorinated groundwater as the test solution. Vessels were covered with an overturned Petri dish. Tests were conducted in an environmental chamber at 23 °C with a 16:8 light: dark schedule. Aeration occurred via a network of airline tubing connected to a compressor via a manifold. A glass Pasteur pipette was attached to the end of each line and placed into each beaker through a hole drilled in the Petri dish lid. Vessels were gently aerated (2-3 bubbles per second) throughout the experiment. On day 0, 20-30 organisms were preserved for determination of head capsule width and ash-free dry mass (AFDM).

Throughout the test, organisms in each beaker were observed and fed 1.5 mL of 4 mg/mL Nutrafin® fish food slurry daily. Aeration rates were also adjusted as necessary. Dissolved oxygen, temperature, pH, ammonia and specific conductivity were measured at the 0 h and at 96 h (using Nutrafin® Master Test Kit #A-7865).

At the end of a test, live and moribund larvae were counted. Any organism that was completely inactive and showed no response when prodded with a sharp point was considered dead.

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2.2.4 Chemical Analysis

Stock (1L) and test solution (125 mL) samples were taken after solutions were prepared (before 0 h), and at 96 h. Samples were stored in amber glass bottles at 4 °C and shipped to the National Hydrology Research Centre (Environment Canada) in Saskatoon, SK, Canada for analysis. Pesticides were isolated using solid-phase (imidacloprid and dimethoate) or liquid-liquid (chlorpyrifos) extraction. The sample extracts were evaporated to dryness and the extract residues re-dissolved in water (1.0 mL) prior to analysis by LC-MS-MS. The LC/MS/MS system consisted of a Waters Micromass Quattro Ultima triple quadrupole mass spectrometer interfaced with a Waters 2695 Alliance HPLC System (Waters Limited, Mississauga, ON, Canada). The mass spectrometer was equipped with an electrospray ionization interface set to positive ion mode. The LC system was used with a Waters Xterra MS C_{18} (100 mm x 2.1 mm i.d., 3.5 µm particle size) analytical column. The mobile phase was aqueous acetonitrile containing 0.1 % formic acid (v /v). The column flow rate was 200 µL/min and injection volumes were 100 µL for chlorpyrifos and imidacloprid and 20 µL for dimethoate.

2.2.5 Data Analysis

Actual exposure concentrations used throughout the study were determined by analysing samples for two treatment concentrations per chemical type (chlorpyrifos, imidacloprid, dimethoate and mixture) using the analytical methods described above. A regression equation was determined for each chemical using the analysed values. The regression equations were then used to calculate the actual concentrations for the remaining treatments to which the organisms were exposed.

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LC_{50} values and their associated 95 % confidence intervals (CI) were calculated using the traditional probit method (USEPA 1990a). If data did not fit the probit model (USEPA 2002), data were analysed using the trimmed Spearman-Karber method (USEPA 1990b).

Mayfly data were adjusted to exclude individuals that emerged during the exposure period. Mayfly 96-h survival results were analyzed using repeated-measures ANOVA. In all survival data, significant differences among treatments were determined using one-way ANOVA along with Bonferroni multiple pairwise comparison ($p>0.05$). All analyses were performed using Systat®, versions 11 and 13 (Systat Software, Inc., Chicago, IL, USA). Mixtures were analysed using their combined toxic unit values.

2.2.6 Modelling

The descriptive MIXTOX model (Jonker *et al.* 2005) was used to model the binary mixture effects by describing any deviations from the traditional concentration addition and independent action models.

The log-logistic equation:

$$y = \frac{max}{1 + \left(\frac{c}{EC_{50}} \right)^{\beta}} \qquad (1)$$

was used in order to estimate *max* (control response at dose 0, or the maximum value for the measured endpoint), EC_{50} (the concentration of a chemical that induces a given effect in 50 % of test organisms) and beta (slope) parameters for individual chemicals. These estimated parameters were then used as starting points to determine the actual values of

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max, EC_{50} and beta for both chemicals in the mixture that best described the mixture response, while minimizing the sum of squared residuals (SS). To determine if mixture response was better described by a reference model alone, or if the response was better described when a deviation was applied to the model, two parameters, a and b, were sequentially added to the model. Parameter "*a*" identified whether antagonism or synergism deviations were present, while parameter "*b*" described ratio effects and dose level deviations (Table 2.3). Synergism/antagonism (S/A), dose-ratio dependant (DR) and dose-level (DL) deviations were compared with the reference model to determine if they were significantly different, as well as if they were significantly different from the other deviations. The mixture response was deemed best represented by the deviation that was significantly different from CA or IA, and subsequent deviations were consistent with this observation, or the reference model itself if no deviations exhibited a significantly better fit. In the current experiment, because the experimental design included only a single ratio in addition to the single insecticide concentrations, testing for the dose-ratio dependent deviation was considered inappropriate (Jonker *et al.* 2005).

2.3 Results

2.3.1 Mayfly Experiment

When Heptageniid mayflies were exposed to chlorpyrifos, imidacloprid or dimethoate, chlorpyrifos was the most toxic. Exposure to the two highest concentrations of chlorpyrifos (7.2 and 15.1 μg/L) resulted in 100 % mortality after only 12 h. A 96-h exposure to chlorpyrifos resulted in a LC_{50} (CI) value of 0.05 (0.01 – 0.15) μg/L while

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the LC_{50} values of imidacloprid and dimethoate were 3.7 (2.8 – 4.8) μg/L and 0.20 (0.18 – 0.21) mg/L respectively (Table 2.4). Higher concentrations of toxicants generally resulted in larger decreases in percent survival over the 96-h exposure period (Figure 2.3 – 2.5). For all three chemicals, except for the two highest concentrations of chlorpyrifos and the highest concentration of dimethoate, decreases in survival were mostly seen after 24 h of exposure.

The LC_{50} values obtained for the Heptageniidae larvae cannot be attributed at genus or species level, because I was unable to distinguish between larvae of two co-occurring Heptageniid mayfly genera (*Leucrocuta* and *Stenonema*) without adversely stressing the organisms. Survival data for both species were combined to determine the LC_{50} values for the individual insecticides. Consequently, no mixture experiments were performed with mayflies, and the individual insecticide results informed the selection of starting concentrations used for the bioassays with *C. tentans*, which could be cultured in the lab.

2.3.2 Chironomid Experiment

2.3.2.1 Single insecticide exposure

Ten-day old *C. tentans* larvae exposed for 96 h to one of the individual insecticides, chlorpyrifos, imidacloprid or dimethoate showed reduced mean percent survival as the concentration of pesticide increased. Chlorpyrifos was the most toxic of the three chemicals to *C. tentans* larvae with a 96-h LC_{50} value of 0.634 (0.309 – 1.05) μg/L (Table 2.5). Mean percent survival was significantly different from the control at values of 0.796 μg/L and greater ($F_{5, 24}$=27.8; $p<0.001$; Figure 2.6A). Imidacloprid was

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less toxic than chlorpyrifos with a 96-h LC_{50} of 2.65 (1.60 – 3.58) µg/L (Table 2.5), and mean percent survival was significantly different from the control at values of 5.76 µg/L and greater ($F_{5,24}$=52.4; p<0.001; Figure 2.6B). Dimethoate was more than three orders of magnitude less toxic than chlorpyrifos with a 96-h LC_{50} of 1.29 (0.740 – 1.97) mg/L (Table 2.5), while mean percent survival was significantly different from the control at values of 2.30 mg/L and greater ($F_{5,24}$=11.5 p<0.001; Figure 2.6C). Compared with mayfly sensitivity, *C. tentans* was less sensitive to chlorpyrifos (*C. tentans* LC_{50} = 0.634 µg/L; mayfly LC_{50} = 0.05 µg/L) and dimethoate (*C. tentans* LC_{50} = 1.29 mg/L; mayfly LC_{50} = 0.20 mg/L), but the midges were slightly more sensitive to imidacloprid (*C. tentans* LC_{50} = 2.65 µg/L; mayfly LC_{50} = 3.7 µg/L).

2.3.2.2 Binary and ternary insecticide exposure

When *C. tentans* larvae were exposed to binary mixtures of the three study insecticides, the trend was the same as for exposure to single insecticides, in that overall, as the concentration of chemicals increased, mean percent survival decreased. However, strict concentration addition was not observed with any mixture combination.

Exposure of *C. tentans* to either a chlorpyrifos-imidacloprid or dimethoate-chlorpyrifos mixture resulted in greater than additive effects, with 96-h LC_{50} values of 0.442 (0.321 – 0.544) TU and 0.51 (0.43 – 0.60) TU, respectively (Table 2.6). In the chlorpyrifos–imidacloprid mixture, mean percent survival was significantly reduced at mixture concentrations of 0.444 TU or greater ($F_{5,25}$=7.83; p<0.001; Figure 2.7A), while the dimethoate–chlorpyrifos mixture resulted in significant reduction in mean percent survival at 0.831 TU or more ($F_{5,25}$=7.59; p<0.001; Figure 2.7B). Alternatively,

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exposure to an imidacloprid–dimethoate mixture had a less than additive effect, with a 96-h LC_{50} value of 2.57 (2.08 – 4.19) TU (Table 2.6) and mean percent survival significantly reduced at the highest test concentration of 2.52 TU ($F_{5, 25}$=14.8; p<0.001; Figure 2.7C).

When *C. tentans* larvae were exposed to a mixture of all three insecticides, a nearly additive effect was observed, although the 96-h LC_{50} value was closer to the predicted LC_{50} value of 1 TU, with an actual value of 0.897 (0.575 – 1.22) TU (Table 2.6). Mean percent survival was significantly reduced at 2.02 TU or greater ($F_{5, 25}$=13.4; p<0.001; Figure 2.8).

2.3.2.3 Modelling

Comparing the effect of the binary mixture of chlorpyrifos–imidacloprid to the CA reference model yielded an SS of 465.4 (Table 2.7). The best fit was achieved by using the synergism/antagonism deviation, which clustered the data points most tightly along the 1:1 line and minimized the SS (SS = 180.7; Figure 2.9A and B). The S/A model was significant at the 5% level (p [χ^2] < 0.001). The value of parameter *a* was less than zero (*a* = -4.730), therefore significant synergism occurred (Table 2.7). The fit of the model was not improved further by adding the dose level deviation (SS = 174.0; p [χ^2] = 0.42). When the mixture was described using the IA reference model, an SS of 738.4 was achieved (Table 2.8). The S/A deviation significantly improved the fit of the model (SS = 417.7; p [χ^2] = 0.002), but the DL deviation further improved the fit (SS = 174.5; p [χ^2] < 0.001), most closely aligning the observed versus modelled data points

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along the 1:1 line (Figure 2.9C and D). Significant synergism was observed (Table 2.8) given that parameter $a < 0$ and $b < 0$.

Fitting the imidacloprid – dimethoate mixture to the CA reference model resulted in an SS of 1943.32 (Table 2.7). The best fit was achieved by the DL deviation model (SS = 661.8; p [χ^2] = 0.018; Figure 2.10A and B). The value of parameter a was greater than zero, therefore antagonism occurred at low concentrations and synergism occurred at high concentrations (Table 2.7), with the change from antagonism to synergism occurring at concentrations two times the LC_{50} ($1/b = 1/0.45 = 2.21 \cdot LC_{50}$). When the mixture was fitted to the IA reference model, an SS of 1323.7 was obtained (Table 2.8). The S/A deviation best improved the fit of the model (SS = 490.0; p [χ^2] < 0.001; Figure 2.10C and D). Parameter a was greater than zero, therefore significant antagonism was observed (Table 2.8).

The CA reference model fit to the dimethoate – chlorpyrifos mixture produced an SS of 2402.0; however the DL deviation model produced the best fit, with an SS minimized to 1487.1 (p[χ^2] = 0.022; Table 2.7; Figure 2.11A and B). The value of a and b were both negative, therefore significant synergism occurred (Table 2.7). When the mixture was fit to the IA reference model, SS = 2197.2 (Table 2.8). This value was minimized best by the DL deviation (SS = 1289.0; p [χ^2] = 0.009; Figure 2.11C and D). The value of a and b were both negative, therefore significant synergism occurred (Table 2.8).

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2.4 Discussion

Regulation of the use of pesticides such as chlorpyrifos, imidacloprid and dimethoate is based on the effects of the individual chemicals despite the high likelihood that non-target organisms are exposed to mixtures of these and other chemicals in aquatic ecosystems. My results indicate that the effects of mixtures of these chemicals are difficult to predict from MOA alone, with complicated synergistic and antagonistic interactions observed. This suggests that detrimental effects cannot necessarily be predicted from looking at the toxicity of individual chemicals alone, but rather that more attention should be given to assessing the potential synergistic and antagonistic effects of chemicals resulting from interactions such as dose-level and dose-ratio interactions (Jonker *et al.* 2005). Investigations of interactions are particularly relevant for low doses of chemicals that are routinely detected in biomonitoring. For example, imidacloprid has been detected in Atlantic Canadian watersheds at concentrations of 0.3 μg/L and dimethoate at 0.4 μg/L (Murphy *et al.* 2006), while studies of US watersheds have detected chlorpyrifos at concentrations between 0.06 and 0.3 μg/L (Hoffman *et al.* 2000; Phillips and Bode 2004; Anderson *et al.* 2006).

C. tentans larvae were most sensitive to chlorpyrifos followed by imidacloprid, and least sensitive to dimethoate by four orders of magnitude. Results for Heptageniid mayflies showed a similar trend. The 96-h LC_{50} estimate of 0.634 μg/L for chlorpyrifos and 2.65 μg/L for imidacloprid were both in the range of estimated values previously determined in other toxicity studies (Chlorpyrifos: Ankley and Collyard 1995; Moore *et al.* 1998; Jin-Clark *et al.* 2002; Mehler *et al.* 2008) (Imidacloprid: Alexander *et al.* 2007; Stoughton *et al.* 2008). The 96-h LC_{50} estimate of 1.29 mg/L for dimethoate was higher

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than other similar studies for *C. tentans* (Anderson and Zhu 2004; Domingues *et al.* 2007), however, Domingues *et al.* (2007) reported an even greater LC_{50} estimate of 1.75 mg/L for a related species, *Chironomus kiefferulus* Calligaster.

When *C. tentans* was exposed to binary mixtures of the insecticides, response to the chlorpyrifos-imidacloprid, and dimethoate-chlorpyrifos mixtures was greater than additive, while the response to the imidacloprid-dimethoate mixture was less than additive. The response to the ternary mixture was also greater than additive, although it was the closest to additive of any of the mixtures (LC_{50} = 0.897 TU). This finding lends support to the currently held hypothesis that as the number of chemicals in a mixture increases, the mixture effects tend towards strict additivity because antagonism between some chemicals may be balanced by synergism between others (Martin *et al.* 2009).

None of the binary mixtures induced an additive response with an LC_{50} of 1 TU, including the binary mixture of chlorpyrifos-dimethoate, which was hypothesized to produce an additive response given the common MOA of the two insecticides. Rather, the LC_{50} values were generally at lower than expected doses for mixtures that contained chlorpyrifos versus the imidacloprid-dimethoate mixture which had a higher than expected LC_{50} value. These results were further substantiated when the data were modelled using the MIXTOX model developed by Jonker *et al.* (2005).

Given that I only investigated the effects of a single ratio (1:1) of the binary mixtures, I did not evaluate the fit of the dose-ratio deviation. Further investigation of multiple ratios with the current binary mixtures would be valuable given that in their investigation of the effects of chlorpyrifos-imidacloprid on the nematode *C. elegans*,

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Svendsen *et al.* (2010) found that the dose-ratio deviation fit the data better than did the synergism/ antagonism deviation, and that CA was a better model than IA.

Both models adequately predicted antagonism and synergism for the three binary mixtures; however, the deviations were not always the same. In fact, CA best described the chlorpyrifos-imidacloprid mixture using the S/A deviation and the DL deviation for the imidacloprid-dimethoate mixture; the IA model reversed the deviations. Both models best described the dimethoate-chlorpyrifos mixture using the DL deviation. These findings support the conclusion that concentration addition, which is known to be more conservative than the independent action model (Syberg *et al.* 2008), may be sufficient for determining the effects of mixtures of toxicants, given that the same effects were described when using the IA model. In his review of mixture studies investigating the use of the CA and IA models, Deneer (2000) concluded that the CA reference model works quite well in most situations. Furthermore, Cedergreen *et al.* (2008) seriously questioned validity of the IA model given the unrealistic assumption that chemicals in a mixture could react with complete independence. Thus, the CA model continues to be the more robust approach for assessing toxicant mixtures.

Despite their common mode of action as acetylcholinesterase inhibitors, chlorpyrifos and dimethoate were not strictly additive as predicted. This was likely due to differences in the complex biochemical pathways by which they exert toxicity. Many mixtures with greater than additive effects occur because one of the chemicals induces a change in toxicokinetics (e.g., alternative MOAs, absorption and metabolism rates, etc.) (Belden and Lydy 2006) in the organism subjected to the mixture. Other studies have similarly found that chemicals with common MOA did not necessarily act by additive

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toxicity as was predicted. In their investigation of the effects of a binary mixture of imidacloprid and thiacloprid on *Caenorhabditis elegans*, Gomez-Eyles *et al.* (2009) found that the effects of these two insecticides were not predicted best by the strictly CA model, but instead by the dose-level dependent deviation, with synergism occurring at low concentrations and antagonism occurring at high concentrations. They did conclude, however, that the mixture was better described by the CA model than the IA model. Additionally, Moser *et al.* (2006) in their study of the effects on rats of a mixture of five organophosphates (including chlorpyrifos and dimethoate), concluded that synergism occurred, and the cause of synergism was likely due to the interactive effects on toxicokinetics and differences in relative potencies between pesticides. Finally, Svendsen *et al.* (2010) found that chlorpyrifos dominated synergism that was elicited in *Caenorhabditis elegans* by a binary mixture of the organophosphates chlorpyrifos and diazinon.

Interestingly, the organophosphate-imidacloprid mixtures (chlorpyrifos-imidacloprid and imidacloprid-dimethoate) exhibited opposing effects. Most surprising was that the imidacloprid-dimethoate mixtures indicated antagonistic effects. One possible explanation of these results could be related to a combination of organophosphate water solubility and cytochrome P450 enzymes (CYP) that are active in metabolizing toxicants in many animals, including insects. Chlorpyrifos is much less soluble (2 mg/L at 25°C) than dimethoate (25 g/L at 21°C) (Anderson *et al.* 2006). When neonicotinoids are catalysed by CYP, the by-products are less toxic, while typically, organophosphate by-products, known as "oxons", are more toxic than the parent molecules. CYP inhibition and induction, which are mediated by toxicants, can influence

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the effects of the chemicals. Inhibition is typically in the form of competitive inhibition of CYP binding sites, while induction results in an increased production of CYP protein, thereby increasing the capacity of CYP to metabolize these chemicals.

In their investigation of the synergistic chlorpyrifos-imidacloprid mixture, Svendsen *et al.* (2010) suggested that if imidacloprid induced CYP, and organophosphates are favoured by CYP as substrate over neonicotinamides, there would be more organophosphate-oxons present in the mixture, which would lead to greater toxicity, as I found with the chlorpyrifos-imidacloprid mixture in the current study. Chlorpyrifos, with it's low water solubility and tendency to accumulate in organisms (log K_{ow} = 3.31 to 5.27), may be the favoured substrate to bind with CYP, therefore an increased amount of chlorpyrifos is oxidized to its more toxic oxon metabolite and synergism is observed. Svendsen *et al.* (2010) also suggested that substances that have a higher substrate affinity with CYP than organophosphates would result in antagonism due to competitive inhibition of the conversion of organophosphates to their toxic oxons. This may be the case for the imidacloprid-dimethoate mixture where I observed antagonistic effects. Dimethoate is highly soluble in water (log K_{ow} = 0.76) and has a low tendency to bioaccumulate (Anderson *et al.* 2006), therefore is more likely to be eliminated from an organism than chlorpyrifos. In the imidacloprid-dimethoate mixture, imidacloprid induces CYP, but given that dimethoate is readily excreted and therefore less available than chlorpyrifos, imidacloprid is metabolized by CYP and a less toxic metabobolite is produced, which results in antagonism.

Alternatively, the opposing effects of the organophosphate-imidacloprid mixtures may have been due to the formulations of insecticides used in this study that differed

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from the purified technical grade insecticides. Formulations were used instead of purified technical grade insecticides in an attempt to ensure environmental relevance, given that it is the formulations that are actually entering aquatic systems. Pesticide formulations also contain adjuvants. Adjuvants make up the bulk of pesticide formulations and are added to enhance the formulations, e.g., enhancing solubility, increasing the effectiveness of the active ingredient or increasing adsorption (Krogh *et al.* 2003). The adjuvants in the chlorpyrifos, imidacloprid and/or dimethoate formulations may have contributed to the toxicity of the active ingredients, or interacted with the other adjuvants or active ingredients in the mixtures to alter the toxicity of the mixtures. Further investigation into the effects of the adjuvant components of the insecticide formulations from this study is warranted.

Finally, while MOA is generally accepted as the common factor by which to predict the effects of mixtures, my results where chlorpyrifos and dimethoate did not behave as expected by their common MOA suggests other factors may play a role in mixture toxicity. While pesticides generally have a known primary MOA by which mixture toxicity predictions are made, such chemicals often act within an organism via a cascade of events; therefore they commonly have secondary MOAs that may very well affect the interactions in mixture toxicity. Small differences in the cascades of events between different pesticides with the same primary MOA could induce differing toxic effects (Pope 1999). Furthermore, chemicals can produce different effects at different doses and exposure durations, or multiple effects by different mechanisms (Borgert *et al.* 2004). More study regarding the use of MOA or alternative methods which take

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mechanisms of toxicity in to account in order to classify pesticides and predict the effects of mixtures on aquatic organisms is warranted.

2.5 Conclusions

While the CA reference model is conservative and overestimates the toxicity of chemicals with dissimilar modes of action, the IA reference model typically underestimates the effects of those with similar modes of action (Syberg *et al.* 2008). Although both models can effectively predict mixture effects, the CA model is more easily calculated. My results agree the growing consensus in the literature (e.g., (Backhaus *et al.* 2000; Belden and Lydy 2000; Broderius *et al.* 2005; Belden *et al.* 2007) that, even though CA may over predict toxicities, it is unlikely to under predict them and is the most appropriate mixture model currently available.

As originally predicted, dimethoate was less toxic to both Heptageniid mayflies and the midge *C. tentans.* The two organophosphates, chlorpyrifos and dimethoate, were not additive despite their common MOA, and resulted in opposing effects (synergism versus antagonism) when mixed with imidacloprid. This may have been due to the difference in their availability to bind with CYP because they have different water solubilities, or to interactions with the adjuvants in the formulations. Furthermore, the opposing effects of chlorpyrifos and dimethoate may highlight a problem with the use of a common MOA to predict mixture toxicities. The reference models of concentration addition and independent action predicted less well the effects of the binary mixtures as

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was expected; instead, S/A and DL deviations provided better descriptions. The ternary mixture was in fact synergistic as originally predicted.

The findings of this study clearly highlight the importance of further investigation into the effects of mixture effects. It is not necessarily adequate to assume that CA and IA are effective models to describe mixture effects, based on MOA alone. Other factors such as water solubility, secondary MOAs and mechanisms of toxicity must be taken into account. Additionally, the extension of the MIXTOX model to describe ternary mixtures and mixtures of even greater numbers of chemicals would be very beneficial in extending our knowledge of mixture effects. A model that teased apart interactive effects that result from mixtures would be beneficial in assessing the current assumption that complex mixtures tend towards additivity due to a balancing of antagonistic effects of some chemicals by synergistic effects of others.

2.6 Acknowledgements

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ED_002896_00040210-00056

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2.8 Tables

Table 2.1 – Exposure concentrations used in 96-h acute bioassays of mayflies. Concentrations were calculated based on the regression equation obtained from two analyzed concentrations per chemical. Values indicated by † were actually negative using the regression equation, and in such cases concentration was rounded to zero.

Chlorpyrifos (µg/L)	Imidacloprid (µg/L)	Dimethoate (mg/L)
0†	0.058	0.16
0.030	0.45	0.20
0.83	0.94	0.29
7.2	4.9	0.53
15.1	9.7	0.82

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Table 2.2 – Exposure concentrations used in 96-h acute bioassays of chironomids. Binary and ternary concentrations are given in TU, with TU of individual mixture components in brackets, in the order listed in mixture title (e.g., chlorpyrifos/imidacloprid mixture = total mixture toxicity (chlorpyrifos toxicity + imidacloprid toxicity). Values were calculated based on the regression equation obtained from two analyzed concentrations per chemical. Values indicated by † were actually negative using the regression equation, and in such cases concentration was rounded to zero.

Test	**Insecticides**		
Individual	*Chlorpyrifos (µg/L)*	*Imidacloprid (µg/L)*	*Dimethoate (mg/L)*
	0†	0.842	0.109
	0.265	1.39	0.474
	0.796	5.76	0.931
	5.04	11.2	2.30
	10.4	22.2	4.59
Binary Mixture	*Chlorpyrifos-Imidacloprid (TU)*	*Imidacloprid-Dimethoate (TU)*	*Dimethoate-Chlorpyrifos (TU)*
	0.167 (0.0575 + 0.110)	0.216 (0.090 + 0.126)	0.283 (0.100 + 0.183)
	0.259 (0.0842 + 0.175)	0.370 (0.161 + 0.209)	0.361 (0.171 + 0.191)
	0.444 (0.138 + 0.306)	0.677 (0.303 + 0.374)	0.518 (0.312 + 0.206)
	0.813 (0.245 + 0.568)	1.29 (0.586 + 0.705)	0.831 (0.594 + 0.237)
	1.55 (0.458 + 1.09)	2.52 (1.15 + 1.37)	1.46 (1.16 + 0.298)
Ternary Mixture	*Chlorpyrifos-Imidacloprid-Dimethoate (TU)*		
	0.252 (0.158 + 0.094 + 0.126)		
	0.504 (0.315 + 0.189 + 0.251)		
	1.01 (0.631 + 0.377 + 0.503)		
	2.02 (1.26 + 0.754 + 1.01)		
	4.03 (2.52 + 1.51 + 2.01)		

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Table 2.3 – Summarized interpretation of additional MIXTOX model parameters for the concentration reference models that help define the functional form of the deviation pattern (Adapted from Jonker *et al.* 2005).

Parameter	Value	Interpretation
		Synergism/Antagonism
A	< 0	Synergism
	> 0	Antagonism
		Dose-level Dependence
A	< 0	Synergism at low concentrations; antagonism at high concentrations
	> 0	Antagonism at low concentrations; synergism at high concentrations
B	> 1	Antagonism/synergism change at concentrations lower than L/EC_{50}
	= 1	Antagonism/synergism change at L/EC_{50} concentration level
	$0 < b < 1$	Antagonism/synergism change at concentrations higher than L/EC_{50}
	< 0	No change, however the magnitude of synergism/antagonism is dose-level (CA) or effect-level (IA) dependent

Table 2.4 –Acute toxicity bioassay endpoints calculated using probit method (except for imidacloprid treatment where trimmed Spearman-Karber method was used) for 96-h static exposures of Heptageniidae mayflies to three individual insecticides.

Insecticide	96-h LC_{50}	95 % confidence interval
Chlorpyrifos	0.05 µg/L	0.01 – 0.15
Imidacloprid	3.7 µg/L[†]	2.8 – 4.8
Dimethoate	0.20 mg/L	0.18 – 0.21

[†] % trim using Trimmed Spearman-Karber method = 1.81%

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Table 2.5 - Acute toxicity bioassay endpoints calculated probit method for 96-h exposures of *C. tentans* to individual insecticides.

Insecticide	96-h LC_{50}	95 % Confidence Interval
Chlorpyrifos	0.634 µg/L	0.309 – 1.05
Imidacloprid	2.65 µg/L	1.60 – 3.58
Dimethoate	1.29 mg/L	0.740 – 1.97

Table 2.6 – Acute toxicity bioassay endpoints calculated using probit method (except for dimethoate-chlorpyrifos treatment where trimmed Spearman-Karber method was used) for 96-h exposures of *C. tentans* to binary and ternary insecticide mixtures. Additionally, combined action classification is indicated based on the expected results of strict concentration addition with an LC_{50} of 1 TU.

Insecticide	LC_{50} (TU)	95% Confidence Interval	Combined Action
Chlorpyrifos-Imidacloprid	0.442	0.321 - 0.544	Greater than additive
Imidacloprid-Dimethoate	2.57	2.08 - 4.19	Less than additive
Dimethoate-Chlorpyrifos	0.51[†]	0.43 - 0.60	Greater than additive
Ternary Mixture	0.897	0.575 - 1.22	Greater than additive

[†] % trim using Trimmed Spearman-Karber method = 17.4 %

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Table 2.7 – Summary of the MIXTOX analysis of the effect of 96-h binary mixture exposure on *C. tentans* compared to the concentration addition (CA) model. S/A = synergism/antagonism deviation and DL = dose level deviation. *a* and *b* = deviation parameters (Table 2.4). SS = sum of squares, χ^2 = chi square value and $p(\chi^2)$ = significance of the likelihood test.

Mixture	Fitted Model	a	b	SS	R^2	vs. Ref. Model		vs. S/A Deviation		Best Fit	Interpretation
						χ^2	$p(\chi^2)$	χ^2	$p(\chi^2)$		
Chlorpyrifos-Imidacloprid	CA			465.4	0.947					S/A	Significant synergism
	S/A	-4.73		180.7	0.990	16.1	<0.001				
	DL	-2.97	-0.93	174.0	0.990	16.7	<0.001	0.64	0.42		
Imidacloprid-Dimethoate	CA			1943.3	0.891					DL	Antagonism at low concentrations, change to synergism at $2.21 \cdot LC_{50}$
	S/A	3.98		940.5	0.947	11.6	<0.001				
	DL	17.0	0.45	661.8	0.963	17.2	<0.001	5.62	0.018		
Dimethoate-Chlorpyrifos	CA			2402.0	0.841					DL	Significant synergism (magnitude of synergism is dose level dependent)
	S/A	-2.60		2059.7	0.863	2.46	0.117				
	DL	-0.004	-1250.7	1487.1	0.902	7.67	0.022	5.21	0.022		

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Table 2.8– Summary of the MIXTOX analysis of the effect of 96-h binary mixture exposure on *C. tentans* compared to the independent action (IA) model. S/A = synergism/antagonism deviation and DL = dose level deviation. *a* and *b* = deviation parameters (Table 2.4). SS = sum of squares, χ^2 = chi square value and $p(\chi^2)$ = significance of the likelihood test.

Mixture	Fitted Model	a	b	SS	R^2	vs. Ref. Model		vs. S/A Deviation		Best Fit	Interpretation
						χ^2	$p(\chi^2)$	χ^2	$p(\chi^2)$		
Chlorpyrifos-Imidacloprid	IA			738.4	0.959					DL	Significant synergism (magnitude of synergism is effect level dependent)
	S/A	-7.48		417.7	0.977	9.68	0.002				
	DL	-1.85	-21.9	174.5	0.990	24.5	<0.001	14.84	<0.001		
Imidacloprid-Dimethoate	IA			1323.7	0.926					S/A	Significant antagonism
	S/A	4.84		490.0	0.972	15.9	<0.001				
	DL	6.48	0.37	478.3	0.973	16.3	<0.001	0.39	0.534		
Dimethoate-Chlorpyrifos	IA			2187.2	0.855					DL	Significant synergism (magnitude of synergism is effect level dependent)

2.9 Figures

Figure 2.1 – Mayfly study exposure set-up. A preparation dish (10-cm diameter) containing 4 ceramic tiles and a stir bar was placed on a stir plate topped with 13 mm thick expanded polystyrene foam to insulate the test solution from heat produced by the stir plate. 200 mL of test solution was used, and 4-7 Heptageniid mayflies. Mayflies clung to the tiles typically for the duration of the experiment.

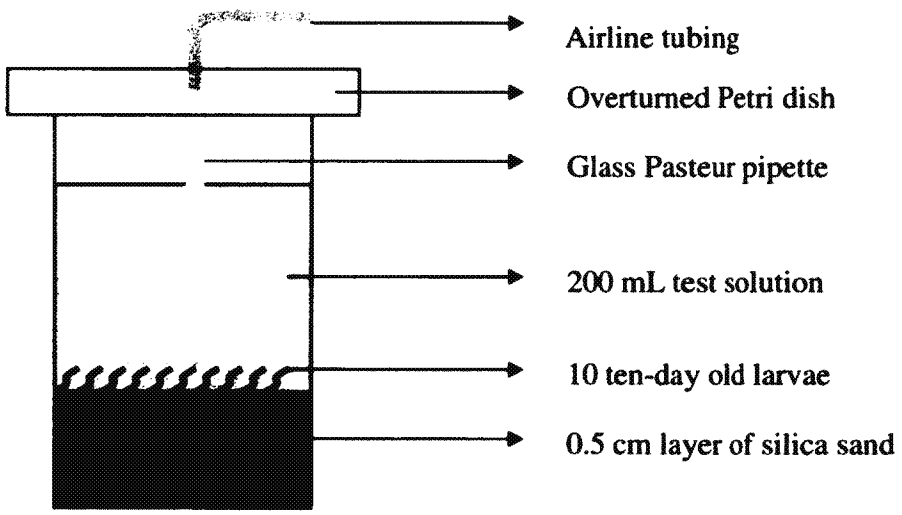

Figure 2.2 – Diagram of *C. tentans* exposure vessel set-up.

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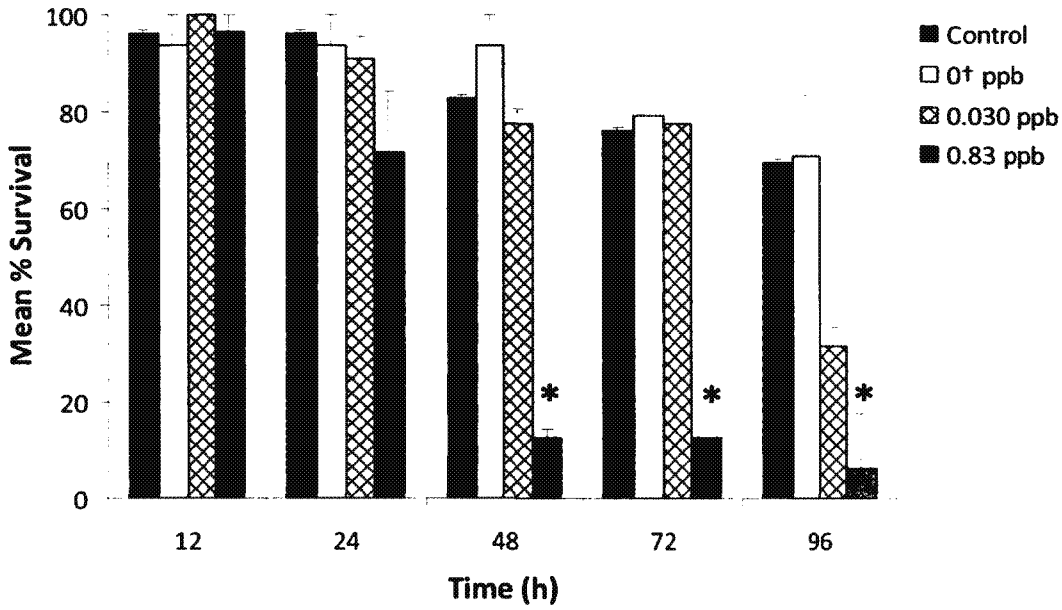

Figure 2.3 – Mean % survival of Heptageniidae mayflies ($n_{mayflies/replicate}$ = 3-7; $k_{replicates/treatment}$ = 4-5) with exposure to chlorpyrifos over 96 h. Asterisks indicate significantly different survival rates ($p<0.05$) when compared to control using Bonferroni post-hoc multiple comparison. Error bars indicate one standard error. Values indicated by † were negative using the regression equation, and in such cases concentration was rounded to zero.

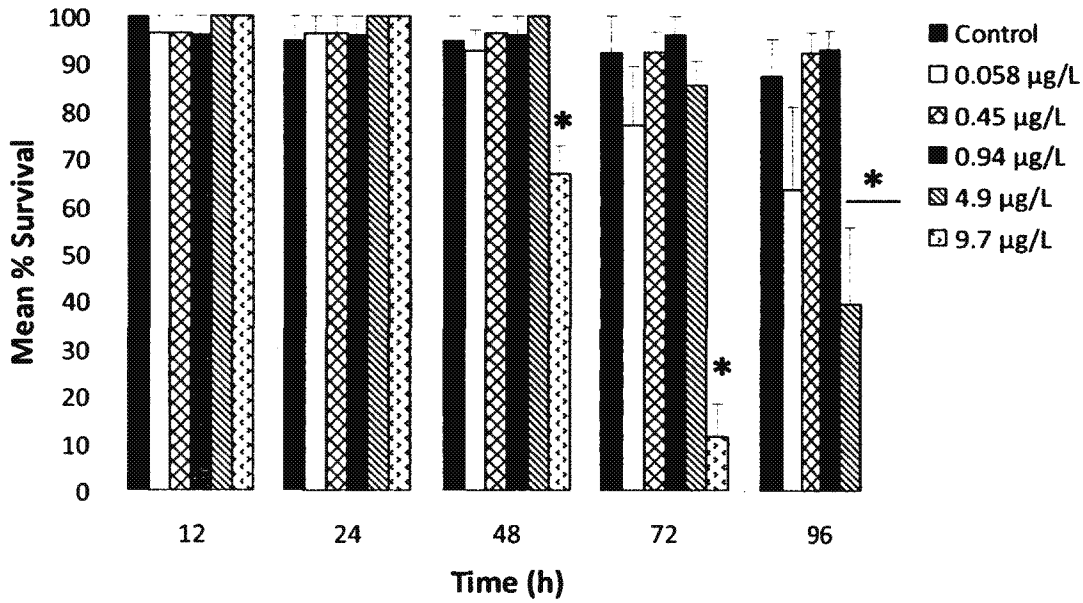

Figure 2.4 – Mean % survival of Heptageniidae mayflies ($n_{mayflies/replicate}$ = 3-7; $k_{replicates/treatment}$ = 4-5) with exposure to imidacloprid over 96 h. Asterisks indicate significantly different survival rates ($p<0.05$) when compared to control using Bonferroni post-hoc multiple comparison. Error bars indicate one standard error.

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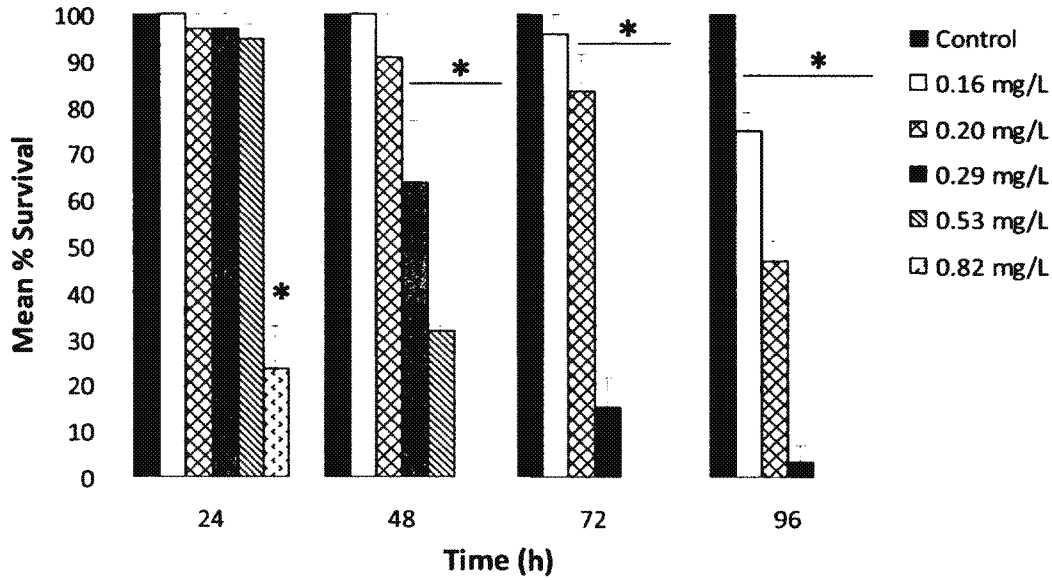

Figure 2.5 – Mean % survival of Heptageniidae mayflies ($n_{mayflies/replicate}$ = 3-8; $k_{replicates/treatment}$ = 3-5) with exposure to dimethoate over 96 h. Asterisks indicate significantly different survival rates ($p<0.05$) when compared to control using Bonferroni post-hoc multiple comparison. Error bars indicate one standard error.

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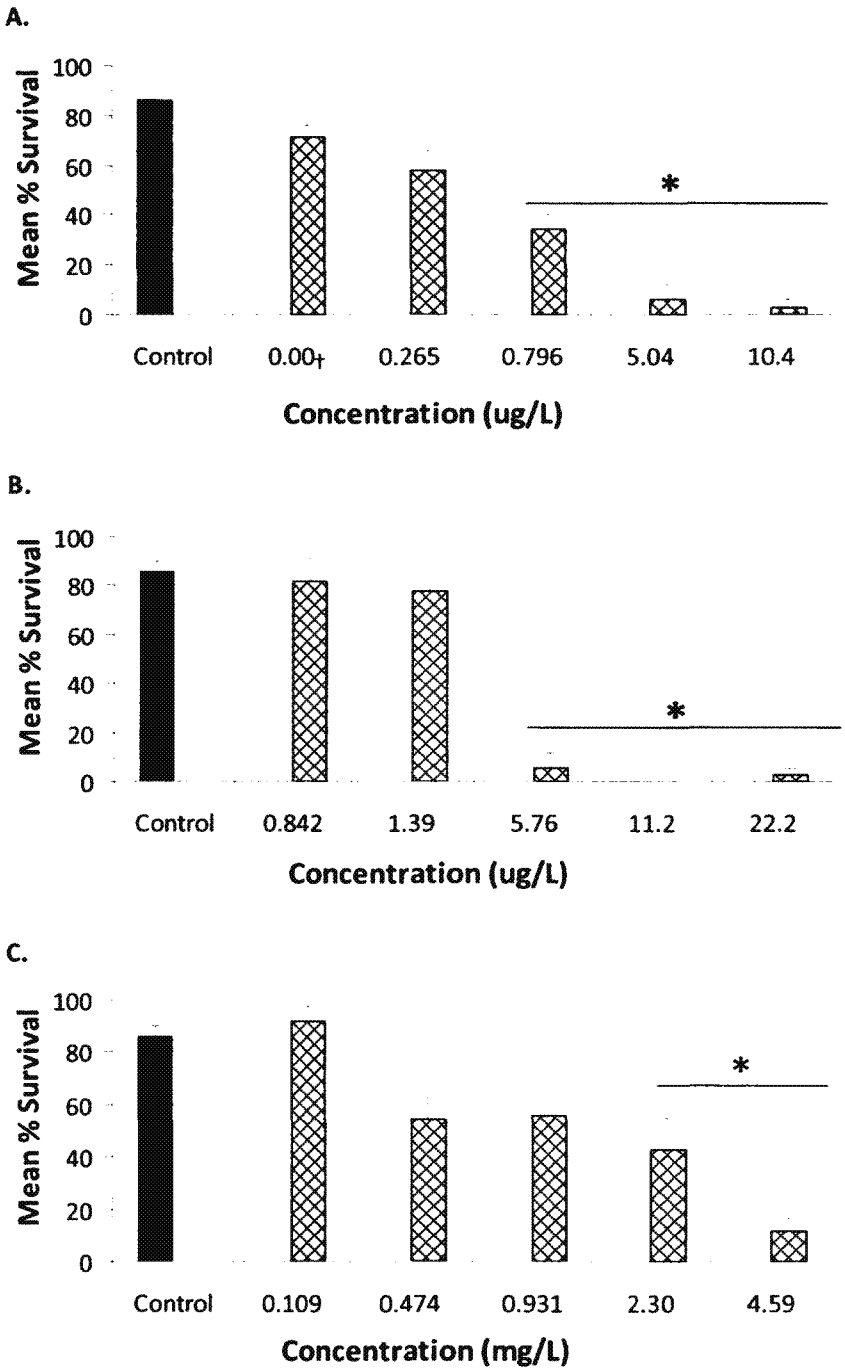

Figure 2.6 - Mean % survival of *C. tentans* larvae per treatment level ($n_{chironomids/replicate}$ = 7-10; $k_{replicates/treatment}$ = 5) after 96-h static exposure to (A) chlorpyrifos, (B) imidacloprid and (C) dimethoate. Asterisks indicate significantly different survival rates ($p<0.05$) when compared to control using Bonferroni post-hoc multiple comparison. Errors bars indicate one standard error. Values indicated by † were actually negative using the regression equation, and in such cases concentration was rounded to zero.

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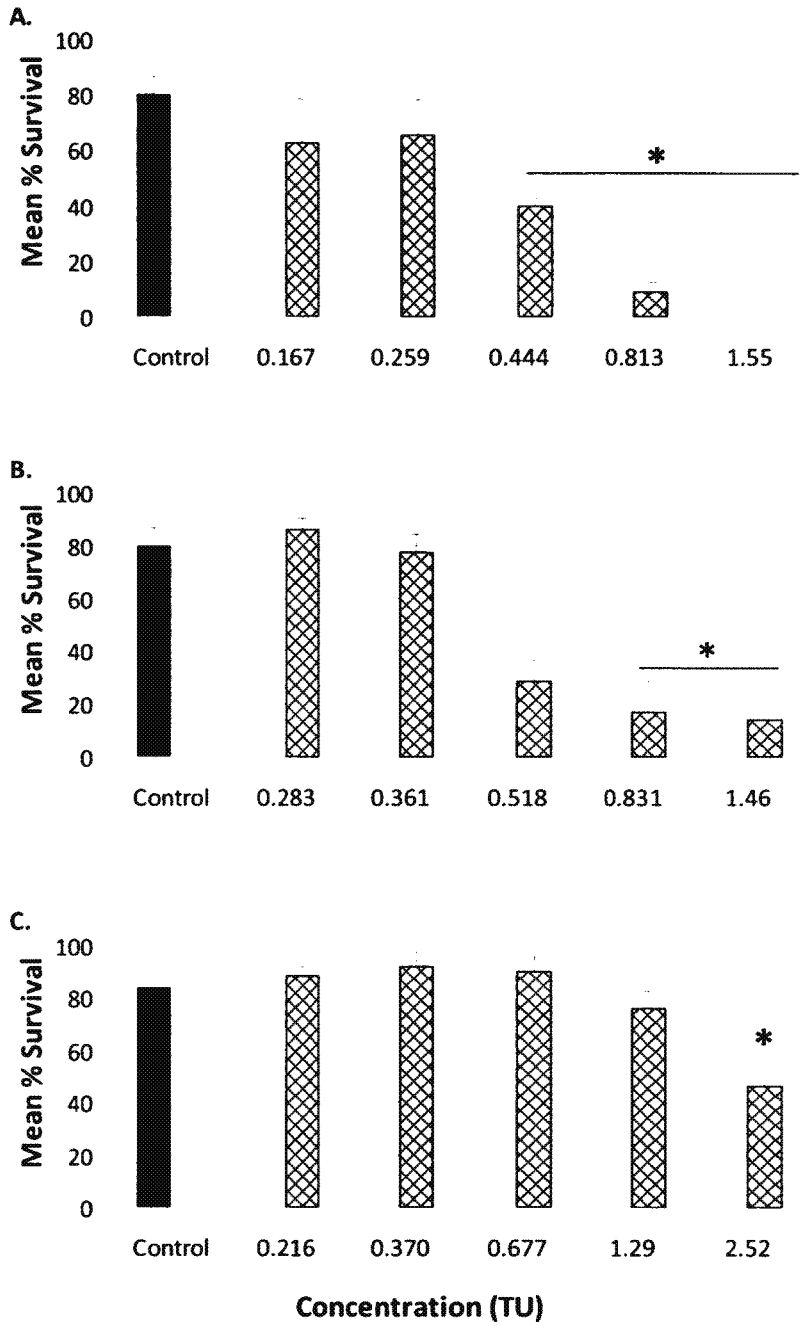

Figure 2.7 - Mean % survival of *C. tentans* larvae per treatment level ($n_{chironomids/replicate}$ = 7-10; $k_{replicates/treatment}$ = 5) after 96-h static exposure to binary mixtures of (A) chlorpyrifos-imidacloprid, (B) dimethoate-chlorpyrifos and (C) imidacloprid-dimethoate. Asterisks indicate significantly different survival rates ($p<0.05$) when compared to control using Bonferroni post-hoc multiple comparison. Errors bars indicate one standard error.

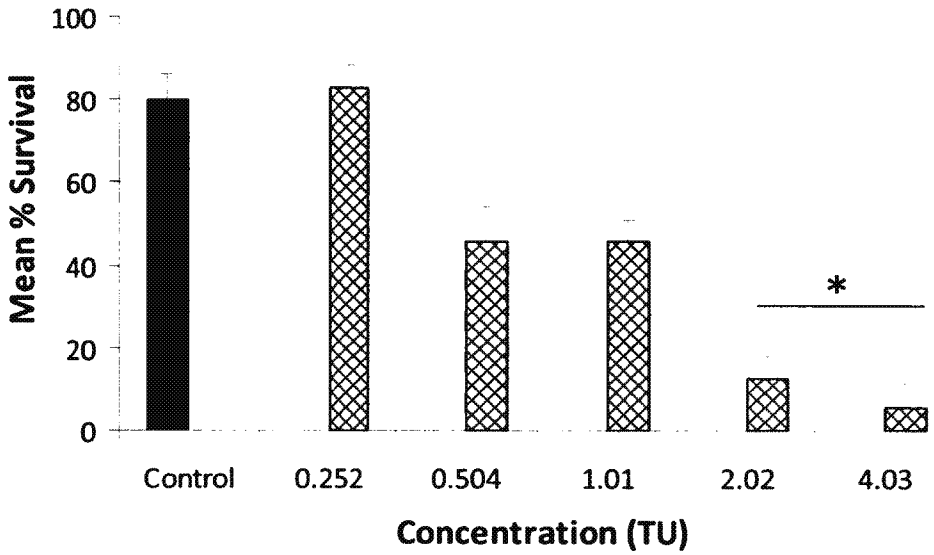

Figure 2.8 - Mean % survival of *C. tentans* larvae per treatment level ($n_{chironomids/replicate}$ = 7-10; $k_{replicates/treatment}$ = 5) after 96-h static exposure to a ternary mixture of chlorpyrifos, imidacloprid and dimethoate. Asterisks indicate significantly different survival rates ($p<0.05$) when compared to control using Bonferroni post-hoc multiple comparison. Errors bars indicate one standard error.

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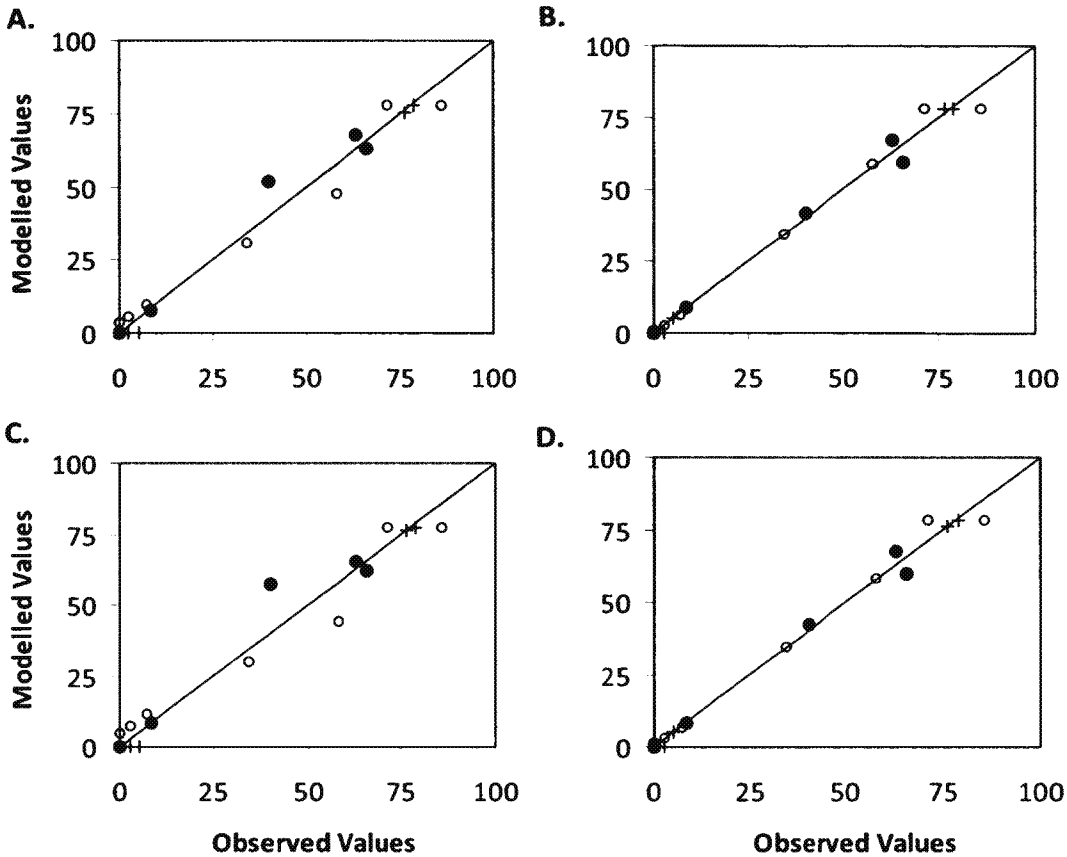

Figure 2.9 – Description of the effect of chlorpyrifos - imidacloprid mixture on mean % survival of *C. tentans*. Observed values related to modeled values calculated using the MIXTOX model for (A) the CA reference model, (B) the CA synergistic/antagonistic model, (C) the IA reference model and (D) the IA dose-level dependence model. Data points are labelled as chlorpyrifos (○), imidacloprid (+) and mixture (●). The points in (B) and (D) are distributed more equally around and draw closer to the 1:1 line than in the reference models (A or C).

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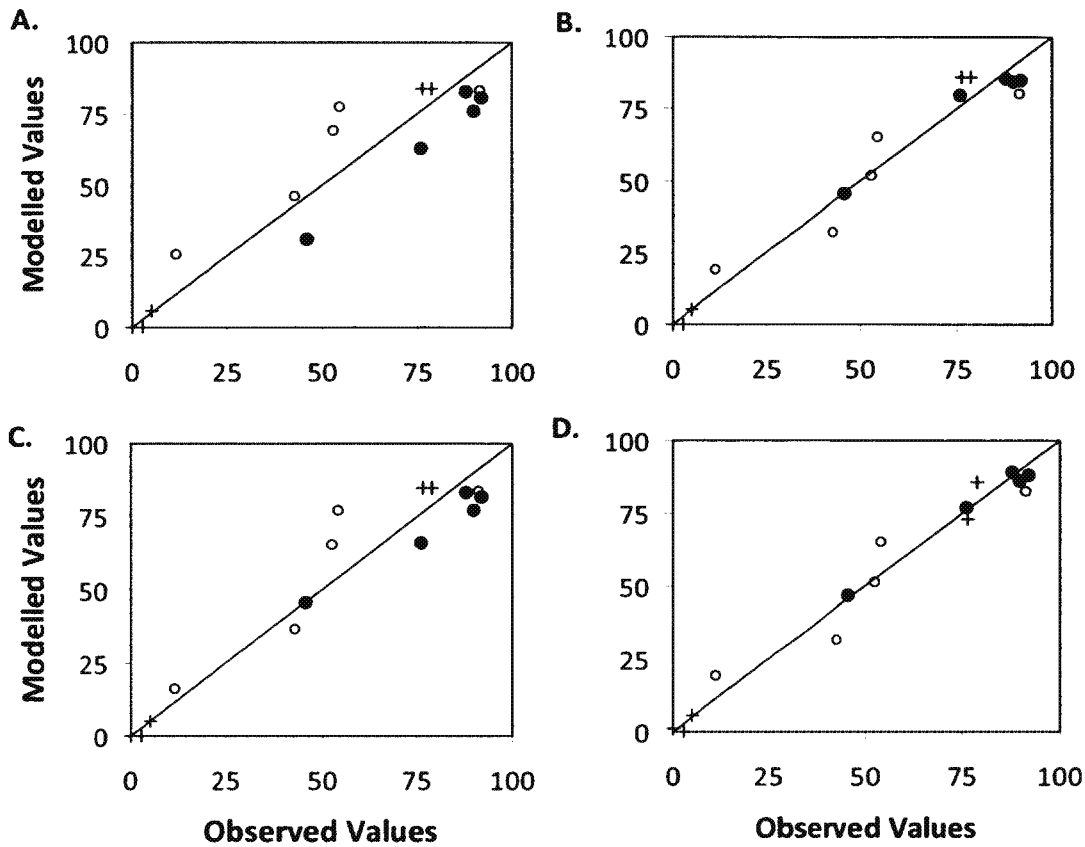

Figure 2.10 - Description of the effect of imidacloprid - dimethoate mixture on mean % survival of *C. tentans*. Observed values related to modeled values calculated using the MIXTOX model for (A) the CA reference model, (B) the CA dose-level dependence model, (C) the IA reference model and (D) the IA synergistic/antagonistic model. Data points are labelled as imidacloprid (○), dimethoate (+) and mixture (●). The points in (B) and (D) are distributed more equally around and draw closer to the 1:1 line than in the reference models (A or C).

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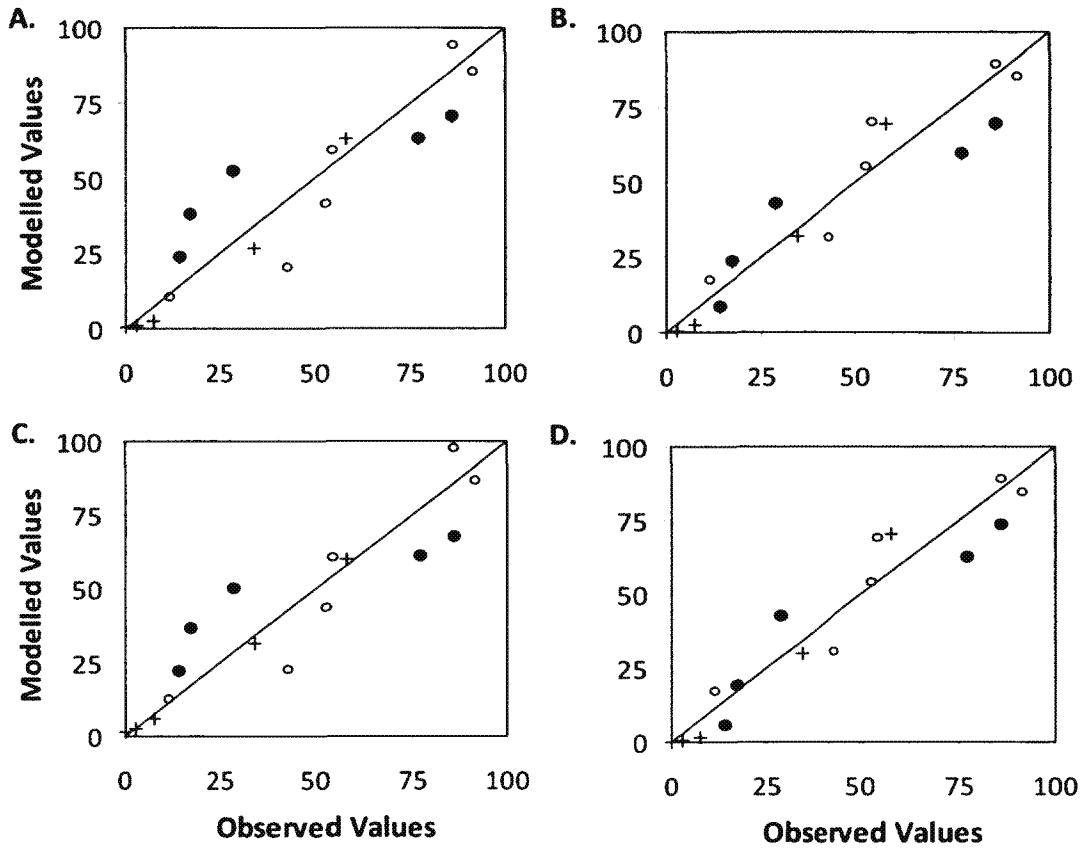

Figure 2.11 - Description of the effect of dimethoate - chlorpyrifos mixture on mean % survival of *C. tentans*. Observed values related to modeled values calculated using the MIXTOX model for (A) the CA reference model, (B) the CA dose-level dependence model, (C) the IA reference model and (D) the IA dose-level dependence model. Data points are labelled as dimethoate (○), chlorpyrifos (+) and mixture (●). The points in (B) and (D) are distributed more equally around and draw closer to the 1:1 line than in the reference models (A or C).

CHAPTER 3: Sublethal effects of a pulsed exposure to a mixture of three agricultural insecticides on the midge *Chironomus tentans*

3.0 Abstract

Given that pesticides typically enter aquatic systems through events such as runoff from precipitation events or spray drift, aquatic organisms are typically exposed to irregular influxes of contaminants through non-continuous pulses that rapidly dissipate throughout the system. This study investigated sublethal effects (growth, emergence and sex ratio) of a pulse of agricultural insecticides – chlorpyrifos, imidacloprid and dimethoate – and subsequent recovery period on the midge *Chironomus tentans*. Seven-day old larvae were exposed to a 4-d pulse of one of the single toxicants or the ternary mixture, followed by a 24-d recovery period. My results show that at the study concentrations, *C. tentans* was able to recover from the single-pulse exposures over the 24-d recovery period. Survival with exposure to chlorpyrifos and imidacloprid, and emergence in the dimethoate and mixture treatments were greater than in the controls, which suggests that hormesis occurred. No significant effects on growth or sex ratio were observed.

3.1 Introduction

Aquatic systems in agricultural settings are frequently exposed to contaminants through such events as runoff from irrigation and precipitation events or from spray drift. These irregular influxes of chemicals into systems often occur as non-continuous pulses

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of contaminants which quickly dissipate throughout the system (Zhao and Newman 2006). Given the complex combinations of chemicals used in agricultural situations, non-target aquatic biota are often exposed to mixtures of these chemicals once they enter the ecosystem. Furthermore, pesticide mixtures often occur at relatively low contaminant concentrations that produce sublethal effects (Teuschler *et al.* 2002). Depending on the length of the pulse and the time between pulses, some studies have shown that aquatic organisms may be able to recover from the effects of such contaminant pulses (Naddy *et al.* 2000; Naddy and Klaine 2001; Zhao and Newman 2006). It is therefore both important and necessary to investigate the sublethal effects of pulses of agricultural chemical mixtures on non-target aquatic organisms, and to assess the potential for biota to recover from pulse exposures.

Three agricultural insecticides currently utilized in potato production in New Brunswick, Canada were chosen for use as the insecticide mixture (Dunn 2004; Murphy *et al.* 2006; GNB 2009). Chlorpyrifos (*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate) and dimethoate (*O,O*-dimethyl *S*-[2-(methylamino)-2-oxoethyl] phosphorodithioate) are both organophosphate insecticides used to control beetles (chlorpyrifos) and aphids (dimethoate), while imidacloprid ((1-[(6-chloro-3-pyridinyl)methyl]-*N*-nitro-2-imidazolidinimine) is a neonicotinoid used to control Colorado potato beetles, flea beetles and aphids (GNB 2009). Both organophosphate insecticides exert their toxic action as acetylcholinesterase inhibitors. Imidacloprid mimics the nicotine molecule and irreversibly blocks insect nicotinic acetylcholine receptors, thereby preventing acetylcholine from binding. All three insecticides disrupt the nervous system of insects, ultimately causing death (Cox 1994; Cox 2001).

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The main objective of the study was to determine the sublethal effects of a pulse of chlorpyrifos, imidacloprid, dimethoate, and a mixture of all three chemicals on the survival, growth, emergence and reproduction of *Chironomus tentans*. I further examined recovery from the effects. This midge is ubiquitous in aquatic systems, has a relatively short lifecycle (23-30 d at 23°C under laboratory conditions (Benoit *et al.* 1997), is easily cultured in the laboratory, and its early instars are especially sensitive to contaminants (Rakotondravelo *et al.* 2006). I hypothesized that *C. tentans* growth would decrease and fewer chironomids would emerge with exposure to the ternary mixture, and that there would be a shift in sex ratio towards the emergence of more adult males than females, as was observed by Rakotondravelo *et al.* (2006). Recovery takes place via processes such as detoxification, elimination and healing of damaged tissues and can differ by chemical (Zhao and Newman 2006). I hypothesized that less recovery from the pulse effects would occur in the mixture treatments as opposed to the single chemical treatments given that the presence of multiple chemicals could require multiple detoxification, elimination and other recovery processes to occur simultaneously.

3.2 Materials and Methods

3.2.1 Exposure Solutions and Dilutions

Test solutions were prepared using agricultural formulations of the three test insecticides (Lorsban 4E® (active ingredient (a.i.): chlorpyrifos; Dow AgroSciences Canada, Calgary, AB, Canada), Admire 240F® (a.i.: imidacloprid; Bayer CropScience, Calgary, AB, Canada), and Lagon 480® (a.i.: dimethoate; United Agri Products Canada,

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Dorchester, ON, Canada)). Stock solutions of each chemical were prepared using the appropriate amount of the chemicals into dechlorinated groundwater (Aquaculture Facility, University of New Brunswick, Fredericton, NB, Canada) which had been acclimated to test conditions and aerated for a minimum of 24 h in a 25-L Nalgene® carboy. Stock solutions were stored at 4 °C in amber glass bottles.

Exposures were conducted for each of the three individual insecticides as well as for the ternary mixture of the same chemicals. Exposure concentrations were prepared by pipetting calculated concentrations of the stock solution into known volumes of test water to achieve desired concentrations. Exposure mixtures were prepared by adding aliquots of nominally equivalent toxic units (TU) of the individual chemicals to the same container of test water. One TU was equivalent to the 96-h LC_{50} of each chemical determined in previous work (see Chapter 2; Table 3.1).

3.2.2 Exposure Organisms

Culturing and exposure procedures were a combination of those outlined by Environment Canada (1997), USEPA (2000) and Stoughton *et al.* (2008).*Chironomus tentans* egg sacs were obtained from laboratory cultures at the Toxicology Centre, University of Saskatchewan, Saskatoon, SK. Egg sacs were acclimated to the culture conditions in an environmental growth chamber (Percival Scientific, Inc., Perry, IA, USA) until hatching was observed. They were then transferred to 28 L culture tanks containing a layer of washed silica sand (212 – 425 µm) approximately 1 cm deep and 12 L of dechlorinated groundwater. Culture tanks were maintained in an environmental

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chamber at 23 °C with a 16:8 light: dark schedule. Cultures were fed a 20 mL of 100 g/L Nutrafin® fish food slurry three times per week.

Ten-day and 28-d tests were conducted with approximately 7-d old larvae. Eleven days prior to the start of a test, adults were collected and bred overnight to produce egg sacs. Eggs sacs were held in hatching chambers until hatching occurred, and were then transferred to a new culture tank, under the same conditions as the main culture tank, although this tank was fed 5 mL of 100 g/L Nutrafin® fish food slurry every second day.

3.2.3 Exposure Procedures

Groups of ten larvae were randomly allocated to each exposure vessel (Kimax® 300 mL tall form beaker without spout) containing a 0.5 cm deep layer of washed silica sand (212 – 425 μm) and 200 mL test solution (Figure 3.1; Table 3.2). 96 h static exposure to treatments was followed by a recovery period in dechlorinated groundwater for the remainder of the test durations. Two concentrations per chemical combination were used, with seven replicates per concentration. Fourteen control vessels were used, with dechlorinated groundwater as the exposure solution. Tests were conducted in an environmental chamber at 23 °C with a 16:8 light: dark schedule. Aeration occurred through a network of airline tubing connected to a compressor via a manifold. A glass Pasteur pipette was attached to the end of each line and placed into each beaker through a hole drilled in the Petri dish lid. Vessels were gently aerated (2-3 bubbles per second) throughout the experiment.

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Throughout the test, organisms in each beaker were fed 1.5 mL of 4 mg/mL Nutrafin® fish food slurry and emergence was monitored daily, as was the sex of all emerged organisms. Emergence was considered complete when organisms shed the pupal exuviae and escaped the surface tension of the water. Partial emergence was recorded when organisms only partially shed the pupal exuviae and did not escape the surface tension of the water, which typically resulted in death within 24 hours. Aeration rates were also adjusted as necessary. Dissolved oxygen, temperature, pH, ammonia and specific conductivity were measured at 0 h and approximately weekly for the duration of the experiment (using Nutrafin® Master Test Kit #A-7865).

After the 96-h exposure period, organisms were transferred to clean vessels containing a 0.5 cm deep layer of washed silica sand (212 – 425 μm) and 200 mL dechlorinated groundwater. During the recovery period, water was partially renewed three times per week by removing 140 mL of vessel water and replacing it with fresh, acclimated and aerated dechlorinated groundwater.

On day 10, three vessels from each treatment, and seven controls were randomly selected and terminally sampled. Survival of organisms was determined, and any living larvae were preserved in ethanol (EtOH) to determine growth by measurement of head capsule width and determination of ash-free dry mass (AFDM). Any pupae recovered were included in survival counts, but not in growth measurements. Any organism that was completely inactive and showed no response when prodded with a sharp point was considered dead.

Remaining beakers were monitored in the same manner for the remainder of the test. At day 28, the remaining four vessels per treatment and seven controls were

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terminally sampled as on day 10. Survival at the end of the test was determined as the total number of larvae and pupa alive at day 28, plus the total number of emerged adults over the duration of the test. Emergence success was determined by totalling the number of chironomids that had successfully emerged over the 28-d test period. Sex ratio was determined by tallying the total number of males and females that successfully emerged in each treatment. AFDM and head capsule width were not measured at 28 d, as the majority of the organisms were no longer in the larval stage but had emerged as adults.

3.2.4 Sample Processing

Head capsule width of preserved chironomids from day 10 was determined. Measured organisms were then placed in pre-ashed aluminum weight boats (all larvae from each replicate were placed in the same boat), oven dried to a constant weight for 24-48 h at 60°C, and then brought to room temperature in a dessicator. Boats and organisms were then weighed to nearest 0.01 mg. Boats containing organisms were ashed for 2 h at 550°C, cooled in a dessicator once again and then reweighed. AFDM was the difference between the weight of the dried larvae in weigh boat and the weight of the ashed larvae in weigh boat.

3.2.5 Chemical Analysis

Stock (1L) and test solution (125 mL) samples were taken after solutions were prepared (before 0 h), and at 96 h. Samples were stored in amber glass bottles at 4 °C and shipped to the National Hydrology Research Centre (Environment Canada) in Saskatoon, SK, Canada for analysis. Pesticides were isolated using solid-phase (imidacloprid and dimethoate) or liquid-liquid (chlorpyrifos) extraction. The sample

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extracts were evaporated to dryness and the extract residues re-dissolved in water (1.0 mL) prior to analysis by LC-MS-MS. The LC/MS/MS system consisted of a Waters Micromass Quattro Ultima triple quadrupole mass spectrometer interfaced with a Waters 2695 Alliance HPLC System (Waters Limited, Mississauga, Ontario, Canada). The mass spectrometer was equipped with an electrospray ionization interface set to positive ion mode. The LC system was used with a Waters Xterra MS C_{18} (100 mm x 2.1 mm i.d., 3.5 μm particle size) analytical column. The mobile phase was aqueous acetonitrile containing 0.1 % formic acid (v /v). The column flow rate was 200 μL/min and injection volumes were 100 μL for chlorpyrifos and imidacloprid and 20 μL for dimethoate.

3.2.6 Data Analysis

Actual exposure concentrations used to determine exposure toxic units were based on chemical analysis results. Stated values are the average of the analysed concentrations at 0 h and 96 h. Survival, growth and reproduction (Table 3.2) treatment effects and interaction terms were assessed by factorial analysis of variance (ANOVA) using Systat® versions 11 and 13 (Systat Software, Inc., Chicago, IL, USA). When assumptions of normality and/or homogeneity of variance were not met, data were transformed using an arcsine square-root transformation. Cumulative emergence was analysed by ANCOVA using a general linear model in Minitab® version 16 (Minitab Inc., State College, PA, USA) to investigate the effects of treatment on the rate of chironomid emergence. Factorial ANOVA was used to determine differences in overall emergence when the regression lines in the ANCOVA did not have homogeneous slopes. Post-hoc comparisons were made using Bonferroni multiple pairwise comparisons. Normality and

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homogeneity of variance were tested, and data were transformed if either assumption were violated. Differences between time to first emergence in males and females were determined using t-tests. An alpha level of 0.05 was used in all statistical analyses.

3.3 Results

Larval survival was significantly affected by an interaction between pesticide type and concentration ($F_{(interaction)\ 6,\ 82} = 4.91, p < 0.001$) using a three-way ANOVA. The main effect of time was also significant ($F_{(main\ effect)\ 1,\ 82} = 17.86, p < 0.001$) and therefore required a two-way ANOVA to test the effect of pesticide types on pesticide concentration and time. Survival was significantly affected by pesticide concentration in both the chlorpyrifos and imidacloprid treatments (Figure 3.2). The low concentration of chlorpyrifos resulted in borderline significantly greater survival as compared to the high concentration (Fig. 3.2; $F_{2,\ 22} = 3.32, p = 0.055$), while survival in the low concentration of imidacloprid was significantly different from both the control and the high concentration ($F_{2,\ 23} = 9.48, p = 0.001$). Survival was significantly affected by time (10 d or 28 d) when larvae were exposed to chlorpyrifos or dimethoate (chlorpyrifos: $F_{1,\ 22} = 5.26, p = 0.032$; dimethoate: $F_{1,\ 22} = 6.66, p = 0.017$). This is not surprising, given that there was similar decrease in survival in the control beakers between the two time periods as well. Larval survival in the pesticide mixture treatment was not affected by concentration or time. Overall, survival in the control treatment was 75% at the end of the test, which is considered acceptable by USEPA (2002) guidelines.

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Larval AFDM at 10 d was not significantly affected by pesticide type or concentration (Figure 3.3). Likewise, larval head capsule width was not significantly affected by either treatment. The controls of both of these endpoints conformed to standard testing requirements, which require that by 20 d post-hatch AFDM be greater than 0.48 mg per organism (USEPA 2000), and that organisms older than 12.5 d post hatch have a mean head capsule width of 0.67 mm (EC 1997).

Slightly more organisms cumulatively emerged in the chlorpyrifos treatment (irrespective of concentration) than in the control treatment (Figure 3.4A), while imidacloprid had no effect on larval emergence (Figure 3.4B). Chironomids exposed to the high dimethoate treatment emerged at a quicker rate than in the control or low concentration (Figure 3.4C). Total percent emergence was borderline significantly different ($F_{2, 10} = 3.94$, $p = 0.055$) in the high treatment compared to the low concentration by Bonferroni's post-hoc comparison ($p = 0.067$), but not from the control. More chironomids emerged in the high concentration of the pesticide mixture and fewer emerged in the low concentration compared to the control, but neither treatment caused emergence to occur at a significantly different rate than the control (Figure 3.4D). Overall, the control had a mean percent emergence of 58 %. Sex ratio was not significantly affected by any of the treatments (Table 3.3; $F \leq 0.92, p \geq 0.431$ for all treatments). Generally, the time to first emergence was shorter for males than for females (Table 3.3), although not significantly so in the low chlorpyrifos and high dimethoate treatments ($t \leq 1.84, p \geq 0.140$ for both treatments).

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3.4 Discussion

Although traditional laboratory-based studies often focus on lethal effects of continuous exposures to contaminants, it is likely more relevant to investigate the effects of pulsed exposures which are typically observed in the environment. Pulses of contaminants at sublethal concentrations followed by adequate recovery periods can provide opportunity for organisms to partially or fully recover from such exposures (Zhao and Newman 2006). The results of the current study indicate that *C. tentans* is able to recover from a 96-h pulse of low dose chlorpyrifos, imidacloprid, dimethoate or a ternary mixture of all three over a subsequent 24-d recovery period.

Based on the chemical analysis results, the actual exposure concentrations for each of the individual treatments were extremely low (0.04 to 0.22 TU; see Table 3.1 for concentrations in μg/L). Furthermore, both the low and high mixture treatments were also low (0.18 and 0.46 TU respectively). While they were low exposure concentrations, both chlorpyrifos and dimethoate treatments and the high imidacloprid treatment, individually and in the mixture treatments exceeded Canadian water quality guidelines for the protection of aquatic life and would therefore be considered dangerous in aquatic systems. The current guideline for chlorpyrifos is 0.0035 μg/L, and 0.23 μg/L and 6.2 μg/L for imidacloprid and dimethoate respectively (CCME 2007). Imidacloprid has been detected in Atlantic Canadian watersheds at concentrations of 0.3 μg/L and dimethoate at 0.4 μg/L (Murphy *et al.* 2006). Studies in the United States have reported the presence of chlorpyrifos in aquatic systems at concentrations between 0.06 and 0.3 μg/L (Hoffman *et al.* 2000; Phillips and Bode 2004; Anderson *et al.* 2006).

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The two endpoints that were most affected by exposure to the study pesticides were survival and emergence. The results were especially interesting given that stimulatory responses instead of inhibitory responses were observed with both chemicals. With both chlorpyrifos and imidacloprid, there was an increase in chironomid survival at the low chemical concentration; at the high chemical concentration, there was a return to control levels of survival in both cases. The concept of hormesis could offer a potential description of the effects found in the current study, although further investigation would be required to confirm this hypothesis.

Hormesis is a biphasic dose-response, where an organism's response is stimulated at low concentrations of a toxicant which produces inhibitory effects at high concentrations (Mattson 2008). Based on previous acute experiments, it is known that chlorpyrifos and imidacloprid are both toxic to *C. tentans* (see Chapter 2), therefore it can be postulated that if higher concentrations had been tested in the current study, there would have been a subsequent decrease in survival below the control levels, resulting in hormesis.

One suggested mechanism for hormesis is that of overcompensation in homeostatic disruption. The application of a low dose of pesticide or other toxin may trigger low levels of stress, which upsets homeostasis. The organism likely overcompensates with certain repair mechanisms that once initiated, are able to repair even defects that were not caused by the toxic, resulting in stimulation. Once the dose exceeds certain a threshold, the toxic effects would then be observed as per usual (Calabrese and Baldwin 1999; Stebbing 2003; Yu *et al.* 2010).

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Studies have found pesticide and other toxin-induced hormesis to occur with various organisms and effects. Yu *et al.* (2010) observed imidacloprid-induced hormesis on the green peach aphid *Myzus persicae* Sulzer, where low concentrations of imidacloprid induced stimulation of fecundity, but fecundity was inhibited at high concentrations. Similarly, at low doses chlorpyrifos was found to induce the number of offspring and time to first brood in *Daphnia carinata* (Zalizniak and Nugegoda 2006). A study of the effects of several explosives on freshwater *C. tentans* and *Hyalella azteca* revealed increased growth at sub-lethal doses of the chemicals, while increased mortality was observed at higher concentrations (Steevens *et al.* 2002).

The concept of hormesis is somewhat contentious. Although many studies suggest that hormesis may be occurring (e.g., Stebbing 1982; Steevens *et al.* 2002; Zanuncio *et al.* 2003; Zalizniak and Nugegoda 2006; Yu *et al.* 2010), hormetic effects are difficult to confirm, because there is a lack of defined, appropriate methodology and no well-defined mechanisms to help describe experimental observations (Yu *et al.* 2010). Often, experimental designs are not robust enough to definitively conclude hormesis is occurring. Stebbing (1982) suggests that hormesis is often overlooked because the focus in toxicology is often on inhibitory and lethal effects. Consequently, experimental designs do not contain enough low doses, adequate dose spacing at the low end of the response curve, or sufficient measurements over time (Calabrese and Baldwin 1999). Alternatively, given that life history traits are subject to strong environmental tradeoffs, a perceived enhanced response in a measured trait may be a trade-off for a reduced response in an unmeasured trait which goes undetected, resulting in no overall stimulatory effect. Furthermore, it is contended that with certain endpoints, the responses

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at low doses below the no observable adverse effects level that differ from the control may simply be normal variation, random background fluctuations in response and not truly stimulatory (Rodricks 2003; Yu *et al.* 2010).

Given these concerns regarding hormesis, and the small number of concentrations investigated in the current study, further investigation with several more low doses would be required to confirm if the results of this study were in fact due to a hormetic effect. Additionally, a greater number of time intervals would also be beneficial. One would also need to further investigate the variation in the control to determine if the observed effects were in fact stimulatory or simply normal variation.

Emergence is considered an important and often sensitive endpoint for studying sublethal toxicity (Benoit *et al.* 1997). I did not observe any effects of chlorpyrifos or imidacloprid on either emergence patterns, or total emergence. Given that our data revealed no significant differences among treatments with respect to growth (AFDM and head capsule width) or sex ratio when compared with the control, this is not surprising. Liber *et al.* (1996) found that reduced growth in *C. tentans* larvae resulted in an approximately one-to-one relationship with reduced cumulative emergence.

I did, however, observe a significantly greater percent emergence in the high treatment compared to the low treatment in both the dimethoate and mixture treatments. This increase in survival may be another instance of hormesis, even though the stimulatory effect is seen at the high concentration as opposed to the low concentration. Given the very small concentrations used in this study, the high concentration is still a sublethal concentration, and if higher concentrations were tested, they would likely at some point have become lethal.

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Overall, the current study reveals only a few significant effects of any of the single or mixture pesticide treatments. The effects I did observe were mainly stimulatory responses of survival and emergence. Given the very low exposure concentrations used, and the relatively short exposure pulse of 96 h, it would appear that *C. tentans* larva for the most part were able to fully recover from any negative effects from exposure to the pesticide treatments. This was even the case in the mixture treatments, where the high treatment had a combined toxic unit of 0.46, indicating that there is no evidence that either antagonism or synergism occurred at any measureable level.

When *Daphnia magna* were exposed to chlorpyrifos, a constant exposure to 0.5 μg/L for 12 hours caused a 50 % lethal effect, while two 6-h pulses of 0.5 μg/L separated by 3 h did not cause any lethal effects (Naddy *et al.* 2000). The recovery process may be able to occur if the time between pulses is long enough. Furthermore, if adequate, recovery time may enable multiple pulses to be less toxic overall than a continuous exposure to a lower concentration. For this reason, it is important to investigate pulsed exposures in addition to constant exposures, seeing as there is the potential for constant exposure experiments to overestimate toxicity of real world exposures to contaminants (Stoughton *et al.* 2008).

The need to understand pesticide-induced hormesis in the agricultural context is very important. A better understanding of this concept could have effects on pest control strategies and pest resistance management practices (Yu *et al.* 2010). Hormesis offers a potential alternative explanation for the pest resurgence phenomenon which often occurs in agricultural situations. After a period of reduced pest occurrence when a pesticide is sprayed on a crop, there is often a resurgence of the pest a short while later. This

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resurgence is often attributed to the pest's predators being susceptible to the pesticide, which reduces the predators, allowing the pest population to increase. As an alternative explanation, increased pest survivorship and maturation could be the result of pesticide-induced hormesis (Morse 1998).

3.5 Conclusions

Although our original predictions of reduced growth and emergence and a shift in sex ratio towards males with exposure to the ternary mixture were not confirmed, this may have been due to the extremely low exposure concentrations used. At the concentrations used, no detectable negative long term effects were observed, despite the exposure concentrations having exceeded several Canadian water quality guidelines. Thus, I conclude that *C. tentans* larva were able to fully recover or experience induced stimulation from the single pulse exposure at the given test concentrations and reach maturity either without measureable negative effects or possibly with measureable positive effects.

The findings of this study highlight the need for further investigation of the effects of contaminant pulses on aquatic systems. Further research into pesticide-induced hormetic effects is also warranted. Furthermore, the question of whether mixtures have differing sublethal effects than the individual mixture components is still a valid research question that merits further investigation with greater exposure concentrations. A valuable pulse regime to investigate would be one with a one or several high pulse concentration to mimic the exposure of aquatic systems during peak runoff events,

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followed by a much lower, or decreasing, extended exposure similar to the contaminants dissipating downstream (Naddy *et al.* 2000).

3.6 Acknowledgements

I would like to acknowledge and thank the Environment Canada (Saskatoon) laboratory, especially Jon Bailey for technical assistance throughout this study, as well as Kristie Heard, Alexa Alexander, and Adam Bliss for their invaluable help in monitoring the experiment. I would also like to thank Dave Hryn for laboratory assistance and Dr. Robert Brua for his statistical expertise.

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3.8 Tables

Table 3.1 - Exposure concentrations used in chronic 10 d and 28 d bioassays of *C. tentans*. LC_{50} values used to calculate the TU values were determined in previous work (see Table 2.5, Chapter 2). Mixture concentrations are given as total TU, with TU (or µg/L) of individual mixture components in brackets (chlorpyrifos + imidacloprid + dimethoate respectively). N/A indicates not applicable.

Chemical	LC_{50} (µg/L)	Low Concentration		High Concentration	
		(µg/L)	(TU)	(µg/L)	(TU)
Chlorpyrifos	0.54	0.022	0.04	0.043	0.08
Imidacloprid	2.65	0.15	0.06	0.44	0.16
Dimethoate	1292.99	102.18	0.08	283.02	0.22
Mixture	N/A	(0.017+0.17 +105.54)	0.18 (0.03+0.06 +0.08)	(0.026+0.49 +278.78)	0.46 (0.05+0.19 +0.22)

Table 3.2 - Summary of investigated effects.

Lethal	Sublethal		
Survival	Growth	Emergence	Reproduction
Larvae (10 d)	Larvae dry weight (10 d)	Time to first emergence	Sex ratio
Adults	Larvae head capsule width (10 d)	Total emergence	
		Cumulative emergence	

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Table 3.3 - Sex ratio (♂:♀) and time to first emergence (in experimental days) ($n_{chironomids/replicate}$ = 10; $k_{replicates/treatment}$ = 4). Sex ratios in all treatments were not significantly different from the control. Asterisks indicate significant differences.

Treatment		Sex Ratio (♂:♀)	Mean Time to First Emergence (± SE)	
Concentration	**Chemical**		**Males**	**Females**
Control	--	1.41	18.25 (1.09)*	23.00 (0.93)
Low	Chlorpyrifos	1.78	18.75 (2.17)	22.25 (0.85)
	Imidacloprid	2.29	17.75 (0.75)*	23.00 (1.68)
	Dimethoate	1.33	18.00 (1.29)*	23.25 (1.44)
	Mixture	1.71	19.25 (1.49)*	24.00 (0.91)
High	Chlorpyrifos	1.86	16.67 (0.88)*	22.00 (1.15)
	Imidacloprid	2.67	18.25 (1.60)*	23.25 (0.95)
	Dimethoate	1.3	18.00 (0.58)	21.00 (1.53)
	Mixture	2.11	16.50 (0.65)*	22.25 (1.49)

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3.9 Figures

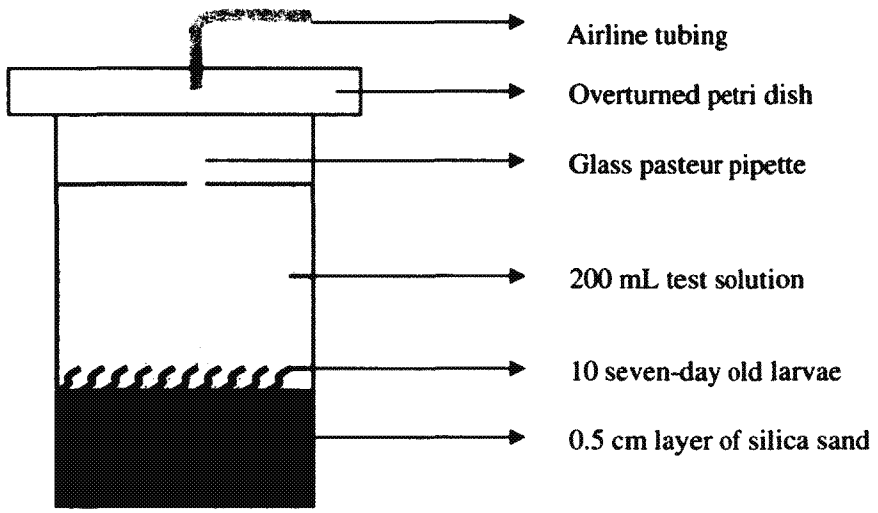

Figure 3.1 – Diagram of *Chironomus tentans* exposure vessel set-up.

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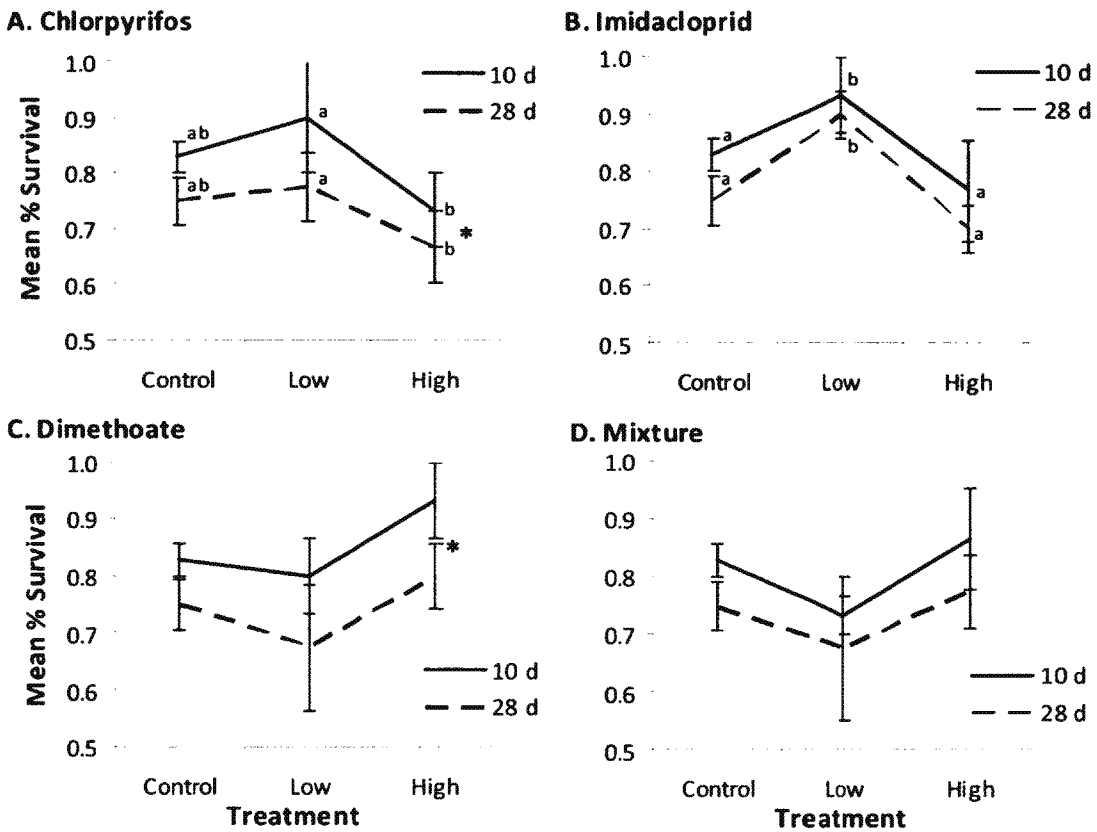

Figure 3.2 – Mean % survival of *C. tentans* per treatment level ($n_{chironomids/replicate}$ = 10; $k_{replicates/treatment}$ = 3-4) at 10 d and 28 d of chronic exposure to 96 h pulse of (a) chlorpyrifos, (b) imidacloprid, (c) dimethoate and (d) ternary mixture of chlorpyrifos, imidacloprid and dimethoate, followed by recovery period. Different letters indicate significant differences between treatments ($p<0.05$), and asterisks indicate significant differences between 10 d and 28 d time periods ($p<0.05$). Error bars indicate ± one standard error.

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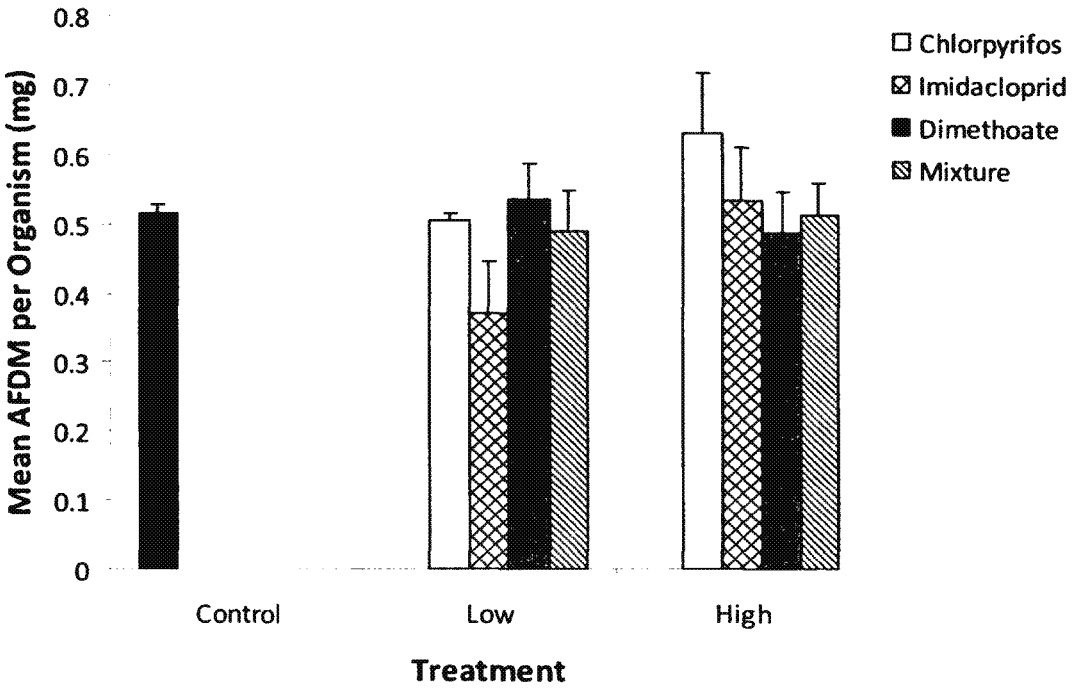

Figure 3.3 - Mean AFDM per *C. tentans* larvae (mg) per treatment level ($n_{chironomids/replicate}$ = 6-10; $k_{replicates/treatment}$ = 3) at day 10 of chronic bioassay (96 h pulse of treatment chemical, followed by 6 d recovery period). None of the treatments were significantly different emergence rates (α=0.05) when compared to control. Error bars indicate one standard error.

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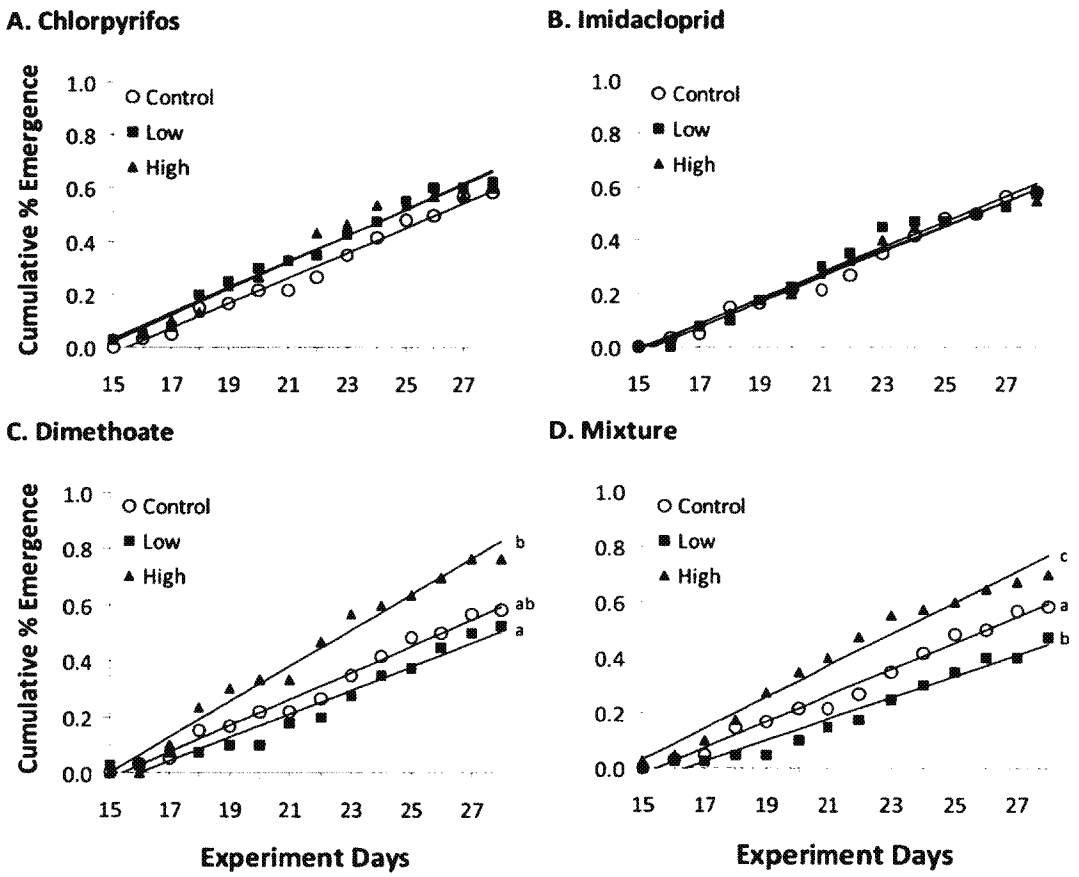

Figure 3.4 - Cumulative % emergence over time of *C. tentans* throughout 96 h pulse of (a) chlorpyrifos, (b) imidacloprid, (c) dimethoate and (d) ternary mixture of chlorpyrifos, imidacloprid and dimethoate, followed by a recovery period. Different letters indicate significant differences in the total percent emergence by two-way ANOVA ($p<0.05$).

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CHAPTER 4: Conclusion

4.1 Introduction

My research has revealed that there are important lethal and sublethal effects of insecticide mixtures on aquatic insects that warrant consideration and further investigation. The main objective was to investigate the lethal and sublethal effects on non-target aquatic insects of chlorpyrifos, imidacloprid and dimethoate, three agricultural insecticides currently in use in potato production in New Brunswick (GNB 2009), as well as mixtures of these chemicals. These are important questions to investigate, given that pesticides are applied to crops in combination, therefore, they are entering aquatic systems and exerting effects in combination as opposed to individually. Despite this reality, pesticides are typically regulated separately, without regard for the joint effects that they may exert in the real world. Furthering our knowledge of mixture effects will allow regulators to better regulate pesticide usage based on realistic effects that occur in the real world.

Chapter 2 investigated the lethal effects of chlorpyrifos, imidacloprid, dimethoate and the binary and ternary mixtures of these same chemicals. Test organisms were exposed to a range of concentrations of these chemicals using 96 h bioassays. LC_{50} values were determined, and used to create binary and ternary mixture combinations using toxic units. Survival data was then modelled using the MIXTOX model (Jonker *et al.* 2005) in an attempt to determine whether the mixtures were antagonistic, additive or synergistic.

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Chapter 3 investigated the sublethal effects of the same chemicals: chlorpyrifos, imidacloprid and dimethoate, as well as a ternary mixture of all three chemicals at sublethal levels. I was interested in the effects of a brief (96-h) sublethal pulse of toxicant followed by a long recovery period (24-d) on the organisms, which generally encompassed the early instar larvae to emergence stage of the organisms. Such a pulse more realistically mimics the brief, high concentration entrance of pesticides into aquatic systems than traditional continuous exposure experiments. During a pulsed exposure, toxicants enter aquatic systems mostly through run-off events (e.g., during rain storm events) that dissipate fairly rapidly in the system, leaving recovery periods where organisms may be able to recover either partially or in full from the effects of the toxicant exposure (Naddy *et al.* 2000).

4.2 Findings and Implications

Chironomus tentans (Diptera: Chironomidae) was chosen as the main study species given that the species is ubiquitous in aquatic systems, readily cultured in the laboratory and has a relatively short lifecycle. *C. tentans* are commonly used in ecotoxicological studies; therefore there was a previous body of work with which to compare the study results. Heptageniid mayfly larvae, used only in the 96-h LC_{50} bioassays for the single toxicant exposures, are common in local stream and river ecosystems. Mayflies are known to be sensitive to environmental stressors such as insecticide exposure (Alexander *et al.* 2007; Alexander *et al.* 2008).

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Chlorpyrifos was found to be the most acutely toxic insecticide (Mayfly 96-h LC_{50} = 0.01 µg/L; *C. tentans* 96-h LC_{50} = 0.54 µg/L), followed by imidacloprid (Mayfly 96-h LC_{50} = 3.67 µg/L; *C. tentans* 96-h LC_{50} = 2.65 µg/L) and then dimethoate for both Heptageniid mayfly and *C. tentans* larvae. Dimethoate was much less toxic than the other two insecticides for both study organisms (Mayfly 96-h LC_{50} = 199.18 µg/L; *C. tentans* 96-h LC_{50} = 1292.99 µg/L). These values are consistent with other literature values (Ankley and Collyard 1995; Moore *et al.* 1998; Jin-Clark *et al.* 2002; Anderson and Zhu 2004; Alexander *et al.* 2007; Domingues *et al.* 2007; Mehler *et al.* 2008; Stoughton *et al.* 2008).

All of these LC_{50} values greatly exceed Canadian Water Quality Guidelines for the protection of aquatic life (CCME 2007), with the exception of the mayfly value for chlorpyrifos. Furthermore, these values also exceeded the values that have been detected in aquatic systems by such studies as Murphy *et al.* (2006), Hoffman *et al.* (2000), Phillips and Bode (2004) and Anderson *et al.* (2006), which means that the guidelines appear to be adequately protecting the study species from lethal toxicity due to exposure to chlorpyrifos, imidacloprid or dimethoate individually.

When the test chemicals were combined into binary mixtures, none of the mixtures were strictly additive, not even the mixture of chlorpyrifos and dimethoate which are both organophosphates and would traditionally be expected to be additive based on their common mode of action (MOA). Both the dimethoate-chlorpyrifos mixture and the ternary mixture of all three insecticides were also synergistic. Furthermore, the two organophosphates resulted in opposing mixture effects when

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combined with imidacloprid. The chlorpyrifos-imidacloprid mixture was synergistic, while the imidacloprid-dimethoate mixture was antagonistic.

The opposing effects of the organophosphorus insecticides chlorpyrifos and dimethoate may lend support to the supposition that MOA is not necessarily the best predictor of mixture effects. MOA traditionally has been used to predict which model, concentration addition (CA) or independent action (IA), will best predict or describe the effects of a pesticide mixture, where chemicals with similar MOA are presumed to behave by CA, and chemicals with differing MOA are presumed to behave by IA. The models, however, only account for the toxicodynamics of mixtures, or the interactions at target sites, while many chemicals might influence factors such as bioavailability, solubility, and secondary modes of action which could alter the interaction of chemicals within a mixture and consequently the mixture's effect on organisms (Lydy *et al.* 2004; Cedergreen *et al.* 2008).

Despite the fact that MOA may not be the best predictor of mixture effects, it is currently a very predominant concept in mixture modeling. Using the MIXTOX model to describe the binary mixtures, the CA and IA reference models predicted less well the effects of the mixtures than did the inclusion of either synergism/antagonism or dose-level deviations. Our results agreed with the growing consensus in the literature that the CA model, although it is more conservative than the IA model, and may in some cases overestimate toxicity, is more easily calculated and is the most appropriate mixture model currently available (e.g., Backhaus *et al.* 2000; Belden and Lydy 2000; Broderius *et al.* 2005; Belden *et al.* 2007).

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Subjecting *C. tentans* to a 96-h sublethal pulse of one of the individual test chemicals, or a mixture of all three, followed by a 24-d recovery period resulted in full recovery for all endpoints. No inhibitory effects of the single exposures or exposures to the ternary mixture were observed, and furthermore, potential stimulation of increased survival was observed with the chlorpyrifos and imidacloprid treatments and increased emergence was observed with the dimethoate and mixture treatments. Such results suggest that at very low concentrations, if the recovery period length is sufficient, *C. tentans* are able to recover from the sublethal insecticide effects, whether or not the organisms were exposed to the individual chemicals or a mixture of all three.

Given the extremely low concentrations used, the observed stimulatory effects of the sublethal exposures may be evidence of hormetic effects, however further work with a greater number of low concentrations would be required to confirm this hypothesis. Several studies investigating contaminant effects on insects have observed similar potential cases of hormesis (Steevens *et al.* 2002; Zalizniak and Nugegoda 2006; Yu *et al.* 2010). Alternatively, theses stimulatory effects may simply be due to normal background variation in the endpoints (Rodricks 2003; Yu *et al.* 2010).

4.3 Recommendations and Conclusions

The current study provides important findings with respect to pesticides, both singly and in mixture, at both lethal and sublethal concentrations. These findings also suggest questions that merit further investigation to more fully enable understanding of the effects of pesticide exposures.

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In order to gain a more complete picture of the effects of mixtures of pesticides and other toxicants, experimental designs that more fully span the concentration spectrum are necessary. This is the case with mixture studies, where a wider range of mixture ratios would allow for a more complete description of the response surfaces of the mixtures, and therefore provide a more complete picture of how mixture effects are affected by differing mixture ratios and concentrations. Sublethal studies would also benefit from a design that more fully encompasses the complete concentration spectrum, including the concentration range below the no observable adverse effect level so that more subtle effects such as hormesis may be detected. The larger scale of such experimental designs would require more resources and funding, but finding ways to accomplish such work is necessary if we are to more fully understand the finer details and more subtle effects of toxicants and their mixtures.

Furthermore, models that are able to handle ternary and higher order mixtures are needed to tease apart the effects of these more complicated mixtures. This is especially important given that mixtures in real-world exposures are more than likely to contain many chemicals (De Zwart and Posthuma 2005) instead of the two chemicals that the MIXTOX model in the current study was able to accommodate. Investigation of the validity of MOA as a mixture effects predictor needs also to be examined, because factors such as bioavailability, water solubility and secondary modes of action may be of equal or greater importance in mixture effects.

Finally, investigation of alternative pulse and recovery schemes to evaluate sublethal effects of both single and mixtures of these pesticides is warranted. While I considered one scheme (single, short, low dose pulse with long recovery), several other

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schemes would be environmentally relevant. For example, such schemes as a series of multiple pulses with recovery periods in between pulses, longer pulses, and brief high doses pulses would all enable greater understanding of sublethal effects. While the current study did not elucidate sublethal effects of the mixture of chlorpyrifos, imidacloprid and dimethoate, mixtures at greater concentrations or different ratios may produce effects worth investigating.

Aquatic systems in agricultural and urban catchments are currently protected with regulatory measures based on the best information currently available (e.g. CCME guidelines for the protection of aquatic life). However, while CA appears to be the best model for predicting mixture effects, the results of the current study also demonstrate that response to pesticide mixtures is complicated and such mixtures do not necessarily behave strictly according to the current model. There are deficiencies which need to be addressed (e.g. validity of using MOA as mixture effect predictor). Furthermore, exposure regimes which can be dictated by such events as runoff effects can alter the effects of pesticide exposure. These factors must be addressed in order to ensure the ongoing, adequate protection of aquatic life.

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CURRICULUM VITAE

Heidi Margareta Kierstead LeBlanc

BSc. University of New Brunswick 2006

Publications:

LeBlanc HMK, Culp JC, Baird D, Alexander AC, Cessna A. *In prep*. Single versus combined lethal effects of three agricultural insecticides on freshwater insect larvae.

Alexander AC, Culp JC, **LeBlanc HMK**, Luiker EA, Baird, DJ, Cessna AJ. *In prep.* Antagonistic and synergistic effects of insecticides in aquatic communities: is mode of action classification sufficiently protective?

Conference Presentations:

Culp J, Cessna A, Baird D, Alexander A, **LeBlanc H.** 2009. Ecological risk assessment of pesticides using realistic mixture scenarios. *In* Proceedings of the 36th Aquatic Toxicity Workshop. September 27-30, 2009. La Malbaie, QC, Canada.

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Message

From: Peck, Charles [Peck.Charles@epa.gov]
Sent: 3/19/2019 6:32:54 PM
To: Panger, Melissa [Panger.Melissa@epa.gov]; Garber, Kristina [Garber.Kristina@epa.gov]; Connolly, Jennifer [Connolly.Jennifer@epa.gov]; Rossmeisl, Colleen [Rossmeisl.Colleen@epa.gov]
CC: Paisley-Jones, Claire [Paisley-Jones.Claire@epa.gov]
Subject: Methomyl Use on Grapes
Attachments: methomyl_draft_rcd_11-10-15_wth_app_ IV.pdf

All,

Deliberative Process / Ex. 5

Chuck Peck
OPP/EFED/ERB VI
Potomac Yard South
Crystal City, VA
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peck.charles@epa.gov

Message

From: Galic Nika USGR [Nika.Galic@syngenta.com]
Sent: 3/25/2019 7:49:30 PM
To: Garber, Kristina [Garber.Kristina@epa.gov]
CC: Valery Forbes [veforbes@umn.edu]
Subject: AQUATOX manuscript
Attachments: STOTEN_Aquatox_Reviewer_comments.docx

Flag: Follow up

Dear Kris,

Hope you are well and all caught up with work from January. I wanted to let you know that our manuscript was returned from STOTEN for revisions which are more than addressable. I am currently working on addressing some of the comments and hoping I could get some clarification and input from you, as some were on PRZM modeling. Would you have a look at the questions and help me respond to the reviewer? For now I am just attaching the review, as am still working on the manuscript.

Also, did you have any reviews come back from your managers that you can share with us? I am hoping you can come back to the authors list soon ☺

Thanks,
Nika

==========
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Message

From: Corbin, Mark [Corbin.Mark@epa.gov]
Sent: 6/5/2018 4:22:11 PM
To: Kanarek, Andrew [Kanarek.Andrew@epa.gov]; Kyle, Lee [Kyle.Lee@epa.gov]
CC: Garber, Kristina [Garber.Kristina@epa.gov]; Sankula, Sujatha [Sankula.Sujatha@epa.gov]
Subject: FW: two papers
Attachments: TroutCaseStudy May 4 clean mc.docx; puruckeretal_171101_amphib_dermal_model mc.docx

Just recirculating because I failed to cc Andrew on the last email.

mark

From: Corbin, Mark
Sent: Friday, June 01, 2018 9:42 AM
To: Garber, Kristina <Garber.Kristina@epa.gov>
Cc: Sankula, Sujatha (Sankula.Sujatha@epa.gov) <Sankula.Sujatha@epa.gov>
Subject: RE: two papers

Here is the last back and forth I have on this (if this is the right paper)

Looks like we were going to meet but then there was some hold up? So I may have been mistaken and these have not gone to OD or AA review

Am attaching my two reviews again, but if these papers have changed since my last review I will need to see them again and they will have to go through OD and AA review. Preferably that closest to final version possible

mark

From: Corbin, Mark
Sent: Friday, May 11, 2018 10:57 AM
To: Garber, Kristina <Garber.Kristina@epa.gov>
Subject: RE: two papers

Here are some comments on the amphibian paper.

Deliberative Process / Ex. 5

I will set up a short meeting next week to talk this one and the pop model paper over.

I will add Sujatha because we want BC's to be tracking on folks publications, presentations, etc..

I am still trying to figure out what papers need a review by anna and which don't. we can discuss that also

mark

From: Garber, Kristina
Sent: Friday, May 11, 2018 8:42 AM
To: Corbin, Mark <Corbin.Mark@epa.gov>
Subject: RE: two papers

I was not planning on asking for HED's input. The focus of the manuscript is on empirical data for amphibians, T-REX and TIM.

From: Corbin, Mark
Sent: Thursday, May 10, 2018 4:59 PM
To: Garber, Kristina <Garber.Kristina@epa.gov>
Subject: RE: two papers

Another question on the dermal exposure paper

Are you soliciting any review from HED? They have lots of experience with mammalian dermal exposure and their take might be beneficial

mark

From: Garber, Kristina
Sent: Tuesday, May 08, 2018 6:15 AM
To: Corbin, Mark <Corbin.Mark@epa.gov>
Subject: Re: two papers

Hi Mark,

I will make sure I have the BC comments first and let you know when to start your review. Sorry for the confusion. I thought that the AD review was concurrent to the BC review.

Kris

On May 7, 2018, at 9:03 AM, Corbin, Mark <Corbin.Mark@epa.gov> wrote:

> Kris
>
> On the two papers you circulated late last week I see you have cc'd several folks on this. Are these for my review as part of the Clearance Process or are you expecting feedback from others on the emails?
>
> I ask because I see you have cc'd Ed on the Purucker paper and if you are looking for technical comments or review by you respective BC's (Sujatha for you and Sujatha and Lee for Pop Model paper) then I will need to wait.
>
> If it is for me can you confirm that Sujatha and Lee have signed off on these?
>
> Mark Corbin
> Acting Associate Division Director
> Environmental Fate and Effects Division (7507P)
> Office of Pesticide Programs
> U.S. Environmental Protection Agency
> Washington DC 20460
> 703-605-0033

A Unified Approach for Protecting Listed Species and Ecosystem Services in Geographically Isolated Wetlands Using Community-Level Protection Goals

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Abstract

Geographically Isolated Wetlands (GIWs) are unique geological features that provide habitat for endemic, endangered, and threatened animal and plant species and ecosystem services (ES). GIWs are threatened by a range of chemical contaminants that vary by geographic region and assessing potential impacts to the fragile communities they embody is a challenge. We propose an approach that develops community-level protection goals for GIWs that includes both listed species and Service Providing Units (SPUs) that drive ES for ecological risk assessments (ERAs). Community-level protection goals are achieved by developing a protection community and weighing lines of evidence to determine a set of focal species within that community upon which to base the assessment. Lines of evidence included chemical mechanism of action, likely routes of exposure, and taxa susceptibility, as well as relationships among species. We demonstrate the process using case studies of chlorpyrifos in California vernal pools and coal ash effluent in Carolina bays. In the California vernal pool case study, listed species were the primary SPUs for the ES provided by the critical habitat. The weight of evidence demonstrated the honey bee as the focal species for terrestrial environment and the vernal pool fairy shrimp as the focal species for the aquatic environment. The protection community within the Carolina Bay case study was more taxonomically diverse than vernal pools for both listed species and SPUs, with amphibians identified as the focal species for which to target mitigation goals and hazard levels. The approach presented here will reduce the time and resource investment required for assessment of risk to listed species in GIWs and adds an ES perspective to demonstrate the value of GIWs beyond listed species concerns.

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1. Introduction

At first glance, protection goals for Ecological Risk Assessments (ERAs) that focus on threatened and endangered (listed) species and ecosystem services (ES) appear contradictory. Under the Endangered Species Act (ESA), private and federal actions must ensure that no adverse effects occur to listed species for the sake of preserving the species, regardless of the benefit to humans. Conversely, by definition, ES benefit society through ecological processes that are valued and used by people. While the protection of listed species often is focused on individuals, protection of ES is enmeshed in community- or ecosystem-level dynamics. ERAs that target the protection of listed species do so on a species-by-species basis, where the current state of ES-based ERAs have been more focused on ES in general habitat types (e.g., urban, grassland, wetlands; Maltby et al. 2017) using generic endpoints (e.g., quantity and quality of food production; Munns et al. 2015). Here, we present an approach to ERA that aims to protect both listed species and ES in geographically isolated wetlands (GIWs) using community-based protection goals.

GIWs are wetlands that are surrounded by uplands which may or may not be ephemeral and whose hydrological input is primarily through rainfall and runoff (Tiner, 2003). Ubiquitous throughout the United States, GIWs vary across ecoregions with a unique combination of geological, climatic, and biotic features. They support a diverse array of species and communities, including many endemic species, listed species, and species of concern (e.g., plants, crustaceans, amphibians), and are critical habitat for several listed species (e.g., vernal pool fairy shrimp; USFWS, 2005). They also provide a variety of ES such as provisioning (e.g., genetic resources, fresh water) and regulatory (e.g., pollination, water regulation, water purification) services (Tiner, 2003; Marton et al., 2015). GIWs face multiple threats including encroaching urbanization, agricultural conversion, hydrological changes, and other climatic stressors. In addition to their unique regional stressors, GIWs are vulnerable to chemical contamination, which also may vary based on region (Tiner et al., 2002).

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Chemical contamination is a threat to both listed species and ES and the associated risks to each are, or could be, evaluated through the ERA process. Traditionally, ERAs are performed to ensure that population and communities of organisms do not experience adverse impacts from chemical stressors and are performed under various statutes, such as Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA; Superfund). While GIWs can be evaluated on a case-by-case basis for protection under the Clean Water Act (CWA) based on their potential to influence the integrity of navigable waters, isolated wetlands in general are not protected under the CWA, significantly increasing the potential for losses or impairment of these wetland ecosystems. In addition to these regulatory statutes, some GIWs are protected under the Endangered Species Act (ESA) as critical habitat for listed species. The geographic isolation and transient nature of many GIWs creates a challenge for assessing chemical impacts to the species that inhabit them and the ES they provide.

Under the ESA, Federal and State agencies consult with the Fish and Wildlife Service (FWS) or the National Oceanic and Atmospheric Administration (NOAA) National Marine Fisheries Service (NMFS) (herein combined, the Services) to make certain that federal, federally-funded, or federally-permitted actions do not jeopardize the continued existence of listed species or result in the destruction or adverse modification of those species' critical habitat. Critical habitat of listed species is delineated by the Services and includes GIWs for several species throughout the US. The ERA process is intended to determine what risks an action poses to listed species and their corresponding critical habitat. In assessing risks of pesticide exposure to listed species, the Services and EPA work in tandem in a three-step process to characterize the risk of an action to listed species' viability (NRC, 2013). While the current process involves a species-by-species approach that often targets protection goals at the individual-level, GIWs are unique in that they typically contain multiple listed species spanning across

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taxa (e.g., plants, crustaceans, amphibians). Community-level protection provide potential to protect multiple listed species across diverse taxa with one process.

As prescriptive as the process is for ERAs of listed species, it is equally vague for conservation of ES. The US EPA Risk Assessment Forum only recently added ES endpoints to a list of generic endpoints for ERA (USEPA, 2016a) with the goals of expanding the value of ERA in environmental decision making and improving the communication of risks to stakeholders (Munns et al., 2015). However, inclusion of ES endpoints is considerably less straight-forward, as additional analytical steps are required to link data typically available to risk assessors to ES. The classification of ES involves the designation of final ES that provide direct benefits to society and human well-being (e.g., the benefits of crop pollination realized by farmers), intermediate ES that are not used directly by beneficiaries but contribute to final ES (e.g., the environmental conditions necessary to support pollinator populations), and service providing units (SPUs), which are the ecological units from which ES originate (e.g., the pollinator populations). Ecological production functions are quantitative relationships between SPUs and stressors or management decisions that can alter SPUs. These production functions may not be linear or correlated with one another and can vary both spatially and temporally (Rodriguez et al., 2006). One of the largest challenges in using ES as endpoints in ERA is linking data that is commonly provided in support of ERAs with production functions, SPUs, or intermediate and final ES (Forbes et al., 2017). As such, frameworks and case studies are emerging to begin working through this challenge (e.g., Faber and van Wensem, 2012; Forbes et al., 2017; Galic et al., 2012; Nienstedt et al., 2012).

Given the ecological and economic importance of GIWs, we propose a unified ERA process that evaluates the risks to all species which rely on them and the ES services they provide through one action. Specific geographical and hydrological features are emblematic of GIWs across all regions and we suggest that these characteristics can be used to standardize an approach to improve ERAs for GIWs protective of the GIW ecosystems, including all listed species that rely on or occupy them. In this

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process, we identify a protection community comprised of all listed species and SPUs followed by a Weight of Evidence (WoE) approach for identifying focal species within the protection community which can be targeted for the ERA (Figure 1). We demonstrate this approach using case studies of vernal pools in the California Central Valley and Carolina Bays in the Southeast US.

2. Protection Goals for GIWs

Protection goals are determined in the problem formulation stage of ERAs by risk assessors and risk managers to define the objective of the assessment. For general ERAs that may be applied to non-specific locations, protection goals may be as broad as ensuring that chemicals will not pose any unreasonable risks to plants, wildlife, and the environment. Protection goals are met through the evaluation of assessment endpoints, which are measurable attributes used by decision makers to evaluate chemical hazards. Assessment endpoints are limited to available data or models and may be at the sub-organismal level (e.g., molecular pathways and adverse outcome pathways), the individual level (e.g., increased mortality, reduced reproduction, reduced growth), population level (e.g., population decline), or community level (e.g., taxa richness) (USEPA, 2016a).

The protection goal for an ERA involving a listed species is to avoid jeopardizing the continued existence of the species while avoiding excessive, adverse economic impact (NRC, 2013). Jeopardy occurs when an action directly or indirectly diminishes a species numbers. Protection goals for listed species are straight forward and may be at the individual level for critically endangered species (e.g., Florida panther) or at the population level where compensation for individual-level impacts is possible (e.g., salmon). Where listed species protection goals are at the population level, it must be demonstrated that impacts will not appreciably reduce the survival and recovery of the species. Regardless of the level of the protection goal, assessment endpoints for listed species may include any evidence that the contaminant will result in harming an individual, including altering behavior that may affect foraging, sheltering, and breeding success. Protection goals for listed species extend to all

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locations of its critical habitat, even if individuals are not currently observed in a specific critical habitat. Since the goals of the ESA are to de-list the species, protecting its critical habitat even in the absence of individuals is essential to secure the potential for recovery. Under this protection goal, ERAs need to ensure that critical habitat maintains the biological integrity required for individuals of listed species to sustain or increase their populations or recolonize their critical habitat.

Protection goals for ES-based ERAs are to sustain the natural resources that provide ES. These goals are beneficiary-specific, habitat-specific, and require knowledge of the relevant stakeholder groups as well as an understanding of the ES provided and how they may be impacted by chemical stressors (Maltby et al., 2017). Nienstedt et al. (2012) provides a conceptual framework for identifying protection goals for ERAs using ES that involves: 1) listing ES of the habitat, 2) identifying which ES could be affected by chemical contaminants, 3) identifying the key drivers of those ES, and 4) developing protection goals for each key driver. There also have been calls for participatory stakeholder engagement to be part of the identification process (Munns et al., 2015). Generic ES assessment endpoints have been recommended by the US EPA Risk Assessment Forum and include quantitative metrics such as mass of wood produced, amount of food produced, volume of ground water recharged, and extent of crops pollinated, to name a few (Munns et al., 2015; USEPA, 2016a). These endpoints are more difficult to assess with data typically available to risk assessors (e.g., molecular initiating events, organism-level laboratory responses) and require models to translate available data into production functions. Such a process requires linking models across various levels of biological organization from molecular, to individuals, to populations, to SPUs, and finally to quantitate changes in the SPU to changes in the ES (Forbes and Galic, 2016; Galic et al., 2012). An ES approach to setting protection goals is one that is highly anthropogenic and utilitarian (Maltby et al., 2017). On its own this could put assessment and protection of ES in conflict with assessment and protection of threatened and endangered species. However, the purpose of the approach outlined here is to identify, incorporate, and

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demonstrate ES thinking into a community-level ERA approach that will strengthen the protection goals for GIWs while articulating benefits that might not otherwise be considered (Munns et al., 2015). Because of this, ES is a useful concept for facilitating communication between risk assessors, risk managers, and stakeholder groups and can be used to explore additional benefits that accrue alongside protection of listed species.

We propose that community-level protection goals can be developed for GIWs that avoid jeopardizing listed species while maintaining the natural resources that support ES. These goals require the identification of a protection community (Section 3) along with an initial review of co-occurrence, exposure, and effects information (Section 4). Interactions among members of the protection community are depicted in conceptual diagrams to identify dependent relationships among community members. A WoE approach uses information obtained on co-occurrence, exposure, effects, and community dynamics to reduce the protection community down to one or two focal species for which more intensive data analysis can be performed to establish hazard levels or mitigation goals (Figure 1).

3. Protection Community

For vernal pools and Carolina Bays, we identified all listed species that have been documented to either use the habitat or rely on it as a critical habitat through review of literature (e.g., Tiner et al., 2002) and habitat conservation plans (USFWS, 2005) to develop a first draft of protected species for each GIW type. All species that were identified from this search as protected under federal or state law, as well as those identified as special concern, were confirmed using the USFWS and respective State conservation agency websites (Supplemental Information A). In a second approach to identify and verify listed species for each GIW type, state conservation websites were reviewed for species that were likely to occur in GIWs located within each state based on life history (e.g., amphibian) and habitat usage. For every species identified as likely to use the GIW type, a literature review was performed to determine if

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there was documented evidence of occurrence in the respective GIW. All federal and state listed species for which the GIW is considered critical habitat were identified as well as any whose geographic range overlapped with GIW distribution if the species could use the GIW for life support. For example, listed wading birds that may use the GIW for fishing, but for which the GIW is not listed as critical habitat, were included. Similarly, species of conservation concern identified by federal or state governments were identified for each GIW. Supplemental Information A provides the full list of protected species and species of concern by GIW type.

The ES provided by each GIW was identified for each wetland type. To properly identify the relevant ES for a community-based risk assessment approach, two aspects of ES were considered: the benefits or services that are being produced by the wetland and the stakeholder groups that are present or otherwise interacting with the wetland in order to receive those benefits. To address the first aspect, we started with the ES identified by Maltby et al. (2017) followed by a literature review for each service by GIW type. In many cases, direct reference to ES was completely lacking for a GIW type, in which case internet searches were performed to identify the potential for a service to be provided by a specific GIW. For example, a search was performed for "medicinal plants" and "cypress domes" to identify species found within this GIW type that provided the ES of biochemical/natural medicines. The potential for ecosystem service production, however, is only half of the equation. For an ecosystem's potential production of services to be realized, the services must be valued by human beneficiaries that are present to receive them (Fisher et al., 2009; Munns et al., 2015). For this reason, the distribution and desires of humans across the ecosystem also must be considered when identifying the right set of ES and SPUs. The Final Ecosystem Goods and Services Classification System (FEGS-CS) then was used to find likely environmental attributes of concern for each beneficiary type (Landers and Nahlik, 2013). The combination of these attributes and the GIW's service potential was used to identify the SPUs to the closest taxonomic level possible. In some cases, ES and/or SPUs were based on best professional

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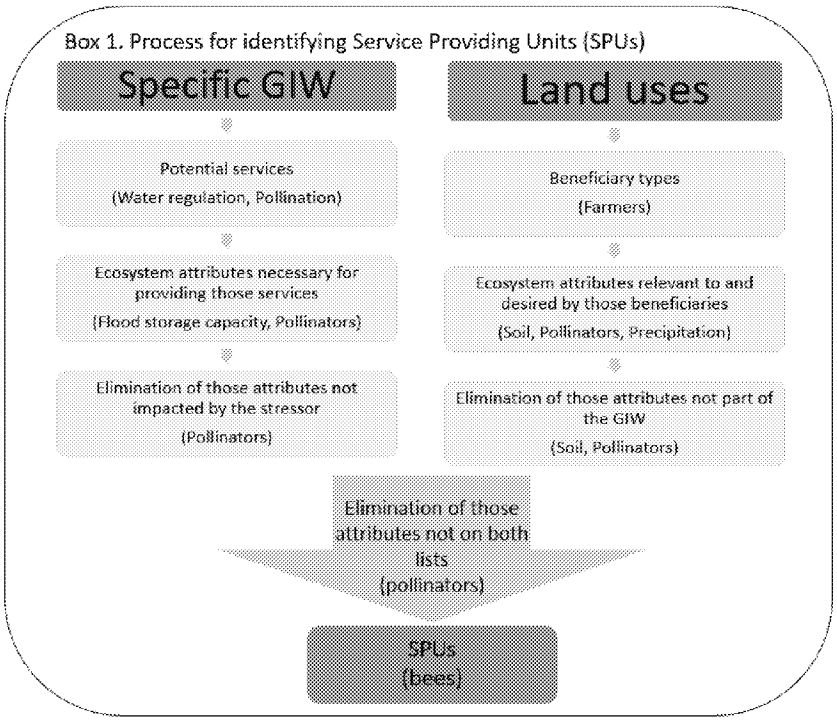
judgement. This process is presented in Box 1. The list of ES provided by each GIW type is listed in Table 1 and their SPUs and associated references are provided in Supplemental Information B. The list of ES in Table 1 should be considered a starting point and should be further refined with the scope of each assessment and regionally-specific knowledge of the GIWs and the stakeholder groups.

For each GIW case study, the interactions among members of the protection community are depicted in conceptual diagrams to identify dependent relationships among community members (Section 5). Visualization of the protection community dynamic is important to identify species or traits-based groups that provide critical ecosystem functions that will inform the lines of evidence described in Section 4.4. We do not prescribe a rigid structure for the conceptual diagrams, as noted by two different approaches used in the case studies in Section 5; however, diagrams should represent elements that are important to the GIW and aid in communication to stakeholders.



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4. Data Analysis

Data limitations are the principle source of uncertainty in all ERAs, particularly for listed species, and gaps between available metrics and their linkage to ES is a noted challenge (Forbes et al., 2017). The analysis phase of an ERA is typically separated into the exposure and effects analyses, which are integrated and evaluated during risk characterization as the co-occurrence of contaminant and receptor (USEPA, 1992). For listed species, however, the potential for co-occurrence may be established prior to data-driven exposure and effects analyses. For example, in the ERA process for listed species exposed to pesticides, the first step requires a determination of whether a pesticide "may affect" a listed species, which occurs when the action area of the pesticide overlaps spatially with the habitats of any listed species (NRC, 2013). Given the ephemeral nature and limited geospatial information on GIWs, approaches for determining co-occurrence with chemical contaminants is discussed here as the first-tier analysis, followed by approaches for estimating exposure, modeling potential effects (including evaluation of chemical mode of action), and applying weight of evidence to achieve the protection goals of GIWs exposed to various contaminants.

4.1 Co-occurrence of GIW and Contaminant of Concern

The co-occurrence of chemical contaminants and members of the protection community within a GIW needs to be considered both spatially and temporally. Spatial co-occurrence is determined by the proximity of the GIW and sources of contaminants and must consider run-off and atmospheric drift potential. Spatial co-occurrence should be determined within the study area by first establishing as precise a spatial boundary for the study area as possible, then searching for GIS data on GIWs in that area. The US Fish and Wildlife Service hosts the National Wetlands Inventory (NWI) dataset which provides wetland locations since the mid-1970s to the public and decision-makers, ([HYPERLINK "https://www.fws.gov/wetlands"]); however, not all GIWs are represented in the NWI. The NWI's

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minimum mapping unit varies among regions from 0.2 – 1.4 hectares which excludes many small GIWs. Photointerpretation of wetlands for the NWI is confounded by leaf cover in spring-summer photographs, and is generally more difficult for forested wetland types (Tiner, 1997). Since 2010, the NWI Plus has answered a need for improved functional assessment information for wetlands by incorporating descriptors such as water flow path and waterbody type to allow wetland position within the landscape and watershed to be better understood (Tiner, 2010b). GIWs in certain areas of the US have been analyzed in greater depth using remote sensing techniques and GIS resources, resulting in higher estimates of GIWs compared to the NWI alone (Vance et al., 2009; Lane and D'Amico, 2016). A nationwide analysis incorporating the NWI and the National Hydrography Dataset used a 10m buffer distance from a wetland to a flowing waterbody to establish a wetland as "isolated", and found that 16% of freshwater resources may be GIWs (Lane and D'Amico, 2016).

Temporal co-occurrence of contaminants and ecological receptors depends on the application, fate, and transport of the contaminant as well as the life history and behavior (e.g., migration) of the receptor. This may be evaluated prior to exposure modeling and used to prioritize exposure modeling scenarios. For example, the timing of pesticide exposure with bird nesting is critical in understanding long term impacts to bird populations (Bennett and Etterson, 2009); if temporal co-occurrence is not likely based on crop type, timing of pesticide application, timing of the nesting season, and chemical fate properties (e.g., half-life, transport potential, bioaccumulation) then estimates of exposure concentration may be considered a lower priority or not needed for that receptor. Temporal co-occurrence is especially challenging with GIWs because their seasonal changes in hydrology result in different ecological communities being present at different times of the year. For example, cycles of pooling and drying ephemeral GIWs are based primarily on the precipitation regime of the region, though groundwater exchange as well as runoff can contribute to water levels in some areas (Brooks, 2005). Not only does pool saturation change throughout the year, the durations of inundation and

desiccation periods can vary greatly between years due to both typical yearly variations as well as atypical droughts or higher than usual precipitation. The presence or absence of standing water influences the life stage of resident species as well as migrants and opportunistic users of the GIW. As such, temporal co-occurrence of GIWs and chemical contaminants needs to consider the life cycle and migratory behavior of resident and non-resident species in the protection community as they correspond to various stages of inundation.

4.2. Estimating Chemical Exposure

Exposure pathways are dependent on the physiochemical properties of the contaminant as well as the environmental factors of the region within which the GIW is located. The principal sources of GIW hydrology are precipitation and run-off, although there can sometimes be groundwater contributions (Brooks, 2004; Zedler, 2003; Golden et al., 2014). Therefore, run-off and erosion are typically the principal exposure pathways for contaminants to enter GIWs. Run-off and erosion can redistribute contaminants deposited into a GIW from other pathways, including near-term spray drift, longer-range atmospheric transport and aquatic point sources. Receptors in a GIW may be exposed to contaminants through relevant media (aqueous and sediment) and food web exposures. Estimated Exposure Concentrations (EECs) are derived via modeling and/or monitoring. Monitoring data are advantageous because they provide a direct measurement of the concentration in the media at a particular time and location, but may not consistently be available at many sites and may not have been collected during times of peak field concentrations. GIW monitoring data are limited and do not provide temporally and spatially data that conform to robust statistical designs. However, databases such as the USEPA's Toxic Release Inventory (TRI; USEPA, 2018b; [HYPERLINK "https://www.epa.gov/toxics-release-inventory-tri-program"]), USEPA's National Wetland Condition Assessment (NWCA; USEPA, 2018c; [HYPERLINK "https://www.epa.gov/national-aquatic-resource-surveys/nwca"]), USEPA's Water Quality Data (WQX; https://www.epa.gov/waterdata/water-quality-data-wqx), USGS National Water Quality Assessment

Program (NAWQA; [HYPERLINK "https://cida.usgs.gov/nawqa_www/nawqa_data_redirect.html?p=nawqa"]), and the California Pesticide Use Reporting (PUR; [HYPERLINK "https://www.cdpr.ca.gov/docs/pur/purmain.htm"]) may provide substantive information to assist in developing EECs. Models are often used to develop EECs for GIWs whether or not field data are available due to their ability to estimate concentrations at times and places where field data are lacking.

Models available to calculate chemical EECs typically focus on a subset of the above exposure routes. Aquatic exposure models focus on chemicals in standing water and benthic sediment pore water, although chemical fluxes entering the system may have been provided by aerial deposition models. Such atmospheric models may be limited to chemical property information, application rates in agricultural areas, and/or released via industrial sources to determine contributions associated with atmospheric transportation from short- or long-term deposition of chemicals. Sediment EECs are more static over time and are more likely to be directly estimated from monitoring data. Food web models vary by habitat type and receptors of interest, and may be limited to the direct use of bioaccumulation factors to estimate the transfer of contaminants through components.

4.2.1 Chemical run-off models: surface water, sediment, groundwater

The degree of groundwater connectivity is a key consideration when considering exposure models for hydrologic transport of chemicals entering a GIW. If there is little or no groundwater exchange, a model which includes runoff, sediment transport via erosion, and shallow subsurface and/or simplified groundwater fluxes is typically sufficient. Two popular semi-distributed watershed hydrologic and water quality models have been modified for use with GIWs: The Soil and Water Assessment Tool (SWAT; Neitsch et al., 2005) and the Hydrological Simulation Program - FORTRAN

(HSPF; Bicknell et al., 2001). A recent advancement in SWAT, the hydrologic equivalent wetland (HEW) concept, improves modeling of wetlands by including attenuation effects reflecting the nonlinear functional relationships between wetlands and runoff, and has been used to predict effects of wetlands restoration efforts in prairie wetlands (Wang et al., 2008; Yang et al., 2009). Further refinements of SWAT for GIWs have included finer scale hydrologic response units (HRUs) to represent distinct GIWs and fill-spill dynamics between neighboring GIWs (Everson et al. 2016). Similar to SWAT, the HSPF model has also been modified to better represent isolated wetland systems. The river reach or reservoir (RCHRES) module in HSPF can describe GIWs through customization of the water body physical dimensions and the hydraulic function table. Using RCHRES, GIWs are typically represented as a conditionally connected reach with no downstream connectivity except at very high water storage (Golden et al., 2014). This method has been used to model GIWs in the Florida coastal plain (Said et al., 2007; Zhang et al., 2009). In addition to hydrological models, more simplified aquatic fate and transport models can be applied to GIWs when the goal is a screening level assessment, or when hydrologic data is scarce. The Pesticide in Water Calculator (PWC) is a fate and transport model which estimates pesticide concentrations in surface water and groundwater bodies (Young, 2016) from nearby land applications. It pairs the Pesticide Root Zone Model (PRZM5), a 1D finite-difference model which estimates daily pesticide runoff, erosion and spray drift fluxes from fields, with the Variable Volume Water Body Model (VVWM), which simulates water column and benthic dynamics in standard and user defined water bodies. This approach has been used to estimate chlorpyrifos, diazinon and malathion exposure to California tiger salamanders and other vulnerable species in California vernal pool habitats (Clemow et al., 2017; Sinnathamby et al., in review).

When groundwater connectivity plays an important role in GIWs, the above models may be unable to properly simulate these dynamics. In these cases, a groundwater flow model such as the U.S. Geological Survey's MODFLOW (McDonald and Harbaugh, 1984) may be necessary. The MODFLOW

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wetlands package (Restrepo et al., 1998) is a promising option for modeling GIWs, as it includes surface flows, wetland-aquifer transfer, evapotranspiration and wet/dry cycles (Golden et al., 2014). Groundwater models such as MODFLOW can also be coupled with surface watershed models such as SWAT, in order to simulate interaction between upland/surface flows and groundwater flow. However, coupled surface water-groundwater models are complicated to parameterize and typically require extensive field data, which may be a key limitation for use on GIWs (Golden et al., 2014).

4.2.2 Agricultural drift and atmospheric transport of industrial pollutants

AgDRIFT (Teske et al., 2002; Bird et al., 2002) and AgDISP (Bilanin et al., 1989) are often used to model pesticide drift processes at relatively short distances from the application site (e.g., 1000s of feet). These models represent improvements on previously implemented empirical curves based on controlled field studies (Himel et al., 1990; Saputro and Smith, 1990). AgDisp provides the computation engine of AgDRIFT and estimates the aerial application of spray droplets with a Lagrangian model to predict downwind drift and deposition. Calibrations with field data have been performed for the primary subroutines, including the drop size distribution, evaporation processes, aircraft and vortex decay effects, and finally ground deposition. Of course, deposition may extend well beyond these relatively short distances. Risk assessments that require longer-range deposition estimates suffer parameterization problems as they scale, and therefore typically rely on monitoring data for assessing long-range transport potential or relatively simple box models (Scheringer, 2015) approaches to capture transport. Models such as Globo-POP (Wania and Mackay, 1999), CliMoChem (Scheringer et al., 2004) and BETR-Global (MacLeod et al., 2005) have been developed for relating global organic chemical loadings to concentrations in relevant media and can be a point of departure for contaminant loading in GIWs. These mass balance approaches use large grid cells or latitudinal zones with emission rate

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parameter estimates, compartment volumes, partitioning and loss processes to provide exposure estimates that can be cross-referenced with available data.

4.2.3 Bioaccumulation

There are many available models that predict EECs based on terrestrial (Armitage and Gobas, 2007; Kelly et al., 2007; Gobas et al., 2016) and aquatic (Mackay and Fraser, 2000; Arnot and Gobas, 2006) bioaccumulation principles. The primary consideration when tailoring these models for use in GIWs is the selection of appropriate species. Aquatic models are generally well-accepted and typically based on BCFs, BAFs, and/or Kows -- all measures of partitioning between biota and water. Attempts to transfer these successful aquatic-derived approaches to terrestrial models is natural but has been shown to be problematic. A number of chemicals have shown different bioaccumulative properties in aquatic versus air-breathing organisms. Therefore, bioaccumulation modeling in terrestrial food webs is an important need, especially for compounds with high environmental persistence and low partitioning to water, properties that may result in significant biomagnification in terrestrial ecosystems. These modeling capabilities are often not currently part of standard regulatory modeling workflows. Other unique terrestrial issues for application in GIWs include linking aquatic and terrestrial food webs, accounting for mobility of animals (i.e., migration, assessing plant reproductive effects and pollinator exposures.

The food web models estimate the cumulative exposure and effects of toxic chemicals and other stressors in order to assess ecological risk across species of interest. The Comprehensive Aquatic Systems Model (CASM) (De Angeleis et al., 1989; Bartell et al., 1999) is a mass-balance aquatic food web modeling platform that has been used to identify the potential exposure effect for a variety of aquatic ecosystems. Bartell et al. (2017) has used a generic wetland form of CASM to identify the potential

effects of neonicotinoid on Midwestern emergent wetlands. AQUATOX can predict the fate of organic chemicals and designate multiple trophic levels to simulate complex food webs. It simulates multiple environmental stressors other than chemicals (e.g., nutrients, temperature) and handles algal, macrophyte, invertebrate, and fish communities (USEPA, 2004; Yang et al., 2012). The three-phase ecological food web analysis (TEFWA) (Preziosi and Pastorok, 2008) guides users through a designed process to construct a food web for risk assessment purposes, this approach may allow for a customized implementation that accounts for GIW receptors and interactions.

4.3 Effects Characterization

Limited effects data are confounding in all ERAs, particularly for listed species and critical habitat assessments. The unique species composition of GIWs requires the application of effects modeling and approaches beyond the use of surrogate species test data for protecting both listed species and ES. Common surrogate species (e.g., *Daphnia magna*, rainbow trout, fathead minnow, bobwhite quail, common rat) do not naturally occupy GIWs, sufficiently relate to the benefits provided by ES, or adequately represent the species adapted to the unique features of GIWs that comprise their protection communities (Supplemental Information A and B). Additionally, there are significant gaps in the methods available to estimate effects from various exposure routes, such as dermal exposure for wading birds and other vertebrates that submerge in aquatic habitats. Regardless, effects data collection for GIW community protection goals will follow the same processes used for other ERAs: 1) collection and evaluation of the most reliable available data for all possible ecological receptors (including incidence reports), 2) understanding the Adverse Outcome Pathway (AOP; Ankley et al., 2010) of the contaminant to the extent that it is available, 3) application of models to evaluate susceptibility and sensitivity of untested taxa (e.g., LaLone et al., 2013; Raimondo et al., 2010; USEPA, 2014; USEPA,

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2017), and 4) development of Species Sensitivity Distributions (SSDs) from measured or extrapolated effects data.

In addition to these steps, our approach employs traits that influence susceptibility for each species in the protection community. Traits are characteristics that influence a population and may be at the individual (e.g., diet, body size), the population (e.g., life span, reproduction strategy), or landscape levels (e.g., dependency on hydroperiod) (van den Brink et al., 2013). Traits may be taxa-specific (e.g., bimodal life history of amphibians) or be shared across broad taxonomic groups (e.g., insectivorous diet). For their application here, we focus on traits that influence the vulnerability of each species relative to its potential co-occurrence with chemical stressors, inherent sensitivity, and potential routes of exposure. We use traits to aggregate species within the protection community into traits-based species groups that will be used in the weight of evidence approach described below. These traits will be specific to each ERA as they will be identified by relevant factors defined in the problem formulation stage of each assessment (e.g., type of contaminant, source of contaminant). The development of traits-based species groups is demonstrated in the case studies.

4.4 Weight of Evidence

The diversity of ecological receptors within a GIW protection community and the various pathways by which they could be affected requires a formal WoE process. A well-designed WoE approach is one that upholds the burden needed to justify regulatory decisions and clearly communicates findings to a wide range of audiences while increasing confidence in, and defensibility of, the ERA (Rhomberg et al., 2013; USEPA, 2016b). WoE approaches typically include three elements: 1) identifying and assembling relevant evidence as discussed in the previous exposure and effects sections,

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2) objectively evaluating the strength, reliability, and relevance of the available evidence, and 3) integrating and interpreting that evidence (USEPA, 2016b).

Here, a WoE approach is used to identify the species or group of species in the GIW protection community that will be either most susceptible to contaminant exposure or otherwise serves as a keystone in the dynamic of the community and the contaminant (referred to here as focal species). The WoE process at this step will demonstrate, based on the evidence collected on exposure routes, species susceptibility, and community dynamics, that protecting the focal species will protect the remaining community members (Lambeck, 1997). Identifying and targeting focal species within the protection community via all potential exposure routes of the contaminant aims to protect the biological integrity of the entire system. The WoE approach traditionally is used in ERA to determine data integration from various pathway models in the exposure analysis, what types of species data is acceptable for the effects analysis, and for integration of exposure-effects relationships for multiple species and stressors at varying spatial and temporal scales. The latter applications have been described thoroughly elsewhere (USEPA, 2016b); WoE as it applies to risk characterization will not be discussed further. Rather, we will focus our discussion and case study demonstrations on WoE application in identifying the focal species of the GIW protection community for which protection should be targeted.

WoE approaches help assessors evaluate the support of a set of alternative causal hypotheses (USEPA, 2016b). In a typical ERA, the alternative hypotheses may be H_0*: the stressor is not likely to affect the species of concern* and H_1*: the stressor is likely to affect the species of concern*. Applying the WoE in this critical habitat approach, there is a much wider set of alternative hypotheses (e.g., H_1*: the stressor is likely to affect listed species A;* H_2*: the stressor is likely to affect listed species B;* H_3*: the stressor is likely to affect SPU species C; etc.)* encompassing the stressor's potential to cause an effect on any species of concern and the null hypothesis would be correspondingly narrower, e.g., H_0*: the stressor is not likely to affect any of the species of concern in the GIW*. Although our approach could initially appear to be more

complex and time-intensive, it allows regulators to avoid spending time on those hypotheses supported by weak or conflicting evidence and base their decisions on those hypotheses that are most strongly supported. Additionally, because the proposed ERA framework is not an ES focused approach, but a community-level ESA approach that includes ES thinking, a WoE process could be used to set differing standards for ES-related evidence and listed species-related evidence that reflect the primary concern of the ERA which is protection of listed species. In this process, the collective hypotheses pose the question: *Which species in the protection community will preserve the biological integrity of the GIW such that no likely adverse effects will occur for the remaining species?* To identify these species, we consider the lines of evidence listed in Box 2. These lines of evidence should be considered for both the parent contaminant as well as its metabolites and/or degradation products or separate toxic components (e.g., mixtures).

Box 2. Lines of evidence to identify focal species:

1. Temporally co-occurrences with the contaminant
2. Exposure pathway present for species uptake
3. Susceptible to the chemical mode of action
4. Demonstrated sensitivity of taxa to chemical
5. Traits that increase susceptibility
6. Traits that provide a critical ecosystem function
7. Provides habitat for other species in the protection community
8. Provides food for other species in the protection community
9. Indirect effects via food web
10. Vulnerable to additional non-chemical stressors (e.g., bacteria/viral prevalence; excluding loss of GIW habitat).

5. Case Studies

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5.1 Chlorpyrifos in California Vernal Pools

This case study focuses on chlorpyrifos exposure to vernal pools located in the California Central Valley. Chlorpyrifos is an organophosphate insecticide applied in the Central Valley to a wide variety of food crops, forage fodder crops, tree nuts, field crops, and fruits. Chlorpyrifos is applied directly to sites as part of integrated pest management strategies and is relied on to control alfalfa weevil, ants, plant bugs, aphids, and whiteflies on alfalfa, almonds, citrus, and cotton (CDPR, 2014). The California Department of Pesticide Regulation reported over 40,000 lbs of chlorpyrifos use in the Central Valley in 2016, with the peak application period occurring between May and August (CDPR, 2018). In 2016, the USEPA submitted a national-scale biological evaluation (BE) for chlorpyrifos and determined that 99% and 100% of listed species and critical habitats, respectively, are likely to be adversely affected by its application based on the action area, its potential distribution, and toxicity (USEPA 2016c). This case study builds off that BE to demonstrate the application of the community-level process described here for California vernal pools.

California vernal pools are characterized by a wet phase, when soils become saturated from rain and/or snowmelt and the pool becomes hydrated (November - April), a terrestrial or flowering phase, during which the pool begins drying and endemic, flowering plants emerge (April - May), and a dry phase, during which egg, cyst, spore, and seed life stages occur (May-November) (USFWS, 2005). The geomorphology of vernal pools makes them insular where they occur, with sharply defined boundaries marked by unique, endemic vegetation that distinctly separates them from surrounding areas (Holland and Jain, 1981). The protection community for these GIWs is depicted in Figure 2, with species and taxa-specific information for listed species and SPUs provided in Supplemental Information A and B, respectively. As demonstrated in Figure 2, the species or taxa within the protection community interact with each other, although many species are active only during one phase of the vernal pool. The

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protection goals are to maintain the biological integrity of the system such that there are no likely adverse effects to listed species or SPUs.

We used the Central Valley vernal pool geospatial data and California's Pesticide Usage Reporting Database (CDPR, 2018) to determine the spatial co-occurrence of chlorpyrifos application near vernal pools. The vernal pool data was developed by a location shapefile (http://www.vernalpools.org, accessed March 13, 2017) that was buffered to 5 km in ArcMap v10.4 (Witham et al., 2014). The 2016 chlorpyrifos data included application to agriculture lands and non-agriculture areas by commercial appliers was summed by COMTRS (County-Meridian-Township-Range-Section) unit and joined to Public Land Survey System COMTRS shapefile. The chlorpyrifos-by-COMTRS shapefile was clipped by the 5 km vernal pool buffer. Almost 4,000 pounds of chloropyrifos was applied to crops within 5 km of any vernal pool feature. The 2016 US Department of Agriculture's Cropland Data Layer or CDL (https://www.nass.usda.gov/Statistics_by_State/index.php, accessed July 17, 2018) was clipped to the 5 km vernal pool buffer. When mapped with vernal pool networks, crops that are labeled for chlorpyrifos application are closest to pools in Tehama, Butte and Nevada counties in Northern California, and pools in San Joaquin, Stanislaus, Merced and Madera counties in central California (Figure 3).

Chlorpyrifos EECs used in this case study were estimated using the Pesticide in Water Calculator (PWC) for vernal pools in Merced County, CA (Sinnathamby et al., in review). In brief, EECs were simulated by parameterizing PWC (version 1.59) for three agricultural vernal pool watersheds located in the San Joaquin River basin in the Central Valley of California. Among watersheds, simulated EECs varied with crop type, crop acreage, soil, application rate, application method etc. Simulated maximum EECs of chlorpyrifos were 13.06 and 6.79 ppb with levels that exceeded the USEPA benchmark for chlorpyrifos (0.04 ppb; USEPA, 2016c) in for 93 and 94% of days when applied to dormant almonds and grains (oats, winter wheat and barley), respectively. Maximum EECs were obtained when chlorpyrifos was applied

during the dormant season (in almonds) or as a pre-planting soil treatment (in grains). The application methods during these crop scenarios were inferred using section-level application dates (CDPR, 2018) and are described in detail in Sinnathamby et al. (in review).

Chlorpyrifos effects were determined through evaluation of the AOP for acetylcholinesterase (AChE) inhibitors, evaluation of species susceptibility, and measured data. The molecular target of chlorpyrifos is AChE, which is present in vertebrate and invertebrate animal species. In the mechanism of action, ACh is blocked from binding to and being degraded by AChE, which results in unregulated excitation at neuromuscular junctions that cause neurotoxic symptoms (e.g., increased respiration, seizures, bradycardia) that can lead to mortality (Russom et al., 2014). Predictions of species susceptibility using Sequence Alignment to Predict Across Species Susceptibility (SeqAPASS) showed widespread susceptibility across the animal kingdom, while plants, fungi, and micro-organisms (e.g., protozoan) were less susceptible (Doering et al., 2018).

The species within the protection community were grouped into traits-based species groups based on broad taxonomic categorization, knowledge of the AOP and predicted species susceptibility, and potential routes of exposure. Listed species and SPUs may be included in the same traits-based group, as was done for plant groups and Lepidoptera in this example (Table 2). For the initial assessment of species susceptibility and sensitivity used in the WoE approach to identify focal species, we used the effects data obtained from the USEPA biological evaluation for chlorpyrifos (Doering et al., 2018; Russom et al., 2014; USEPA, 2016c).

The weights for each line of evidence for each traits-based species group are presented in Table 2. Weights were assigned as high (+++), medium (++), low (+), or no evidence available (0). Negative weights (-) were assigned where a line of evidence may indicate reduced vulnerability of a species group. Since there is the potential for chlorpyrifos to be applied in the Central Valley year-round, all species had a medium to high weight for temporal co-occurrence, with a higher weight given to those

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species that are present during the peak application period (May-August). Exposure pathways also were present for all species occupying the vernal pools full time (e.g., algae, cyst forming crustaceans), with lower weights applied to species that might encounter chlorpyrifos only via food web exposure routes, and no weights applied to higher, opportunistic trophic groups that do not use vernal pools as habitat and do not rely on vernal pool species for food. Susceptibility to chemical mode of action were based on Doering et al (2018). Demonstrated sensitivity was based on the availability and value of toxicity effects data available from USEPA (2016c), with higher weights given to relatively sensitive organisms in their respective habitats (aquatic, terrestrial) and no weight given to taxa where no toxicity data were available. Traits that increase susceptibility included permeable skin (e.g., amphibians) or behavior that increases potential for exposure (e.g., pollinators visiting multiple plants on which with potential drift may occur). In this line of evidence, negative weights were assigned to species developing cysts or seeds that provide protection during application. Traits that provide critical ecosystem function include primary production, nutrient cycling, and pollination. Lines of evidence for providing habitat and food for other members of the protection community were based on community dynamics of vernal pools (Zedler, 1987; USFWS 2005) and do not include other species outside of the protection community. Indirect effects via food web consider whether a species food source, regardless of its inclusion in the protection community (e.g., insectivorous birds) would be impacted. Lastly, high weights were assigned to Hymenoptera and amphibians for the line of evidence for vulnerability to additional non-chemical stressors for their susceptibility to varroa mites and chytrid fungus, respectively.

Based on the weights assigned to the lines of evidence, Lepidoptera and Hymenoptera represent the focal species for the terrestrial and dry phase species; cyst-forming crustaceans are the focal species for aquatic phase species. Four listed species of cyst-forming crustaceans occur within the Central Valley case study area and two species are endemic to the area, providing SPUs of genetic diversity as an ES. Four of these are in the family Branchinectidae, one in the family Triopsidae, and one

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in the family Chirocephalidae (Supplemental Information A). USEPA (2016c) contained three acute mortality data points for cyst-forming crustaceans in the families Thamnocephalidae (*Thamnocephalus platyurus*; LC50 = 0.52 ug/L) and Streptocephalidae (3.48 and 8.25 ug/L). Because no data were available for any of the focal species families, the Web-based Interspecies Correlation Estimation (Web-ICE; [HYPERLINK "https://www3.epa.gov/ceampubl/fchain/webice"], accessed 7/27/2018; Raimondo et al., 2015) was used to estimate sensitivity to the family Branchinectidae (1.29 ug/L, confidence interval 0.227 – 7.40 ug/L) using the value obtained for *Thamnocephalus platyurus* reference above as a surrogate. Web-ICE contains log-linear regressions developed from existing data that predict an acute value of an untested species from a surrogate (Raimondo et al., 2010). A single data point was available for a butterfly in the family Nymphalidae (0.25 kg/ha) while several studies reported toxicity to bees at levels orders of magnitude lower than that reported for the butterfly (0.05 kg/ha; USEPA, 2016c). Since the measured effects endpoints for the focal species is less than the maximum EECs modeled, an additional WoE approach may be applied to the available data for bees and cyst-forming crustaceans to evaluate data reliability and robustness to complete the risk characterization of this case study.

5.2 Coal Ash in Carolina Bays

This case study focuses on coal ash deposited into Carolina Bays in the southeast US (US EPA Region 4). Coal combustion is the principle process used to produce energy in the US and produces approximately 110 million tons of residuals annually (Harkness et al., 2016; Rowe et al., 2002). Coal ash is the primary constituent of a coal combustion residues; it is deposited into surface impoundments and landfills, is highly toxic to plants and animals, and leaches into ground water from disposal sites (Harkness et al., 2016; Rowe et al., 2002). The principle contaminants in coal ash are heavy metals, which readily bind to sediment and are toxic to both plants and animals (Lance et al., 2012; Rowe et al., 2002). A historical precedent has established using isolated wetlands, including Carolina Bays, as settling

basins to remove contaminants from effluent (Lance et al., 2012). The use of GIWs for coal ash water filtration and the associated environmental impacts and remediation is well documented for the Savannah River Site (SRS) in Aiken, SC (Rowe et al., 2002). This case study utilizes data assembled from SRS and represents a scenario in which the contaminant is introduced into the environment to obtain an ES (water purification via adsorption to sediment and uptake by biota) that is contrary to the protection of vulnerable populations. In scenarios such as these, assessments will inform management of effluent treatment to minimize impacts to focal species prior to establishment, as well as remediation of legacy sites that are no longer able to support diverse communities.

Carolina bays are elliptical depressions located from New Jersey to northern Florida with distinct geologic boundaries separating them from surrounding uplands. Many Carolina bays are ephemeral, with inundation and water levels dependent on rainfall and evapotranspiration rates, resulting in a community of diverse species that are well adapted to the habitat and its surrounding ecotome (Sharitz, 2003). Due to their size and diversity, Carolina Bays support a large number of listed species and SPUs. The protection community and the interactions among species is depicted in Figure 4, with species and taxa-specific information for listed species and SPUs provided in Supplemental Information A and B, respectively. In this case study, protection goals are to determine a threshold by which mitigation efforts may be targeted to increase the biological integrity of the system to support vulnerable populations.

Since coal ash is a point-source pollutant, determining the spatial co-occurrence of contaminant and Carolina Bays is more straight forward than it is for non-point source contaminants. For example, on the SRS, coal ash both passively flows into Carolina Bay systems (e.g., Dunbarton Bay; Lance et al., 2012) and is actively pumped into a series of settling ponds and wetlands prior to entering the Savannah River watershed (Rowe et al., 2002). In these cases, spatial co-occurrence is simplified to physical overlap of the coal plume and the Carolina Bays.

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Heavy metals have been measured in the surface water, sediment, and biota of SRS impoundments (Rowe et al., 2002). Twenty-one metals were identified as constituents of concern, with As, Ba, Cd, Cr, Cu, Se, Pb, and Hg identified as the most common contaminants in Carolina Bay surface water and sediment (Lance et al., 2012; Rowe et al., 2002; RTI 2007). Rowe et al. (2002) compiled a review of the ecotoxicological implications of coal ash combustion and reported the following relative occurrence data for As, Cd, Cr, Cu, Pb, and Se measured on SRS basins: As was found in the highest concentration in settling basin surface water, followed by Se and Cu; As and Cu were found in the highest concentrations in sediment, followed by Se and Cr; all were reported in overlapping ranges in plant tissue; all were reported in animals, with the majority of studies reporting on Se, which was typically in the highest concentration in animal tissue when other metals were detected. Levels of As and Cu in biota were also reported in relatively high concentrations in some studies.

While metals were measured in plant tissues from SRS sites, little is documented on potential adverse effects. Conversely, plants are noted for their tolerance to heavy metals with multiple lines of defense engaging prior to the synthesis of stress-related proteins and signaling molecules (Manara, 2012). To assess the relative sensitivity of animal taxa to the three metals that were reported in the highest concentrations in water, sediment, and biota (Ar, Cu, Se), the Web-based Interspecies Correlation Estimation (Web-ICE; [HYPERLINK "https://www3.epa.gov/ceampubl/fchain/webice"], accessed 8/07/18) application was used to either obtain a compilation of measured acute sensitivity or to predict acute sensitivity across a diversity of taxa. Web-ICE uses log-linear least square regressions developed from a database of existing acute toxicity to estimate sensitivity to aquatic organisms (EC/LC50) and wildlife (LC50) from the measured toxicity of surrogate species (Raimondo et al., 2007; 2010). Where Web-ICE did not provide sufficient sensitivity information for a taxonomic group, we referred to Rowe et al. (2002) to complete WoE process below. Assessment of relative species sensitivity was performed separately for aquatic and terrestrial species.

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For aquatic species sensitivity to Cu, the Web-ICE database contained geometric mean acute values for 87 species (Supplemental Information C Table C-1). The Web-ICE database contained toxicity data for 10 aquatic species exposed As, which were used as surrogate species to predict toxicity to 58 additional species using the Species Sensitivity Distribution (SSD) module of Web-ICE (Supplemental Information C Table C-2). The Web-ICE database contained toxicity values for 12 aquatic species exposed to Se, which were used as surrogates to predict sensitivity to 49 additional species (Supplemental Information C Table C-3). For terrestrial animals, the Web-ICE database contained geometric mean acute sensitivity values for 5 bird species exposed to As, which were used to predict to 17 birds and 1 mammal species (Supplemental Information C Table C-4). The Web-ICE database contained acute toxicity for two birds and one mammal exposed to Cu, which were used to predict sensitivity of 21 birds and 6 mammals (Supplemental Information C Table C-5).

The species within the protection community were grouped into traits-based species groups based on broad taxonomic group, diet, and habitat type (Figure 4). Plankton included all phytoplankton and zooplankton. Plants were separated as monocots, herbaceous dicots, and woody dicots and all assumed to grow within the inundation boundaries of the wetland. Arthropods were only represented by terrestrial flying insects. Both bimodal amphibians as well as fully aquatic (unimodal) amphibians were within the protection community. Only aquatic-dependent reptiles were included in the protection community for that taxa. Birds were separated by diet and habitat. Bird diets included insectivorous, omnivorous, and carnivorous (including piscivorous species), which includes birds that eat both vertebrate and invertebrate prey. Their habitat types were lumped as swimmers (e.g., waterfowl), waders (e.g., egrets), and terrestrial (e.g., birds that hunt aquatic prey but spend the rest of their time in terrestrial habitats. Mammals were all primarily terrestrial insectivores or herbivores that were associated, but not dependent, on Carolina Bays. Because Carolina Bays contain a diversity of ES that

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will be important for stakeholder communication, inclusion of these services is depicted in the conceptual diagram of the protection community (Figure 4).

The weights for each line of evidence the traits-based species group are presented in Table 3. Temporal co-occurrence was weighed high (+++) for groups that lived or hunted in the water for their as their primary habitat. Medium weights (++) were given for omnivorous groups who entered the wetland temporarily to obtain food or those whose diets included, but were not limited to, species within the wetland. Low weights (+) for temporal co-occurrence were assigned to those group that opportunistically use the wetland. Exposure pathway was weighted high for species that lived in wetland water or sediment or consumed organisms living in the wetland, with weights decreasing for groups that only opportunistically used the wetland. A weight of zero was assigned to terrestrial insects and insectivores that are unlikely to eat from or come in direct contact with the wetland water or sediment. Based on documented uptake of heavy metals in all taxa and their bioaccumulation potential, all groups were weighed high for susceptibility. Demonstrated sensitivity of metals was weighted lower for plants than animals based on their ability to assimilate high levels of metals without adverse effects (Manara 2012). The SSDs developed for acute sensitivity of aquatic organisms exposed to Cu, As, and Se showed molluscs, aquatic arthropods (crustaceans and aquatic insects), and plankton as the most sensitive traits-based groups for all three metals. Of these, only plankton is listed in the protection community, so that group was weighted high for demonstrated sensitivity. Amphibians were in the middle to upper range of the SSDs, so were assigned a medium weight. Rowe et al. (2002) reported no mortality for aquatic-dependent reptiles collected from SRS sites, so this group was weighted with a low sensitivity. Sensitivity data to the selected metals were only available for omnivorous waterfowl, omnivorous terrestrial birds, herbivorous mammals, and omnivorous mammals. Based on their relative position in the SSDs, omnivorous mammals and omnivorous waterfowl were weighted with a medium sensitivity while omnivorous terrestrial birds and herbivorous mammals were weighted with a high sensitivity.

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Other terrestrial traits-based groups were assumed to have similar relative sensitivity as other birds and mammals, so were assigned a medium weight for this line of evidence. Traits that were considered to increase susceptibility included living in the wetland for all or part of its lifecycle (e.g., amphibians) or carnivorous diets that relied on food items supplied by the wetland. Plants were assigned a negative weight for traits increasing susceptibility based on their adaptations that provide multiple lines of physiological defense against metal toxicity (Manara 2012). Traits that provided critical ecosystem functions included providing food, habitat, and pollination for other members of the protection community. Weights were assigned for a groups potential to provide habitat and food for other species within the protection community based on the relationships depicted in Figure 4. Since the contaminant of this case study has high bioaccumulation potential, all non-herbivorous animals had high weights for indirect effects via the foodweb, with medium weights assigned to omnivorous animals. Of the species within the protection community, the only ones that were assigned high weights for vulnerability to additional non-chemical stressors were amphibians, which are susceptible to chytrid fungus.

Based on the weights assigned to the lines of evidence, amphibians (both unimodal and bimodal) were identified as the focal species. Despite the higher, relative toxicity of plankton in available laboratory measured studies, amphibians possess traits that increase their susceptibility to coal ash, are a critical component of the foodweb, and are vulnerable to pathogens and indirect effects via prey items not included in the protection community (Table 3). Managing mitigation goals to preserve and protect amphibian communities would involve establishing levels low enough to protect amphibians from sublethal effects, including those imposed by a potential reduction in prey, their ability to forage, and functional alterations that may impact long-term survival (Raimondo et al., 1998). Further analysis of amphibian effects data relative to exposure concentrations will provide a final risk characterization or mitigation goal that will be protective of the whole community.

6. Discussion

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We demonstrate a community-level approach to protect critical habitat of GIWs that also protects ES. GIWs are unique in that they often contain multiple species and taxa of concern in a single, spatially-explicit area. Many GIWs, such as vernal pools and pond swamps, are subjects of multi-species recovery plans for conservation management (e.g., USFWS 1998, 2005), supporting their protection under a multi-species risk assessment. The community-level approach demonstrated here will reduce the time investment required of species-by-species assessments and adds an ES perspective to demonstrate the value of GIWs beyond listed species concerns; essentially moving towards the "do more, quicker, with less" ideology that is advocated in our resource-limited era. The approach demonstrated here can be used at various levels of governance, and but will be particularly effective at regional, state, and local scales where protection communities can be more accurately defined for specific sites and stakeholders are more actively engaged in site-specific assessments. While GIWs are unique to their ecoregions (Tiner et al., 2002), they share characteristics that make the approach demonstrated here transferrable across different GIW types.

Hydrologic isolation is a keystone characteristic of GIWs, which creates systems of rare, endemic species and makes estimating exposure concentrations challenging. As discussed here, several exposure models are well suited to estimate the EEC in GIWs from run-off, drift, industrial point source, and food-webs (e.g., Armitage and Gobas 2007; Neitsch et al., 2005; Young, 2016), with potential exposure from atmospheric transport and deposition less understood. The influence of additional sources of potential exposure, such as re-introduction of contaminated groundwater from irrigation practices are also not well understood for GIWs. Pesticides are the most common chemical threat to GIWs across regions (Tiner et al., 2002), and the models described here are widely applicable to estimate pesticide exposure concentration in all GIWs. Other prospective ERAs or environmental impact statements may be performed prior to the permitted use of GIWs to treat waste water (e.g., USEPA, 1993) or disposal of other point source pollution. In other cases of unintentional or accidental release of chemicals such as

metals, petroleum products and industrial pollutants as well as urban runoff, ERAs are most likely to occur retrospectively such that monitoring data may be used to determine concentration levels. The approach demonstrated here can be used to achieve mitigation goals by prioritizing effective mitigation efforts.

Although the species that occupy GIWs vary across regions, the general taxonomic composition within GIWs is somewhat consistent (Supporting Information A, B), as are the traits that allow species to persist in these highly variable environments. Traits such as desiccation-resistant dormancy (e.g., cysts, seeds), high levels of sensitivity to environmental cues, quick release from dormancy, and short maturation and reproduction periods are present in species that occupy ephemeral GIWs (Bliss and Zedler, 1997; Zedler, 1987; Zedler, 2003). These adaptations to the unique environmental conditions along with relative geographic and hydrologic isolation, have led to high rates of endemism in GIW communities [ADDIN EN.CITE ADDIN EN.CITE.DATA], making their populations particularly vulnerable to anthropogenic stressors. All GIWs also are ecologically significant as important breeding sites for many amphibians, as the ephemeral nature of many GIWs precludes the establishment of predatory fish populations (Battaglin et al., 2009; Tiner, 2003; Zedler, 2003). In addition to inherent species sensitivity, traits can be used to evaluate a population's susceptibility to chemical contaminants (e.g., Awkerman et al., in review). The application of traits-based risk assessment approaches is becoming more widespread as a means by which to compensate for species-specific data deficiencies (Baird and Van den Brink, 2007; Van den Brink et al., 2013). As demonstrated by our case studies, traits-based species groups can be used to maximize the representativeness of available toxicity information.

GIWs tend to be consistent in the ES they provide (Supporting Information B). Maintaining some ES can enhance the protection of listed species, and in some cases, such as the one demonstrated here for vernal pools, listed species make up most of the SPUs that deliver the ES. Alternatively, some ES are contrary to the protection of listed species and SPUs of other ES, such as the one demonstrated here for

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Carolina Bays. Management practices that use GIWs (e.g., pond swamps, Carolina bays) to treat waste water intentionally introduce contaminants into the habitat to utilize the water purification properties of the sediments (USEPA, 1993). As demonstrated in the case study of Carolina Bays, coal ash has been previously discarded into the wetlands to filter heavy metals and other industrial contaminants prior to recharging ground water (Rowe et al., 2002). This increases the bioavailability of contaminants, particularly for benthic organisms and those with increased potential for chemical absorption (i.e., amphibians), increasing risks to the unique communities within the Carolina bay ecosystems. With recent increased flexibility in the discretion for coal ash waste water treatment (USEPA, 2018) and lack of protection of these wetlands under the CWA, it is critical to establish protection goals for selected habitats and engage stakeholders to ensure conservation of listed species communities and related ESs within the context of regional- or state-specific priorities.

Characterization of chemical effects on ecological receptors is a source of significant uncertainty in ERAs stemming from limited data availability. This is particularly true for listed species, for which performing laboratory tests is either illegal, impractical, or requires permits that are time consuming to obtain. Understanding potential effects to ES is more complex as modeling impacts across multiple levels of biological organization is required (Forbes and Galic, 2016; Galic et al., 2012). Much of this uncertainty is due to the limited number of surrogate species to represent effects to diverse ecological communities. Even in the few cases of data rich chemicals, species diversity is poorly represented and extrapolation to targeted receptors is the only way to understand impacts to untested species. For the species that are tested, measured endpoints may be limited to acute estimates of lethal concentrations to 50% of tested organisms (e.g., LC50) or effects measured on survival, growth, or reproduction on longer term exposures, typically representing a single life cycle or less (i.e. partial life cycle tests). While models are used regularly to estimate exposure concentrations for ERA, effects models, including interspecies extrapolation and population models, are not regularly applied in ERA (Raimondo et al.,

2018). Despite the recommendation to move towards effects modeling to reduce the uncertainty around the use of surrogate species, little has historically been done with surrogate species data in ERA beyond the application of safety factors (NRC, 2013). Our approach reduces the requirement on data collection for all species within the protection community by identifying the focal species for which models and extrapolation efforts may be targeted.

The WoE process is an integral component of ERA and we demonstrate an additional application of this approach to identify focal species within the protection community. Using a WoE approach as demonstrated here allows environmental resource managers to maximize resources in constrained environments and set explicit levels of uncertainty and provide unequivocal justification for the use of focal species. We provide 10 lines of evidence for focal species (Box 2) that may be applied or used as a guide for a specific assessment. Some lines are loosely defined here, e.g., vulnerable to additional non-chemical stressors, and may be more narrowly defined based on the protection community. For example, populations of many GIW species operate as metapopulations, such that the subpopulation of a species that is present in one GIW is connected to a subpopulation in another GIW through dispersal. Listed species metapopulation dynamics in GIWs may be an important consideration when evaluating chemical risks, as more reliably-inundated pools may act as "sources" or genetic rescues to those that are less inundated (Zedler, 2003). Such dynamics should be considered in this approach if they are suspected to occur for one or more species within the protection community.

The approach described here is demonstrated using case studies of two different GIWs (Section 5). Compared to Carolina Bays and other GIWs, vernal pools are simple systems with a small protection community, allowing linkages among the species to be more clearly described. The distinct phases of vernal pools are critical components of these GIWs that should be considered in achieving protection goals. Thus, the conceptual diagram for this case study highlighted vernal pool phase (Figure 2) and focal species were identified for both aquatic and terrestrial phases. The Carolina bay case study is an

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example of a GIW that provides a diversity of ES and contains a large, inter-connected protection community (Figure 4). Due to their size, biological diversity, and irregular ephemeral nature, inundation of the wetland was not highlighted as a critical component in the conceptual diagram. Rather, we included ES provided by the SPUs in the conceptual diagram to aid in communicating the value of the focal species to stakeholders.

We have highlighted several advantages associated with implementing community-level protections goals using traits-based WoE analysis and demonstrated a process that is ecologically comprehensive while considering limited data availability. The benefits of using a network or community-level approach for ERA include the identification of a wider range of protection. A larger, networked approach also can help explain some ambiguities and discrepancies in the evidence; different species and stressors found within a single critical habitat interact with one another and effects on one species may be the indirect cause of effects on another. These relationships are harder to discern when examining each species in isolation. GIWs are also vulnerable to other stressors, such as habitat conversion and degradation, further emphasizing the importance of maintaining the biological integrity of these unique ecosystems through a comprehensive and holistic ERA process. While multispecies recovery plans have been found to be less effective than single species recovery plans (Clark and Harvey, 2002), chemical modes of action and adverse outcome pathways allow a more targeted evaluation of chemical impacts in complex communities (Russom et al., 2014). Therefore, this multi-species protection approach will establish comprehensive threshold values that not only protect individual listed species, but also conserve the ecological integrity that continues to provide ecosystem services for human benefit.

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Message

From: Purucker, Tom [Purucker.Tom@epa.gov]
Sent: 5/2/2018 8:01:25 PM
To: Garber, Kristina [Garber.Kristina@epa.gov]
Subject: FW: review of revised amphibian manuscript
Attachments: puruckeretal_171101_amphib_dermal_model.docx

Hi Kris-
I am just confirming that this is the latest version of the manuscript.
Thanks,
Tom

From: Purucker, Tom
Sent: Wednesday, November 1, 2017 11:34 AM
To: Garber, Kristina <Garber.Kristina@epa.gov>
Subject: RE: review of revised amphibian manuscript

Hi Kris-
Attached is the latest version of the manuscript that includes your latest comments as well as ORD comments. Please take a look and send through the EFED review process. Once I hear back from you I will submit.
thanks,
Tom

From: Garber, Kristina
Sent: Thursday, September 28, 2017 8:17 AM
To: Purucker, Tom <Purucker.Tom@epa.gov>
Subject: RE: review of revised amphibian manuscript

Tom,

As we discussed, I will need to send this manuscript through EFED management review prior to submission to IEAM. Please let me know when you are ready for the EFED review to occur and I can send the manuscript to my management.

Thanks,
Kris

From: Garber, Kristina
Sent: Wednesday, September 27, 2017 5:22 PM
To: Purucker, Tom <Purucker.Tom@epa.gov>
Subject: review of revised amphibian manuscript

Tom,

My comments on the revised manuscript are attached. I think that there should be some additional discussion of the foliar contact route (see specific comments).

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Let me know if you want to discuss this tomorrow.

Kris

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Neonicotinoid Pesticides In Wisconsin Groundwater and Surface Water

Wisconsin Department of Agriculture, Trade and Consumer Protection

Agricultural Chemical Management Bureau

Environmental Quality Unit

July 2019

A summary of groundwater and surface water test results for neonicotinoid insecticides from 2008 through 2016

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Neonicotinoid Pesticides In Wisconsin Groundwater and Surface Water

Contents

Executive Summary 3

Groundwater and Surface Water Use in Wisconsin 4

Major Crops Grown in Wisconsin 6

Neonicotinoid Insecticides 8

Major Crop Uses in Wisconsin 8

Physiochemical Properties of Neonicotinoids 10

DATCP Groundwater and Surface Water Testing Programs 11

- Private Potable Well Sampling 11
- Non-Potable Well Sampling 12
- Surface Water Sampling 13
- Laboratory Analyses 13

Monitoring Results and Discussion 14

- Results of All Private Potable Wells 14
- Breakdown of All Private Potable Well Results by Program Areas 15
- Non-Potable Wells 22
- Comparison of Groundwater Results across Well Types 27
- Surface Water Monitoring Results 29

Implications for Important Receptors 30

Conclusions 42

Recommendations 43

Appendix 1 44

Appendix 2 45

Appendix 3 46

References 47

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Executive Summary

It has been estimated that agriculture generates $88 billion per year for Wisconsin's economy. The Wisconsin Department of Agriculture, Trade and Consumer Protection (DATCP) manages groundwater and surface water sampling programs designed to assess and protect water resources from chemicals used in agriculture. This report presents a summary of DATCP groundwater and surface water testing programs for neonicotinoid insecticides from 2008 through 2016.

Neonicotinoid insecticides are used widely in agriculture. Their high toxicity to insects, low toxicity to mammals, long lasting systemic activity and wide range of application methods make them the most widely used class of insecticides worldwide. In Wisconsin, more than 500 products containing neonicotinoid active ingredients are registered for use. They are labelled for use on most major crops, including corn, soybeans, forage, small grains, vegetables and cranberries. They have become the subject of extensive research into possible effects on pollinator populations across the country.

DATCP test results show that three commonly used neonicotinoids (clothianidin, imidacloprid and thiamethoxam) were detected in samples collected from private potable wells, field-edge monitoring wells, and irrigation wells. Two neonicotinoid compounds (imidacloprid and thiamethoxam) were detected in stream water samples. In addition to neonicotinoids, DATCP detected numerous other agricultural chemicals, including nitrate in all types of wells sampled as well as in streams.

For the targeted well sampling program, sampling revealed 5.7 percent of potable wells in agricultural areas contained one or more neonicotinoid compounds. The exceedance well sampling program includes potable wells that once contained a pesticide above a drinking water quality standard (chapter NR 140, Wisconsin Admin Code). For this program, the likelihood of neonicotinoid detection increased to 14.3 percent. An evaluation of field-edge monitoring wells revealed that 53.1 percent of sites had samples test positive for neonicotinoids. For irrigation wells, University studies reported up to 69 percent of irrigation wells with one or more detections of the compound thiamethoxam. The majority of neonicotinoid detections occurred where major agricultural crops are grown in areas with coarse, well-drained surface soils and shallow groundwater (less than about 20 feet deep). Many detections coincided with sandy agricultural areas within the Central Sands region and the Wisconsin River Valley.

Neonicotinoids and other pesticides detected in irrigation wells sampled in the Central Sands and Lower Wisconsin River Valley (LWRV), as well as those detected in stream samples in the Central Sands, have implications for target and non-target insects, honeybees, wild bees and other pollinators, as well as aquatic invertebrates. Aside from concerns over direct exposures to these organisms during application and use of these products, there is additional concern over chronic exposures resulting from residual concentrations in soil and plant tissues, and in contaminated groundwater used for irrigation and occurring as base flow discharge to streams.

Based on results presented in this report, additional research is needed in the Central Sands and other areas where neonicotinoid compounds were detected to evaluate measurable effects on organisms from the long term presence of neonicotinoids and other compounds in groundwater and surface water.

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Groundwater and Surface Water Use in Wisconsin

The State of Wisconsin, located in the western Great Lakes Region of North America, has abundant fresh water resources that are extremely important to its residents, businesses, agriculture and recreation. The state borders two Great Lakes, Lake Michigan and Lake Superior. Wisconsin has more than 15,000 inland lakes (WDNR, Water Topics, 2017), most of which were formed as the great Laurentide Ice Sheet receded during the Late Pleistocene Epoch about 13,000 years ago (Flint, 1971). Abundant fresh groundwater exists across much of the state in Paleozoic age sedimentary rocks and Pleistocene glacial sediments (Luczaj & Masarik, 2015).

In 2014, just under 2 trillion gallons of water were withdrawn from Wisconsin's surface waters and from groundwater wells. Surface water withdrawals represent about 88 percent of this total, or about 1.73 trillion gallons. Most surface water withdrawals are used for cooling water in power generation (85 percent). Aside from power generation, the largest users of surface water include paper production (5.6 percent), municipal water supplies (4.4 percent), cranberry production (3.6 percent) and mining or other uses (1.8 percent) (WDNR, Wisconsin Water Use Withdrawal Summary 2014, 2016).

As for groundwater uses, it is estimated that two thirds of Wisconsin's population of 5.7 million people (Census, 2010) rely on groundwater as their primary source of drinking water (WDNR, Groundwater Coordinating Council Annual Report to Legislature, 2016). An estimated 800,000 low capacity wells serve private homes, farms and businesses across the state. These wells withdraw approximately 50 to 75 billion gallons of water per year.

In addition to withdrawals from low capacity wells, more than 11,000 registered high capacity wells withdraw groundwater for agricultural irrigation, municipal water supply systems, industrial and other high volume water uses. A high capacity well is defined as one or more wells on a property where the collective withdrawal capacity is 100,000 gallons or more per day. In 2014 (Figure 1), Department of Natural Resources (DNR) reported 224 billion gallons of groundwater withdrawn from high capacity wells for municipal, irrigation and other uses (WDNR, Wisconsin Water Use Withdrawal Summary 2014, 2016).

Agricultural irrigation and municipal water withdrawals represent two of the largest annual groundwater use categories in Wisconsin. Portage, Waushara and Adams Counties have the largest groundwater withdrawals for agricultural irrigation (Figure 2). These counties are centrally located in the state and are home to a large and globally significant potato and vegetable growing area. This area coincides with a central sand plain, or Central Sands region, an area east of the Wisconsin River where glacially derived sand and gravel deposits extend from the surface to depths greater than 50 feet. The area is known for highly permeable sandy soils and abundant shallow groundwater.

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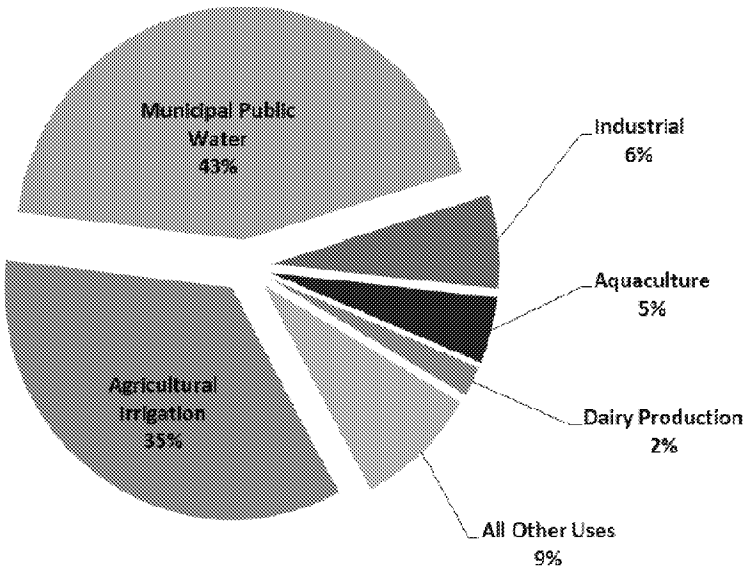
Figure 1 Groundwater Withdrawals by Water Use Type, 2014



Left: In 2014, DNR reported 224 billion gallons of groundwater withdrawn from high capacity wells for municipal, agricultural irrigation, industrial and other purposes.

Municipal public water supplies withdrew about 98 billion gallons (43 percent) for residential, commercial, industrial and institutional uses. Agricultural irrigation withdrawals totaled about 78 billion gallons (35 percent), while industrial, aquaculture, dairy and all other uses totaled about 49 billion gallons (22 percent).

Low capacity wells for private homes, farms and businesses withdraw an additional 50 to 75 billion gallons per year.

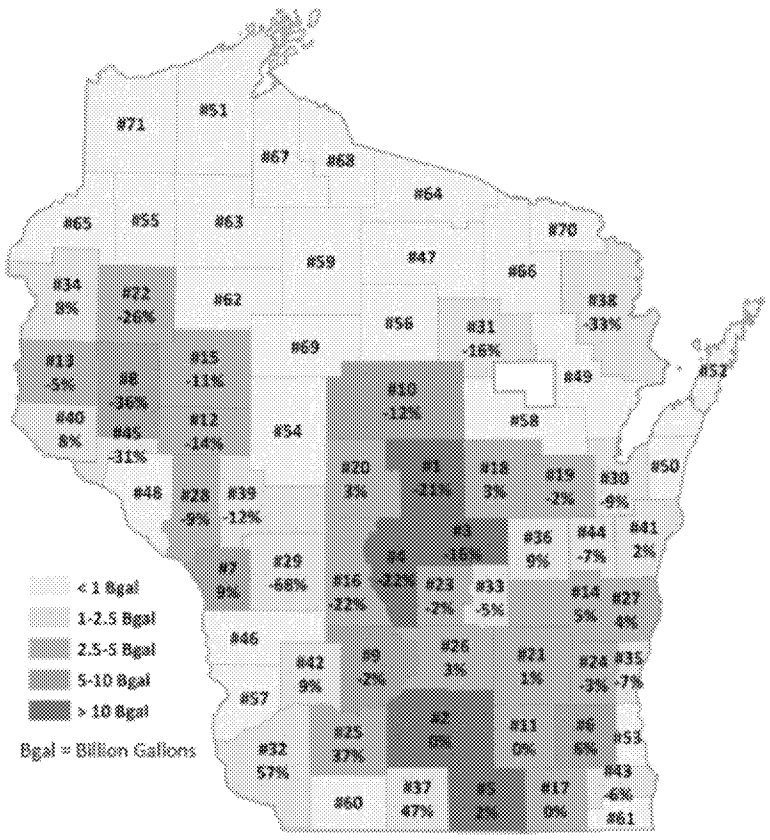
Figure 2 Groundwater Withdrawals by County, 2014

Right: the top number indicates ranking of total withdrawal by county (#1 = highest, #71 = lowest). For counties having withdrawals exceeding 1 billion gallons, the bottom number shows the percent change from 2013.

In the center of the state, Portage, Waushara and Adams Counties have high agricultural irrigation withdrawals (ranking #1, #3 and #4 respectively). These counties are home to the State's globally significant vegetable and potato producing area. Withdrawals in these counties decreased significantly due to increased precipitation during the 2014 growing season.

Dane and Rock Counties (#2 and #5 respectively) have large urban/suburban populations that rely on groundwater for residential, industrial and commercial needs. Groundwater withdrawals remained similar from 2013 through 2014.

Groundwater withdrawals are lower in the north due to greater forest cover and lower population and agricultural needs.



(WDNR, Wisconsin Water Use Withdrawal Summary 2014, 2016)

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Major Crops Grown in Wisconsin

Different crops have different pesticides labeled for various crop protection needs. DATCP uses knowledge about the types of agricultural crops grown across the state to better understand the types of pesticides used on the landscape. This knowledge helps staff design groundwater testing programs to detect pesticides that may contaminate groundwater and surface water resources.

Table 1 shows a summary of the major commodity crops grown in Wisconsin in 2015, as reported by the U.S. Department of Agriculture (USDA) National Agricultural Statistics Service (NASS). Wisconsin often ranks ninth annually among states in the nation for corn grown for grain, with approximately 3 million acres planted in 2015. Corn for silage added about 970,000 acres, for a total of about 4 million acres of corn grown annually. Acres devoted to all forage (alfalfa and hay, dry equivalent) included about 2.6 million acres, and soybeans about 1.9 million acres. Winter wheat, oats and barley combined to represent another 420,000 acres. Potatoes for processing and fresh market were grown on about 62,500 acres, while peas, sweet corn and snap beans combined for another 160,300 acres. Wisconsin routinely ranks as the nation's top producer of cranberries, with production occurring on 20,200 acres in 2015 (USDA-NASS & DATCP, 2016).

TABLE 1 MAJOR CROPS AND APPROXIMATE ACRES UNDER PRODUCTION IN WISCONSIN IN 2015

Major Crops Grown in 2015	Approximate Acreage
Corn (includes for grain and sileage)	3,970,000
Forage Crops (alfalfa and hay, dry equivalent)	2,600,000
Soybeans	1,900,000
Winter Wheat, Oats and Barley	420,000
Vegetables (peas, sweet corn, snapbeans and potatoes for processing and fresh market)	222,800
Cranberries	20,200

(USDA-NASS & DATCP, 2016)

Figure 3 represents geographic information system (GIS) data from NASS showing the distribution of corn, soybean, potato and other vegetable crops grown in 2015 (USDA-National Agricultural Statistics Service, 2016). This figure helps to illustrate agricultural cropping patterns that typically occur across the state. The image shows corn and soybeans are grown widely across the southern two-thirds of the state. These crops are typically interspersed and often in a rotation with each other, and with forage crops or small grains. Potatoes, sweet corn and other vegetables are specialty crops that are also grown in rotation with corn, soybeans and other crops. The areas that favor vegetable cropping can be seen in clusters across the state, the most notable of which occurs in the center of the state (Central Sands). Smaller areas favorable to vegetable growing can be seen on the figure, including an area within the LWRV. Both the Central Sands Region and the LWRV are shown on the figure.

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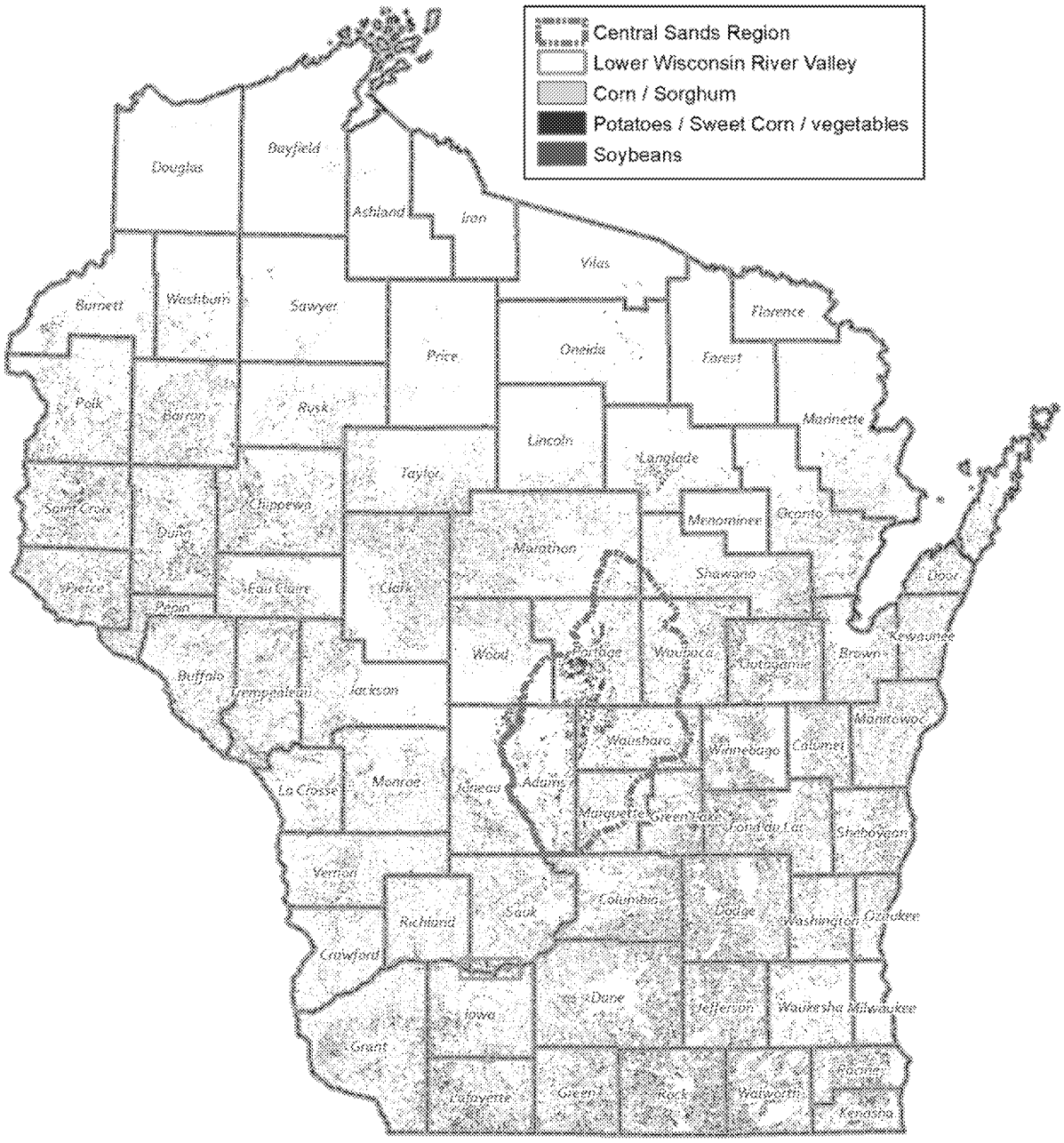
FIGURE 3 CORN, SOYBEAN, POTATO AND VEGETABLE PRODUCTION IN WISCONSIN, 2015



Above: NASS imagery shows where major crops are typically grown in Wisconsin, including corn, soybeans, potatoes and other vegetables. A sand plain located in the center of the state (brown outline) is known as the Central Sands Region (Luczaj & Masarik, 2015). Marked by deep sandy soils and abundant shallow groundwater, it is where much of the state's vegetable production occurs. Similar conditions exist in the LWRV to the south (blue outline), and in other areas not specifically called out on the figure.

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Neonicotinoid Insecticides

Neonicotinoid insecticides are nicotinic acetylcholine receptor agonists. They mimic the action of the neurotransmitter acetylcholine, and bind strongly to nicotinic acetylcholine receptors (nAChRs) in the central nervous system of insects, causing nervous stimulation at low concentrations, and at higher concentrations, receptor blockage, paralysis and death (Tomizawa & Casida, 2005). They bind more strongly to receptors in insects than to those of vertebrates, so they are selectively more toxic to insects (Goulson, 2013). First introduced in 1994 (imidacloprid), they've been widely viewed as safer alternatives to more toxic insecticides in the organochlorine, organophosphate and carbamate classes. They offer advantages of low toxicity to vertebrates, high toxicity to insects, and a broad range of application types and use options over insecticides in other classes. Neonicotinoid insecticides are water soluble and readily absorbed by plant roots or leaves and are readily transported throughout plant tissues to provide long lasting systemic activity to all parts of the plant. These advantages have helped make them the most popular class of insecticides worldwide, with neonicotinoids representing 25 percent of all insecticides used globally (Goulson, 2013).

There are seven compounds classified as neonicotinoids that are commonly used in agriculture. These seven compounds can be further broken out into one of three chemical groups. They include the *N*-nitroguanadines (clothianidin, imidacloprid, thiamethoxam and dinotefuran), the N-cyanoamidines (acetamiprid and thiacloprid), and the nitromethylenes (nitenpyram). Two compounds (nitenpyram and thiacloprid) are not contained in any products registered for use in Wisconsin and they will not be discussed further in this report (one product containing thiacloprid was registered, but the registrant voluntarily canceled the registration in 2016). The five remaining neonicotinoids clothianidin, imidacloprid, thiamethoxam, acetamiprid and dinotefuran, are active ingredients in 600 products registered for use in Wisconsin (Kelly Products Inc., 2017).

There are some important differences in uses of products that contain acetamiprid and dinotefuran that set them apart from clothianidin, imidacloprid and thiamethoxam. Acetamiprid and dinotefuran have products labeled for use on agricultural crops, but they are more widely used in turf, lawn and landscape products, structural pest applications, companion and other animal products, greenhouse and nursery uses, and in bait applications used in homes and other structures. Their crop uses tend to be more limited and do not include use as seed treatments. Their uses are less widespread on the landscape than other neonicotinoid products. Furthermore, DATCP has not detected acetamiprid or dinotefuran in any groundwater or surface water samples collected through 2016. For these reasons, these two compounds are not discussed further in this report.

Major Crop Uses in Wisconsin

Clothianidin, imidacloprid and thiamethoxam are registered in a total of 502 products sold in Wisconsin. Clothianidin is the active ingredient in 29 products registered, imidacloprid in 423 and thiamethoxam in 50 registered products. Out of these total numbers, each active ingredient is found in products labeled for use on major agricultural crops grown in Wisconsin, like corn, soybeans, potatoes, sweet corn, snap beans and other high value vegetables. Clothianidin is in about 15 product formulations, imidacloprid is in about 105, and thiamethoxam is in about 36 product formulations labeled for one or more major agricultural crop uses (Kelly Products, Inc., 2017). Most commonly, these products are used as seed treatments on corn, soybeans and small grains, but they also have formulations that allow their use as soil treatments or as foliar sprays on

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certain crops. Table 2 lists names of some of the major manufacturers or suppliers, common product names, and common uses and formulations containing clothianidin, imidacloprid and thiamethoxam. The table contains examples and is not intended to be an exhaustive list.

TABLE 2 SOME COMMON EXAMPLES OF MANUFACTURERS, PRODUCTS AND FORMULATIONS CONTAINING CLOTHIANIDIN, IMIDACLOPRID AND THIAMETHOXAM

Neonicotinoid Active Ingredient	Manufacturers and Suppliers	Registrations and Some Product Names	Common Formulations and Applications
Clothianidin	Bayer CropScience Sumitomo Chemical Co Takeda Chemicals	A total of 29 products registered in Wisconsin, about 15 for major agricultural crop uses. Some product names include Deter, Modesto, and Poncho.	Commonly supplied as a flowable concentrate prepared for uses as a seed treatment.
Imidacloprid	Bayer CropScience Makhteshim-Agan Scotts	A total of 423 products registered in Wisconsin, about 105 for major agricultural crop uses. Some products include Admire, Gaucho, Raxil, and Chinook Blue.	Often supplied as granules that are mixed with water and applied as a spray, used as a seed treatment or applied directly to compost. For animal use it is usually supplied as a spot-on solution.
Thiamethoxam	Syngenta AgroCare Kingtai Chemicals	A total of 50 products registered in Wisconsin, about 36 for major agricultural crop uses. Some products include Actara, Cruiser SB, Optigard and Platinum.	Formulations include flowable concentrates for seed treatments, water dispersible granules and suspension concentrates.

(Lewis, 2016) (Kelly Products, Inc., 2017)

With 502 products registered in Wisconsin, it is clear that neonicotinoid products are widely available for sale and use in the state. But actual amounts of neonicotinoids used in agriculture is difficult to determine. NASS conducts grower surveys and publishes data on pesticides used in Wisconsin and other states. A 2014 NASS survey of pesticides and insecticides used on corn and potatoes in Wisconsin and several other states reported that about 8 percent of Wisconsin corn acres had an insecticide applied, for a total of about 22,000 pounds of insecticides. Eight percent of Wisconsin's annual 4 million corn acres amounts to 320,000 acres treated. But the same survey further reports that 10 percent of corn growing operations in Wisconsin treat their own seed *after* purchase for insect or disease control (USDA-NASS, 2014). It is unknown if the NASS estimates counted seed that had been treated prior to purchase.

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Meanwhile, a 2015 study that evaluated pesticides applied to treated seed reported that national estimates of neonicotinoid uses on corn and soybean were likely much higher. Douglas and Tooker estimated the use of neonicotinoids and other pesticides on treated seed may be as high as 79-100 percent of corn seed and 34-44 percent of soybean seed planted nationally (Douglas, 2015).

For potatoes grown in Wisconsin, the 2014 NASS survey provides some detail on neonicotinoids used. Growers used insecticides on 99 percent of acres planted, with 56 percent of operations treating their own seed *after* purchase. Acres treated broke down as follows: clothianidin was used on 36 percent, imidacloprid on 15 percent, and thiamethoxam on 56 percent of potato acres. A total of 16,000 pounds of insecticides were reported used on potatoes, with 2,000 pounds used for each of the three neonicotinoids clothianidin, imidacloprid and thiamethoxam.

In summary, it is difficult to accurately estimate the actual amount of neonicotinoids used year to year in Wisconsin agriculture. A large number of products are registered and available for use. Combined with NASS grower-surveys and reports by others, the available data suggest that neonicotinoid use is common on high value vegetable crops like potatoes, as well as other major crop types grown in the state. It may be that the NASS grower surveys provide a conservative estimate of overall use when one considers the use of these products on seed that is treated prior to the grower's purchase. Other estimates suggest that as high as 79-100 percent of corn seed and 34-44 percent of soybean seed planted annually may receive neonicotinoid treatments.

Physiochemical Properties of Neonicotinoids

In plants, clothianidin, imidacloprid and thiamethoxam are all taken up via the roots or across plant stems and leaves. All three are considered xylem mobile, with dominant uptake routes following the transpiration stream (*i.e.*, no downward transport from leaves to roots). Numerous field studies have demonstrated that clothianidin, imidacloprid and thiamethoxam applied via foliar, soil or seed treatment methods can result in residues in pollen and nectar of blooming plants (U.S. EPA, January 4, 2016). Available data also suggests that thiamethoxam is metabolized within plants to form clothianidin (U.S. EPA, January 5, 2017).

Clothianidin, imidacloprid and thiamethoxam also have similar physical and chemical properties. They are all highly soluble in water, have low vapor pressures, low Henry's Law Constants and low octanol-water partition (Kow) coefficients. Compounds with these properties are readily soluble in water, mobile in groundwater, and not likely to volatilize. In addition, their organic carbon partition coefficient (Koc) values are consistent with compounds that are highly leachable. A list of physical and chemical properties of these three compounds is included in Appendix 1.

The dominant transformation process for all three of these compounds is photolysis, or chemical breakdown that occurs under direct exposure to the sun's rays. Photolysis may occur on soil surfaces following soil application and on wet foliage in the case of foliar application. Photolysis on foliage tends to occur more rapidly than on soil. Photolysis can occur in days to weeks in water and months on the soil. Aerobic transformation processes within the soil biome for each of these compounds is very slow, with half- life values on the order of months to more than a year. Imidacloprid persists very long in soil, with an aerobic soil half-life as long as 1600 days or more (U.S. EPA, January 4, 2016).

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DATCP Groundwater and Surface Water Testing Programs

DATCP collects groundwater and surface water samples and tests for agricultural chemicals under a number of routine sampling programs housed within the Agricultural Resource Management Division (ARM). ARM sampling programs are designed to meet groundwater protection obligations required under Ch. 160, Wisconsin Statutes. The statute requires state regulatory agencies to determine whether activities, facilities or practices under their regulation impact groundwater, and to evaluate contamination of groundwater relative to established numeric groundwater quality standards (i.e. Preventive Action Limits (PALs) and Enforcement Standards (ESs) per Wisconsin Admin Code). To meet these statutory obligations, ARM conducts sampling programs that test private potable wells and non-potable wells for agricultural chemical contaminants resulting from routine agricultural use. Table 3 includes a listing of routine ARM groundwater monitoring programs.

TABLE 3 ARM SAMPLING PROGRAMS, WELLS SAMPLED AND PROGRAM FREQUENCY

Sampling Program	Wells Sampled	Purpose	Frequency / Number
Statewide Survey	Private potable	Randomly distributed	Occasional / ≈400 per event
Targeted	Private potable	At-risk, near agricultural area	Annual / 50-120 per year
Exceedance	Private potable	Trend, environmental fate	Annual / 20-30 per year
Field Edge	Monitoring	Surveillance/early warning	Annual / 30-90 per year
Irrigation	High Capacity	Surveillance/early warning	New & evolving

Sampling of private potable wells generally occurs under three sampling programs: statewide random sampling, targeted sampling, and exceedance-well sampling. Potable wells can also be sampled outside of routine sampling programs. Sampling of non-potable wells occurs via field edge monitoring wells and irrigation wells. ARM also performs some annual surface water testing on a small percentage of stream samples DNR collects annually under their surface water monitoring programs. A description of sampling programs is provided below.

Private Potable Well Sampling

Statewide Survey

The statewide survey is a random sampling of private wells for pesticides and nitrate. It is performed on about 400 private wells on an occasional basis, typically once every 5 to 10 years. The purpose of this sampling program is to obtain "snapshots" of statewide water quality over time. Survey wells are selected using a statistically random approach. Half of the wells in each survey are selected using a stratified random sampling procedure. The other half of wells included in the survey are repeats of the randomly selected wells from the prior survey. Benefits of this approach are that statistical comparisons can be made showing the distribution of agricultural chemicals in groundwater statewide as a function of agricultural intensity, and to show water quality changes with time. The three most recent statewide surveys were conducted in 2016, 2007, and 2000. Those completed prior to 2016 did not analyze for neonicotinoid compounds.

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Targeted Sampling

Targeted sampling occurs annually with 50 to 120 wells sampled per year. Unlike the statewide random survey, targeted sampling intentionally uses a biased well selection approach. Under targeted sampling, areas are selected for sampling based on factors that reflect an increased likelihood of pesticides and nitrate leaching to groundwater. Factors like the amounts and types of pesticides used, crop density, lack of crop rotations year after year, depth to groundwater, depth to bedrock and soil texture are all considerations that can go into targeted sample planning. Targeted sampling focuses on sampling wells that are "at-risk" of being contaminated by pesticides through agricultural use.

Exceedance Sampling

State administrative rules require that DATCP take action to prevent further degradation of groundwater quality. For potable wells with a pesticide ES exceedance, this response typically involves restrictions or full prohibitions placed on local uses of the offending pesticide through special orders or administrative rules that apply to growers and landowners near the impacted well. Owners of any impacted wells are issued drinking water advisories to inform them of known health risks associated with exposures so they can take action to limit ongoing exposures. Impacted well owners may purchase and install a water treatment device, or install a replacement well. The state cannot require owners of contaminated private water wells to treat or replace their water supply, but many owners elect to do so. Exceedance sampling involves re-testing potable wells that have exceeded an ES for a pesticide, but which remain in service after contaminant discovery. For wells with an ES exceedance that remain in service, ARM selects 20 to 30 each year for repeat testing to evaluate trends over time. Exceedance sampling allows ARM to determine if pesticide use restrictions are effective at reducing groundwater contamination. Monitoring also helps document the time needed for an agricultural contaminant to attenuate following implementation of pesticide use restrictions for each area.

Potable Wells Sampled for Other Purposes

Aside from the potable well sampling programs identified above, potable wells can be sampled for other regulatory purposes. An example of this is when a groundwater contaminant investigation is performed around a private well that has been found to contain a pesticide above an ES or nitrate significantly above the ES. When this occurs, the impacted well and possibly others in the area of the impacted well are sampled so that appropriate management strategies can be considered. Between April 2011 and September 2016, about 87 potable wells were sampled for purposes not related to another specified sampling program.

Non-Potable Well Sampling

Field-Edge Monitoring

ARM also tests non-potable wells for agricultural chemicals at select field edge monitoring sites as part of the field edge monitoring program. Between October 2006 and December 2016, the agency collected groundwater samples from monitoring wells located on approximately 32 field-edge monitoring sites across the state, representing a total of about 65 wells. The number of sites and wells has varied over time as sites and wells are added or removed and as growers enter and leave the program.

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Field-edge monitoring sites are typically installed under agreements with growers who allow ARM to install monitoring wells to test shallow groundwater for agricultural chemicals applied to fields. Over time, monitoring well sites have been installed in a variety of geologic settings, often within areas susceptible to groundwater contamination (i.e. areas with sandy soil and shallow bedrock or shallow groundwater). Typically one to three groundwater monitoring wells are installed at each field edge site. Sites typically have a shallow well intersecting the water table, and one or more adjacent wells screened at deeper intervals. ARM collects 30 to 90 samples from these wells annually. Testing of these wells is used to identify contaminants that may be present in shallow groundwater at fields where agricultural chemicals are used as intended. Testing of these well networks provides an early warning about pesticides that leach to groundwater and are more likely to impact streams or down gradient potable water wells.

Irrigation Well Monitoring

Non-potable well sampling also performed by ARM includes irrigation wells. In 2015 and 2016, ARM collected samples from 22 high-capacity irrigation wells that extract groundwater from underlying aquifers in the Central Sands growing area. Irrigation wells are typically large diameter deep wells with well screens that intersect a large section of aquifer strata to meet high-capacity pumping needs. While field edge monitoring wells provide information on water quality within the upper portion of an aquifer, irrigation wells provide information on groundwater quality from larger and deeper sections of the aquifer. This is a relatively new type of site sampling that ARM intends to expand in future years.

Surface Water Sampling

In addition to groundwater sampling programs, ARM collaborates with DNR to collect surface water samples for pesticide testing. DNR performs annual surface water sampling on hundreds of streams and rivers statewide to monitor a variety of stream water quality parameters. ARM works with DNR to obtain samples from a small subset of the streams that DNR samples to help evaluate surface water quality impacts from agricultural land use. Under this program, the two agencies identify streams of interest for pesticide testing. DNR sets a sampling schedule and collects the samples. ARM staff coordinates pesticide testing at the agency's Bureau of Laboratory Services (BLS), and communicates results with DNR to supplement DNR surface water quality programs. The number and type of streams sampled, as well as the frequency of sampling varies annually. BLS analyzes approximately 80 stream samples per year under this program.

Laboratory Analyses

Laboratory testing for ARM sampling programs is provided by BLS. BLS is ISO 17025 accredited and performs gas chromatography, high-pressure liquid chromatography and mass spectrometric analytical services consistent with their accreditation. A full listing of BLS groundwater analytes and laboratory reporting limits is listed in Appendix 2 and includes 101 pesticides and nitrate.

Changes to BLS equipment and testing capabilities have occurred over time. BLS first began testing for thiamethoxam in 2008. In 2010, clothianidin and imidacloprid were added to the groundwater analyte list, and the three remaining neonicotinoids (acetamiprid, dinotefuran and thiacloprid) were added in 2011. Since 2011, all six compounds have been included in all ARM surface water and groundwater analyses. ARM

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sampling programs have detected clothianidin, imidacloprid and thiamethoxam numerous times, but acetamiprid, dinotefuran and thiacloprid have not been detected in any samples collected through 2016.

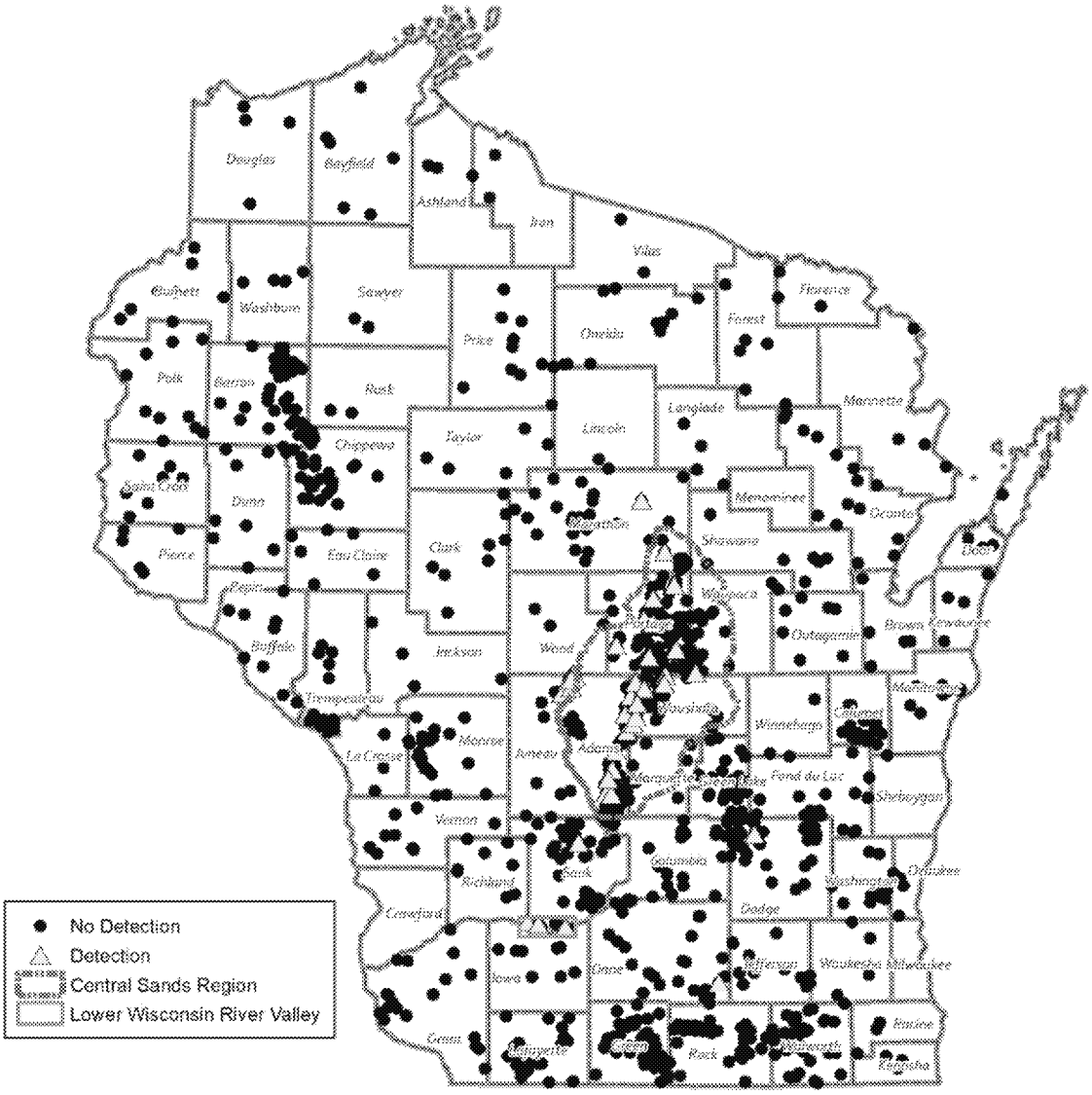
Monitoring Results and Discussion

Results of All Private Potable Wells

Summary of All Potable Well Results

In total, ARM programs sampled 1,048 potable wells over 9 years, from 2008 through 2016. Locations of all potable wells sampled, including those having detectable concentrations of neonicotinoids are shown in Figure 4.

FIGURE 4 LOCATIONS OF NEONICOTINOID DETECTIONS IN ALL POTABLE WELLS SAMPLED - 2008 THROUGH 2016



Out of 1,048 private potable wells sampled, 41 had a detection of one or more neonicotinoid compounds yielding an overall neonicotinoid detection frequency of 3.9 percent of potable wells sampled.[1] Imidacloprid was the most frequent neonicotinoid compound detected in potable wells. It was detected in samples from 35 wells (3.3-percent detection frequency) at concentrations ranging from 0.052 to 1.59 micrograms per liter (µg/L). The average imidacloprid concentration was 0.500 µg/L. Clothianidin was detected 28 times (2.7-percent detection frequency) and included the highest concentrations of the neonicotinoids. Clothianidin concentrations ranged from 0.069 to 3.88 µg/L, with an average of 0.608 µg/L. Thiamethoxam was detected 25 times (2.4-percent detection frequency) at concentrations ranging from 0.141 to 1.61 µg/L with an average of 0.699 µg/L.

All potable well results included testing related to programmatic sampling in the following areas: a statewide sampling survey performed in 2016; targeted and exceedance program sampling performed annually, and some sampling for other purposes, like groundwater investigations. Most wells were sampled just one time, but a few were sampled more than once, such as when samples revealed a pesticide detect above a standard, triggering confirmation testing or additional investigation. A more detailed breakdown of test results by program type is provided below.

The majority of potable wells having detectable concentrations of neonicotinoids are located near agricultural areas with coarse grained soils and shallow groundwater. Other commonalities included crop rotations of corn and/or soybeans intermixed with vegetable crops like potatoes, peas and sweet corn, and areas under irrigation.

Breakdown of All Private Potable Well Results by Program Areas

Statewide Survey Results

ARM conducted random sampling survey of 401 potable wells in 2016 for the Statewide Survey. All wells were tested for 101 pesticide compounds and nitrate. Although nitrate and numerous pesticide compounds were detected statewide, just one sample collected from a well in Marathon County had a detection of the compound imidacloprid (0.0798 µg/L). The detection frequency for neonicotinoids was 0.25 percent of samples collected on a statewide, random basis. The two most commonly detected pesticide compounds were the ethane sulfonic acid breakdown products of the herbicides metolachlor and alachlor. Metolachlor ESA and alachlor ESA were present in 32.2 and 21.5 percent of samples, respectively. Figure 5 shows 2016 Statewide Survey well locations.

Because the 2016 Statewide Survey was the first survey to include analyses for neonicotinoid compounds, comparisons to previous surveys is not possible. However, the 2016 sampling report does make comparisons with prior surveys for nitrate and other pesticides detected (Wisconsin Department of Agriculture, Trade and Consumer Protection, April 2017).

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[1] Some potable wells were sampled more than once. Unless specified otherwise in a figure or text, wells sampled more than once that have multiple detects of a neonicotinoid compound are counted once for purposes of reporting detection frequency by well. Where the average or a range of concentrations is reported for a compound, the sample having the highest total neonicotinoid result is used to represent the data from that well.

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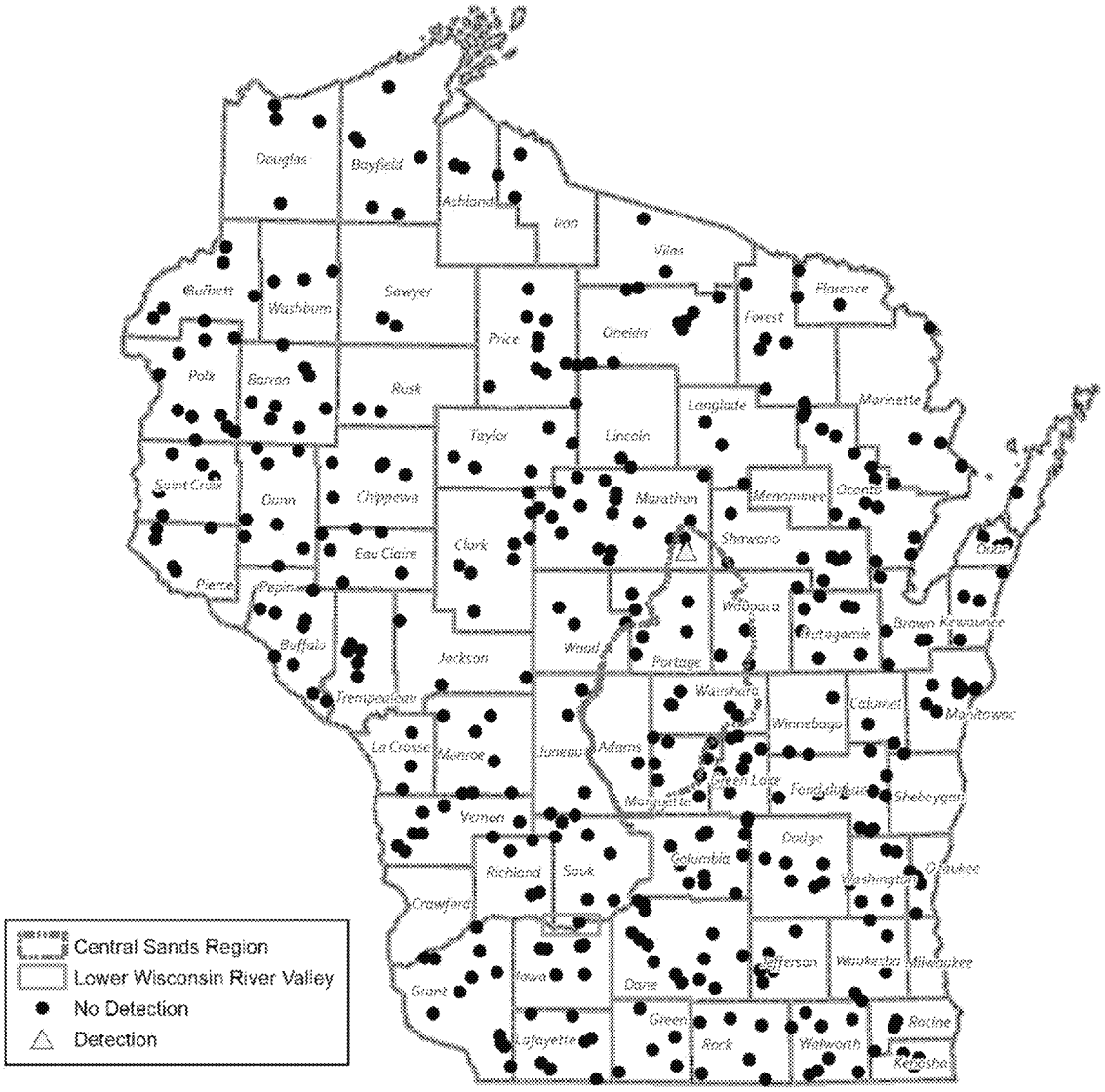
FIGURE 5 LOCATIONS OF NEONICOTINOID DETECTIONS IN POTABLE WELLS -- 2016 STATEWIDE SURVEY



Above: Out of 401 samples collected during random sampling survey in 2016, imidacloprid was detected in one Marathon County well.

Targeted Well Sampling Program Results

Unlike the statewide random sampling survey, the targeted sampling approach focuses on wells in known agricultural areas. Water samples are collected from private wells in areas where major crops like corn, soy beans, potatoes, sweet corn and other vegetables are grown year after year. Targeted sampling areas vary by program year, and wells are typically not resampled unless a test result approaches an ES for a pesticide, triggering verification sampling and a possible investigation.

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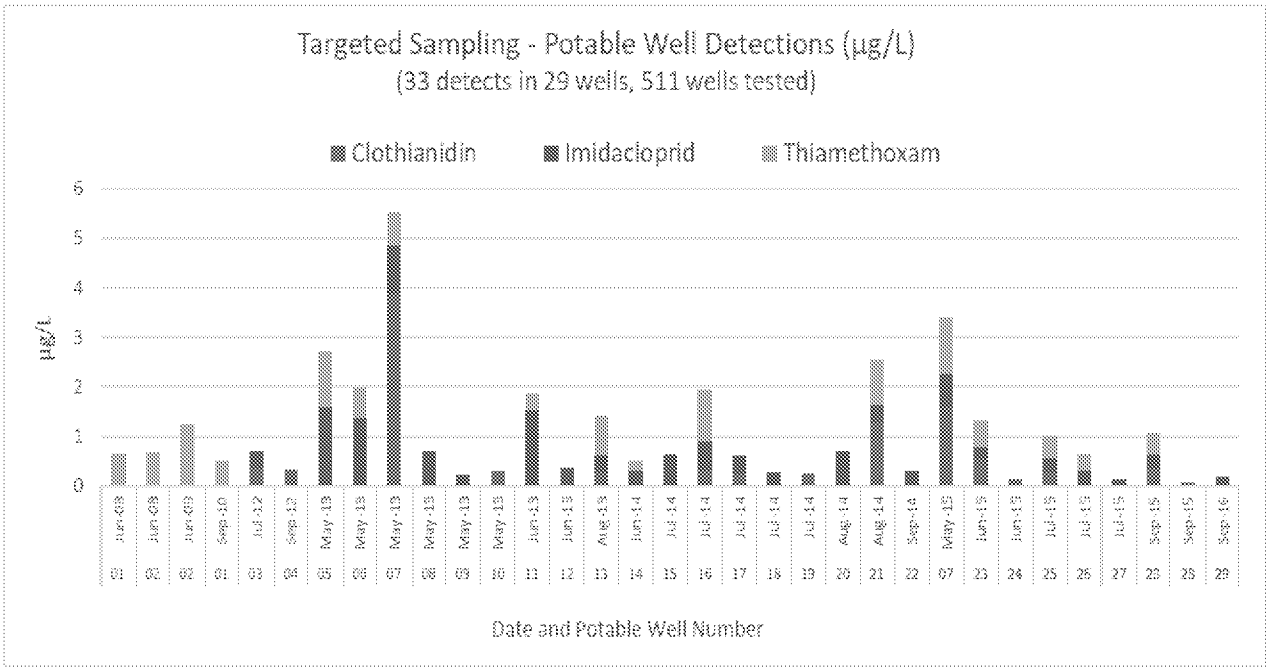
Between June 2008 and October 2016, 525 samples were collected from 511 private potable wells for the targeted sampling program. All samples were tested for pesticides and nitrate. One or more of the compounds clothianidin, imidacloprid and thiamethoxam were detected in 33 samples collected from 29 wells. The overall neonicotinoid detection frequency for wells tested under the targeted program is 5.7 percent of wells (or 6.2 percent of samples), a sharp contrast to the 0.25 percent of wells sampled on a statewide random basis. The detections are shown below in Figure 6.

FIGURE 6 RESULTS FROM TARGETED SAMPLING (525 SAMPLES FROM 511 WELLS)



Because these compounds are in the same chemical class and share similar physiochemical characteristics and toxicity to insects, the neonicotinoid data is presented in a stacked format to allow total combined concentrations to be easily visualized. Three neonicotinoid compounds were detected in eight samples, and two compounds were detected in seven samples. One compound was detected in the remaining samples. Imidacloprid was the most frequently detected compound, detected in 24 samples at concentrations ranging from 0.147 to 1.59 µg/L. Thiamethoxam was detected in 17 samples at concentrations ranging from 0.206 to 1.26 µg/L, and clothianidin was detected in 15 samples at concentrations ranging from 0.0686 to 3.88 µg/L.

The highest cumulative concentration of neonicotinoids detected was 5.525 µg/L in the May 2013 Well 07 sample. Well 07 had all three compounds present, and contained clothianidin at a concentration greater than the combined concentrations detected in any of the remaining 28 wells with detections. A second sample collected from this well two years later (May 2015) exhibited a cumulative concentration of 3.41 µg/L. Other wells with multiple detections included Well 01 (June 2008 and September 2010), Well 02 (June 2008 and June 2009), and Well 23 (June 2015 and September 2015). No State or Federal drinking water standards have been established for any of these detected neonicotinoids.

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The black dots on Figure 7 show the locations of wells where targeted sampling occurred from 2008 through 2016. Yellow triangles represent locations where one or more neonicotinoids were detected.

FIGURE 7 LOCATIONS OF NEONICOTINOID DETECTIONS IN POTABLE WELLS -- TARGETED SAMPLING PROGRAM

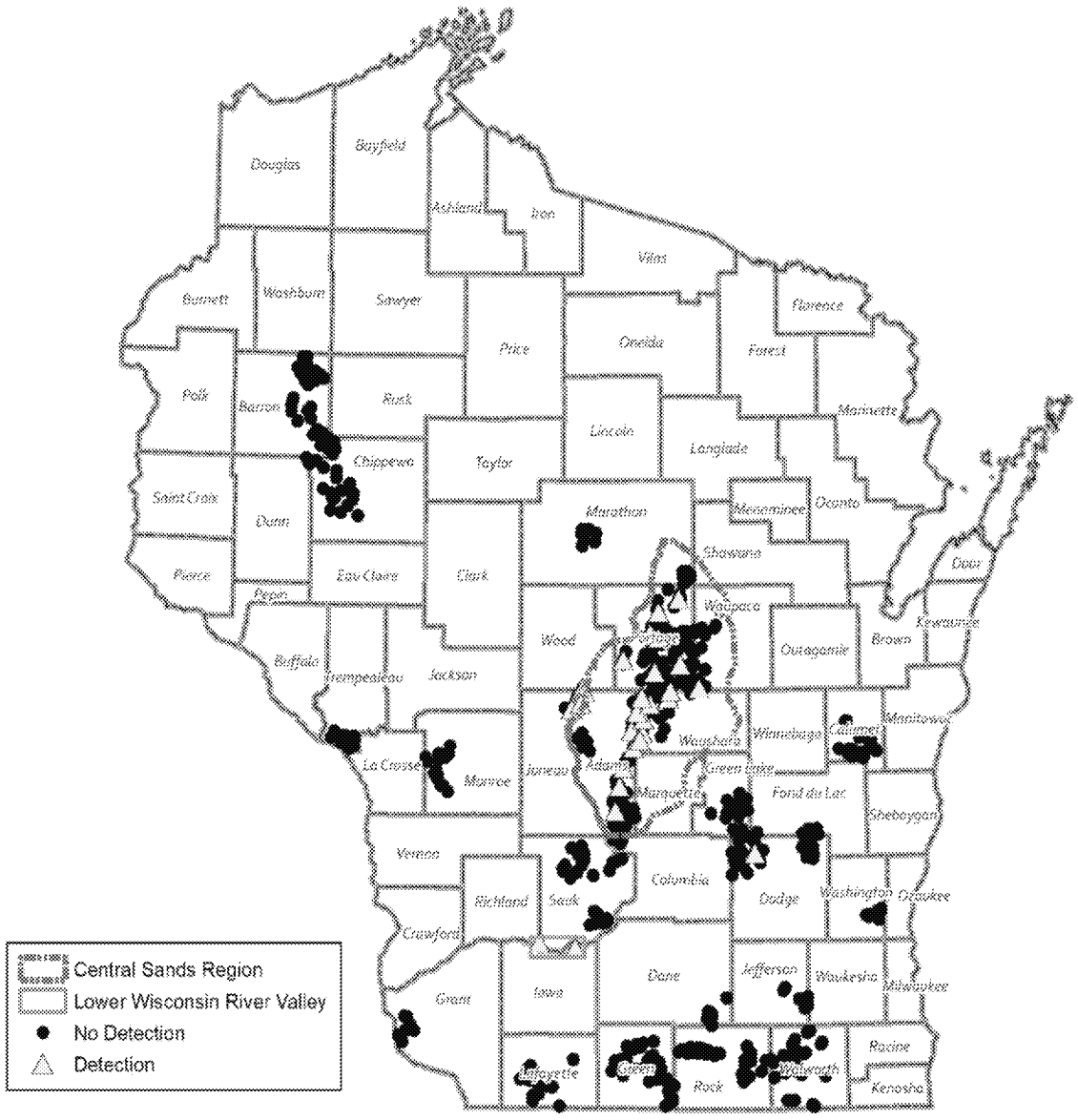

Figure 7 shows a higher incidence of detects in potable well samples collected from the Central Sands region (Portage, Waushara, and Adams Counties). Detections were also observed at wells along the Wisconsin River in northern Adams and Juneau Counties and further south in the Lower Wisconsin River Valley in Sauk, Iowa and Richland counties. The hydrogeologic setting for these areas consists of sandy soil with shallow groundwater. Irrigation is also widely used in these areas. Crops grown in the Central Sands and LWRV include potato and vegetable crops in rotation with corn and soybeans. It is likely that routine labelled use of

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products containing neonicotinoid active ingredients on crops grown in this hydrogeologic setting combined with large areas under irrigation contributes to the contaminants detected in potable wells.

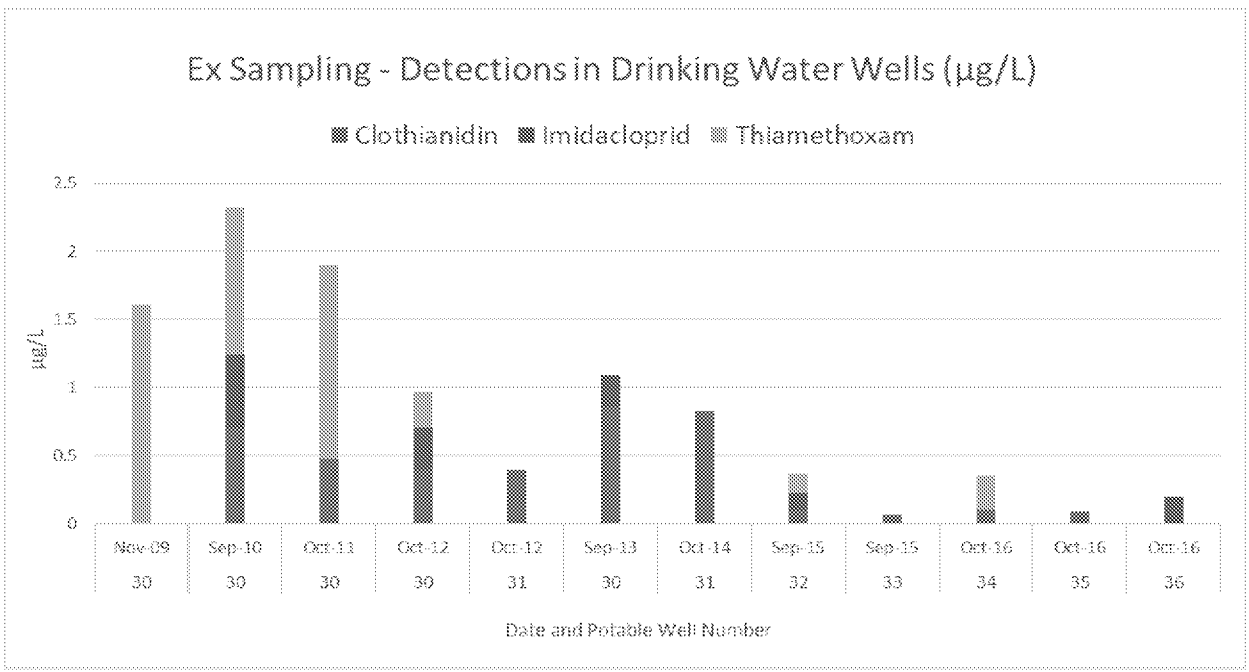
One neonicotinoid detection occurred outside the Central Sands and LWRV areas. Clothianidin was detected at 0.131 µg/L in a sample collected from a potable well in Dodge County. The sample was collected from Well 24 in June 2015. Crops grown on fields near this well are predominantly rotations of corn, soy and forage crops, with soil types within ½-mile consisting of predominantly silt loam (Plano and Markesan) on 0-6-percent slopes (USDA-NRCS, October 2017).

Exceedance Well (Ex) Sampling Program Results

Exceedance wells are private potable wells that have had two or more samples with pesticide contaminants present in excess of an ES during past sampling events. Most wells in the exceedance program are in the program as a result of elevated detections of the compounds atrazine or alachlor, two herbicides that have been used extensively on corn crops statewide. Neonicotinoids are commonly used on corn seed, but no wells are in the exceedance sampling program as a result of past neonicotinoid detects. Through prior testing, this set of wells is known to be susceptible to leaching of contaminants used in agriculture.

Between November 2009 and October 2016, 140 samples were collected from 49 different exceedance wells. Neonicotinoid compounds were detected in 12 samples collected from seven wells, a 14.3 percent detection frequency for wells in this dataset (8.6 percent of samples). Figure 8 shows detected neonicotinoid compounds by well.

FIGURE 8 DETECTIONS OF NEONICOTINOIDS IN POTABLE WELLS--EXCEEDANCE SAMPLING PROGRAM



Concentrations detected were generally less than 1.0 µg/L with the exception of thiamethoxam. Thiamethoxam was present in three samples collected from Well 30 at 1.61 µg/L in November 2009, 1.08 µg/L in September 2010, and at 1.43 µg/L in October 2011. Clothianidin was detected most frequently (six wells, 10 samples) at concentrations ranging from 0.0698 to 0.859 µg/L. Imidacloprid was detected in three

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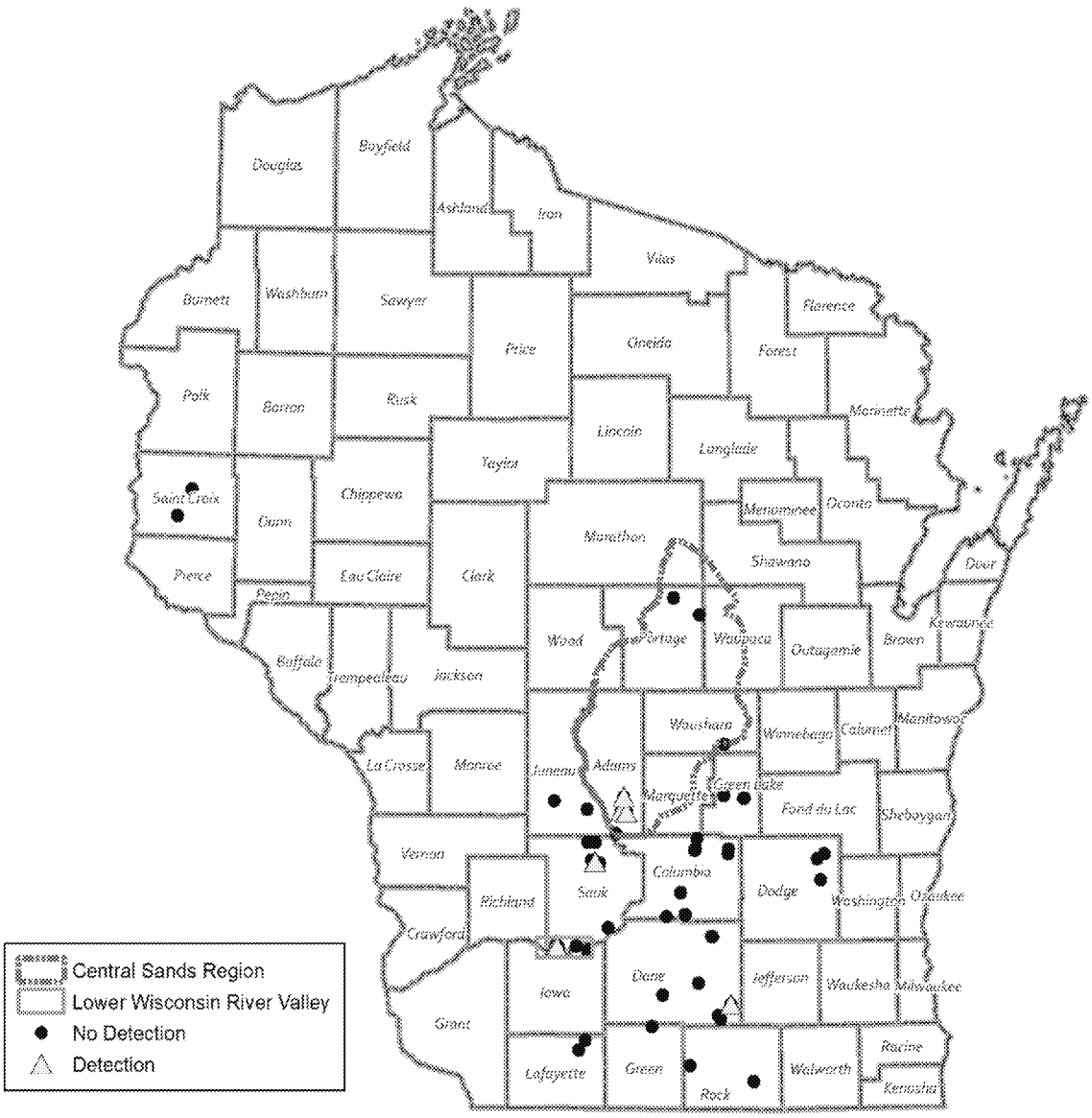
wells (five samples) at concentrations ranging from 0.118 to 0.537 µg/L. Thiamethoxam was detected in five wells (six samples) at concentrations ranging from 0.141 to 1.61 µg/L. All three compounds were detected in three samples (Well 30 in September 2010 and October 2012 and Well 32 in September 2015). The highest total combined concentration for all three compounds was 2.3 µg/L at Well 30 in September 2010.

Figure 9 shows the locations of exceedance wells sampled. Similar to wells sampled under the targeted program, four of the six wells sampled that have detections (yellow triangles) are located in the Central Sands and LWRV. The two remaining wells are located in southeastern Dane County (Well 30) and in north central Sauk County (Well 32).

FIGURE 9 LOCATIONS OF NEONICOTINOID DETECTIONS IN POTABLE WELLS -- EXCEEDANCE SAMPLING PROGRAM



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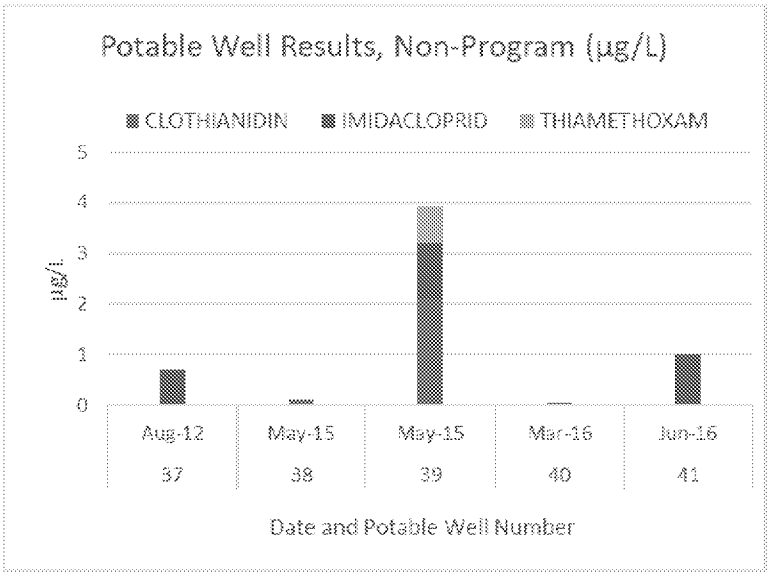
Non-Program Potable Well Sample Results

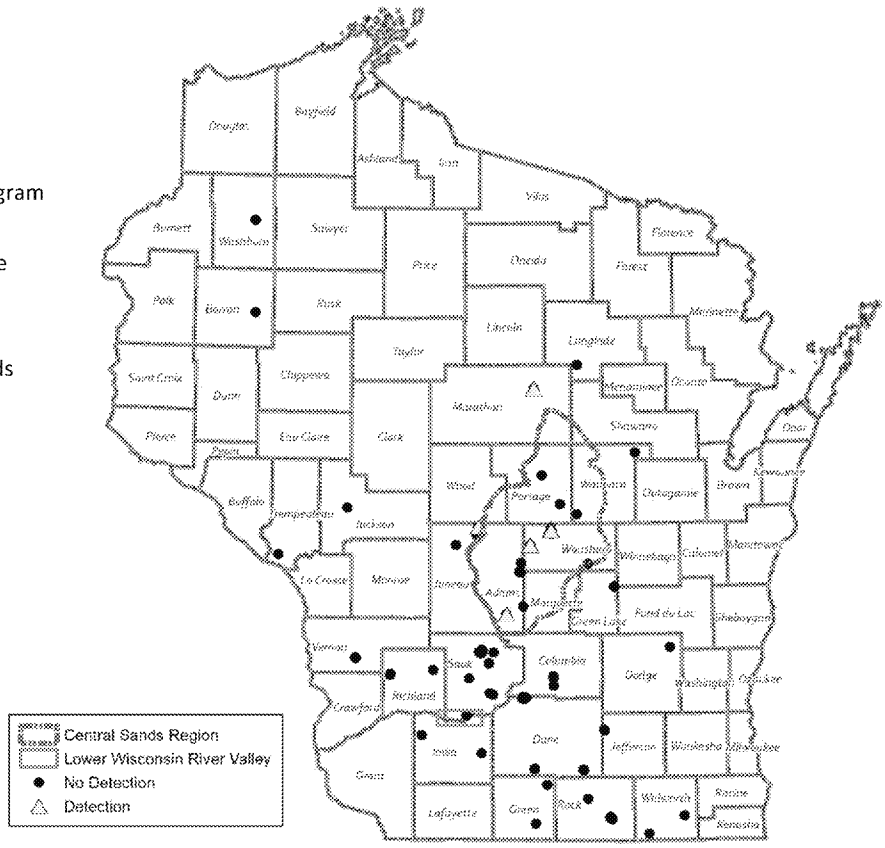
In addition to samples collected for ARM programs shown in Table 3, between April 2011 and September 2016, 107 samples were also collected from 87 potable wells. One or more neonicotinoid compounds were detected in 5 of the 87 non-program wells. Figure 10 includes a graphical presentation of the concentrations of these compounds as well as locations where sampling occurred.

FIGURE 10 DETECTIONS OF NEONICOTINOIDS AND LOCATIONS OF NON-PROGRAM POTABLE WELLS SAMPLED



Left: Out of 87 non-program potable wells sampled over a 10-year period, one or more neonicotinoid compounds were detected in five samples (well numbers 37 through 41).

Right: Locations of 87 non-program potable wells. One or more neonicotinoid compounds were detected at five wells (yellow triangles). Four of the five are located within the Central Sands Region, while one is located in northern Marathon County.



Four out of five of the non-program wells with neonicotinoid detections are located within the Central Sands. Concentrations of individual neonicotinoid compounds detected in these non-program wells was generally less than 1 µg/L. Imidacloprid was detected most frequently in this group of wells (four times) at concentrations ranging from 0.052 to 1.06 µg/L. Clothianidin was detected at two wells at concentrations ranging from 0.112 to 2.13 µg/L, and thiamethoxam was detected at one well at 0.743 µg/L. All three neonicotinoid compounds were detected at a combined concentration of 3.93 µg/L at Well 39 (a well in Waushara County). A well in Marathon County (Well 40) had a very low detection of imidacloprid (0.112 µg/L).

Non-Potable Wells

Field-Edge Monitoring Program

The locations of field edge monitoring sites are shown on Figure 11. Between November 2006 and October 2016, 479 samples were collected from monitoring wells at 32 different field sites around the state.

FIGURE 11 FIELD-EDGE MONITORING WELL LOCATIONS WITH NEONICOTINOID DETECTIONS

Neonicotinoid compounds were detected in samples collected at 17 of the 32 sites. One or more neonicotinoid compounds were detected in 198 samples.

The compound clothianidin was detected in 120 samples collected from 32 monitoring wells at 17 sites. Clothianidin concentrations ranged from 0.0762 to 3.43 µg/L, and the average was 0.540 µg/L. Imidacloprid was detected in 109 samples from 28 monitoring wells at 12 sites. Concentrations of imidacloprid ranged from 0.0594 to 4.54 µg/L, and the average was 0.764 µg/L. Thiamethoxam was detected 129 times from 23 monitoring wells at 15 sites. Thiamethoxam concentrations ranged from 0.0864 to 8.93 µg/L, and the average concentration was 1.28 µg/L. Thiamethoxam was detected at the highest concentration and had the highest average concentration relative to the other compounds.

The field monitoring sites with neonicotinoid detections generally coincided with locations of potable wells that had neonicotinoid detects (Figure 4). These monitoring sites generally include sites located in the Central Sands counties of Adams, Portage and Waushara, and in locations near the Wisconsin River (Juneau, Sauk, Iowa, Dane and Grant Counties). All monitoring well sites having neonicotinoid detections also have coarse grained soils and shallow groundwater. The highest concentration of any neonicotinoid compound detected was thiamethoxam at a concentration of 8.93 µg/L in a sample collected from a monitoring well located at Adams Site 2.

The data from Adams Site 2 and Adams Site 5, are shown in Figure 12. These locations generally had some of the highest concentrations of thiamethoxam detected. Also presented in the figure is Waushara County Site 6, another Central Sands site that had the highest concentration of imidacloprid detected in ARM samples. Crops grown at Adams Sites 2 and 5, and Waushara Site 6 typically includes rotations of high value vegetable crops like sweet corn, snap beans and potatoes. The crop rotation at Waushara Site 6 includes carrots and peas with the other high value vegetable crops.

For comparison to the Central Sands sites, the results of monitoring at three sites located within the LWRV are shown in Figure 13 (Dane, Iowa and Sauk Counties). Sites located outside of the Central Sands and Wisconsin River Valley that had neonicotinoid detects included sites in Langlade and Dunn Counties. These sites also have coarse grained soils and shallow groundwater. At the Langlade site, the neonicotinoids clothianidin and thiamethoxam were detected in three of eight samples collected from one well: clothianidin concentrations ranged from 0.431 to 0.57 µg/L while thiamethoxam ranged from 0.479 to 1.33 µg/L. In Dunn County, two sites had detections. One Dunn County site had detects in four out of eight samples with clothianidin only ranging from 0.076 to 0.12 µg/L. The second Dunn County site had detects of clothianidin and imidacloprid ranging from 0.128 to 0.263 µg/L and 0.069 to 0.297 µg/L respectively.

The Brown County site with detections of neonicotinoids is located in a different geologic setting than the other field monitoring sites with detections. This site has gravelly silt soil overlying dolomite bedrock at about 12 feet. Groundwater is encountered in the bedrock approximately 28 feet below grade, and the monitoring well is 40 feet deep. At this site, thiamethoxam was detected at concentrations of 0.524 µg/L and 0.123 µg/L in two out of eight samples collected. Neither clothianidin nor imidacloprid were detected in any samples here. This site was initially used for a separate study that evaluated the influence of fracture flow infiltration following precipitation events. Researchers reported that fracture flow was evident at the site, with significant changes in water chemistry and temperature occurring in the well about two days following a precipitation event. The main crop grown at this location is corn. It is possible that thiamethoxam

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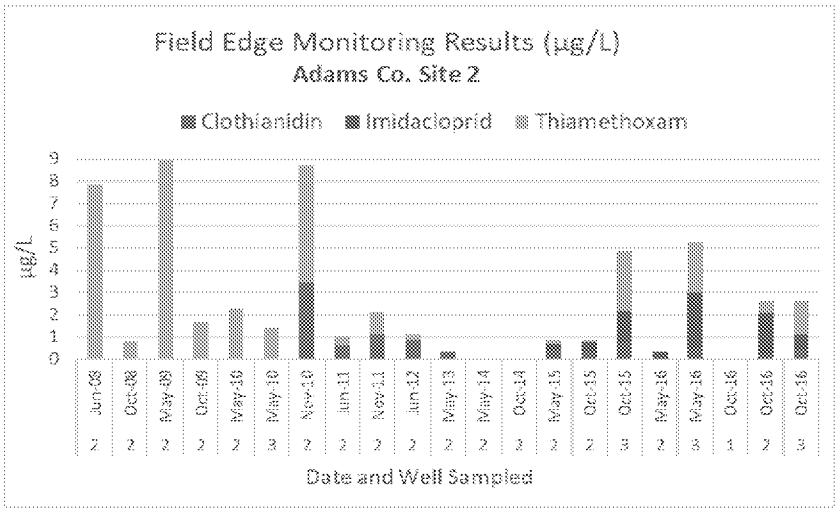
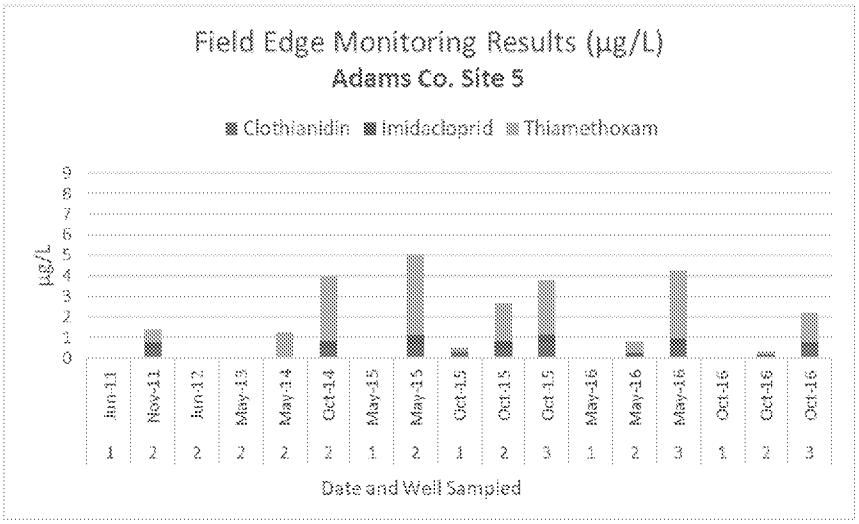
Figure 12 Results from Three Central Sands Monitoring Well Sites

Right: Adams County Site 2, 21 samples were collected from wells 1, 2, and 3 at this site. Samples are typically collected from the shallowest well with groundwater present, but in October 2016, samples were collected from all three wells at this site. Well 2 had the greatest detect of thiamethoxam at 8.93 µg/L in May 2009. Clothianidin and imidacloprid were not analyzed until November 2010. Imidacloprid was first detected in 2015. Crops grown include a rotation of sweet corn, snap beans and potatoes.





Left: Adams County Site 5, 17 samples were collected from wells 1, 2 and 3 between June 2011 and October 2016 at this site. Samples are typically collected from the shallowest well with groundwater present. Note that samples were collected from all three wells in 2016. Similar to Adams Site 2, thiamethoxam is present at significantly greater concentrations than clothianidin or imidacloprid. Crops grown include a rotation of sweet corn, snap beans and potatoes.

Right: Waushara County Site 6, showing results of 20 samples collected from two wells between May 2011 and October 2016. Well 2 had just one detect of imidacloprid in October 2015, while well 1 had detects of imidacloprid in every sample collected, including the highest concentration of imidacloprid detected at any monitoring well site, 4.54 µg/L in October 2014. Crops grown are similar to the rotation of vegetables at the Adams sites, but also includes regular rotations of carrots and peas.

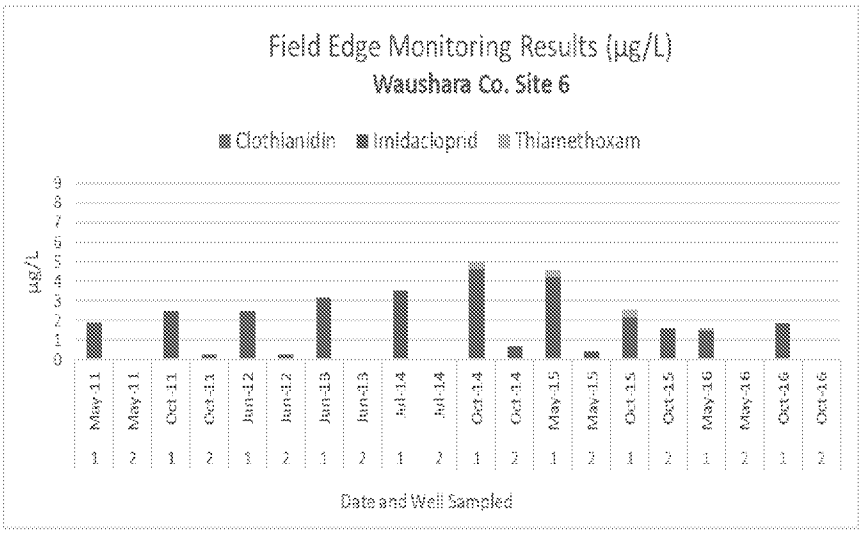


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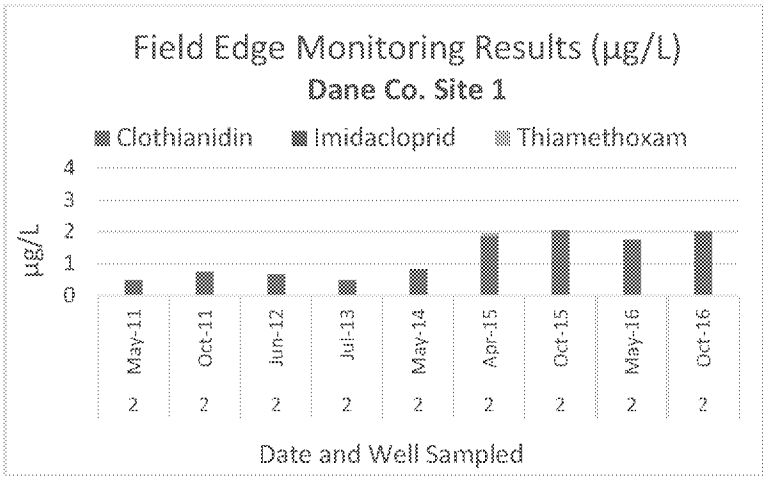
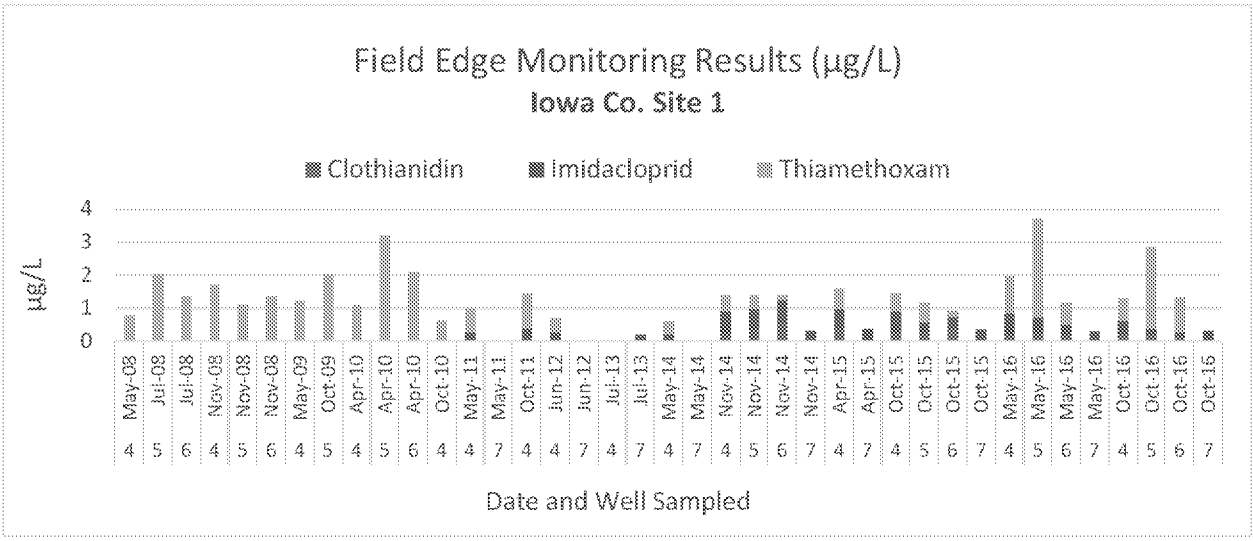
FIGURE 13 RESULTS FROM SELECT DANE, IOWA AND SAUK COUNTY FIELD EDGE SITES (LWRV)

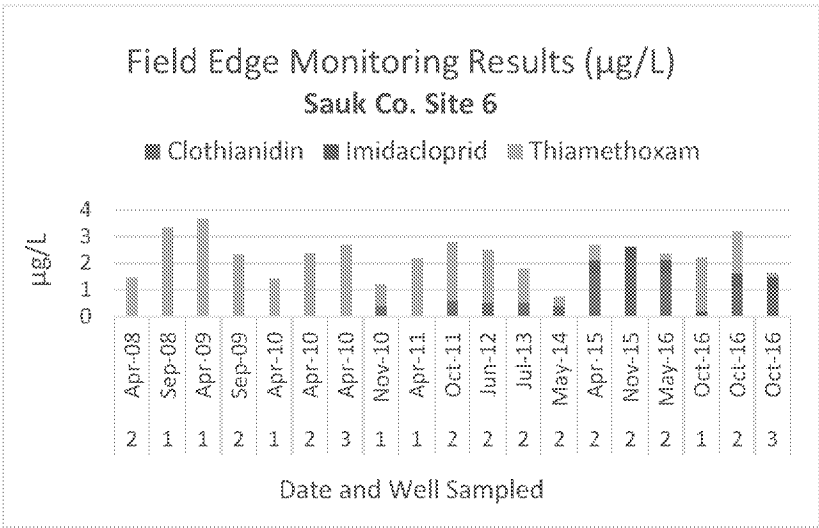


Left: Dane County Site 1, showing results for 9 samples collected from one monitoring well between May 2011 and October 2016. All samples contained clothianidin. The highest concentration was 2.05 µg/L (October 2015) and just one sample (April 2015) had a trace of thiamethoxam detected. This site has coarse soils with shallow groundwater and is equipped with irrigation. The grower reports only corn grown at this site year after year.



Above: Iowa County Site 1 results for 22 samples collected from four wells. Note that this site has four wells, all of which were tested on four separate events. Field is coarse grained, shallow to groundwater, and irrigated. Crops include rotations of potatoes and other vegetables with corn and cover crops.

Right: Sauk County Site 6, showing results for 12 samples collected from three wells. Field is coarse grained, shallow to water, and irrigated. Crops include rotations of potatoes and other vegetables with corn and cover crops.



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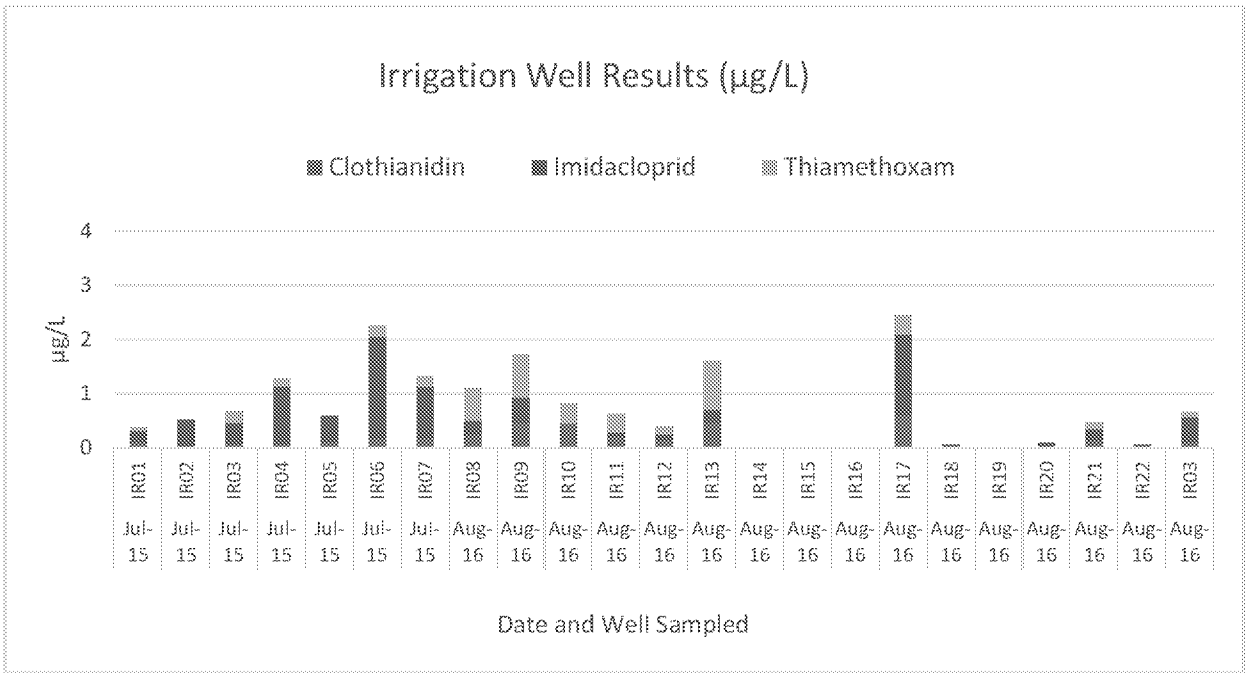
treated corn seed had been planted at the site, but this was not confirmed with the grower.

The monitoring sites in Kewaunee, Manitowoc and Calumet Counties are in geologic settings similar to the Brown County site, but neonicotinoids were not detected in seven samples collected at each of these sites.

Irrigation Well Sample Results

Between 2015 and 2016, ARM collected 23 samples from 22 irrigation wells owned by three cooperating growers within the Central Sands growing area. One or more neonicotinoid compounds were detected in 18 irrigation well samples. The results of testing for neonicotinoids in irrigation wells is shown in Figure 14.

FIGURE 14 NEONICOTINOID DETECTIONS IN IRRIGATION WELL SAMPLES



Clothianidin was detected in 15 samples ranging in concentration from 0.0815 to 0.602 µg/L. The average detection for clothianidin was 0.254 µg/L. Imidacloprid was detected in 18 samples ranging in concentration from 0.0592 to 1.87 µg/L. The average detection for imidacloprid was 0.500 µg/L. Thiamethoxam was detected in 14 samples ranging in concentration from 0.0746 to 0.904 µg/L. The average concentration of thiamethoxam detected was 0.325 µg/L. Generally, the sum of neonicotinoids detected in samples was less than 2 µg/L: only two samples exceeded 2 µg/L.

ARM only recently began sampling irrigation wells, so these results are from a small number of wells, all of which are located within the Central Sands. For comparison, University of Wisconsin-Madison (UW) researchers have published results of testing for neonicotinoids in a much larger set of irrigation wells that included both Central Sands and LWRV wells. Between 2013 and 2015, UW researchers analyzed 289 samples from 92 high capacity irrigation wells for the presence of thiamethoxam. Wells tested were widely located across the Central Sands and the LWRV vegetable growing areas. Each well was tested one to six times over three years. The results showed detections of thiamethoxam over the limit of quantitation (0.5

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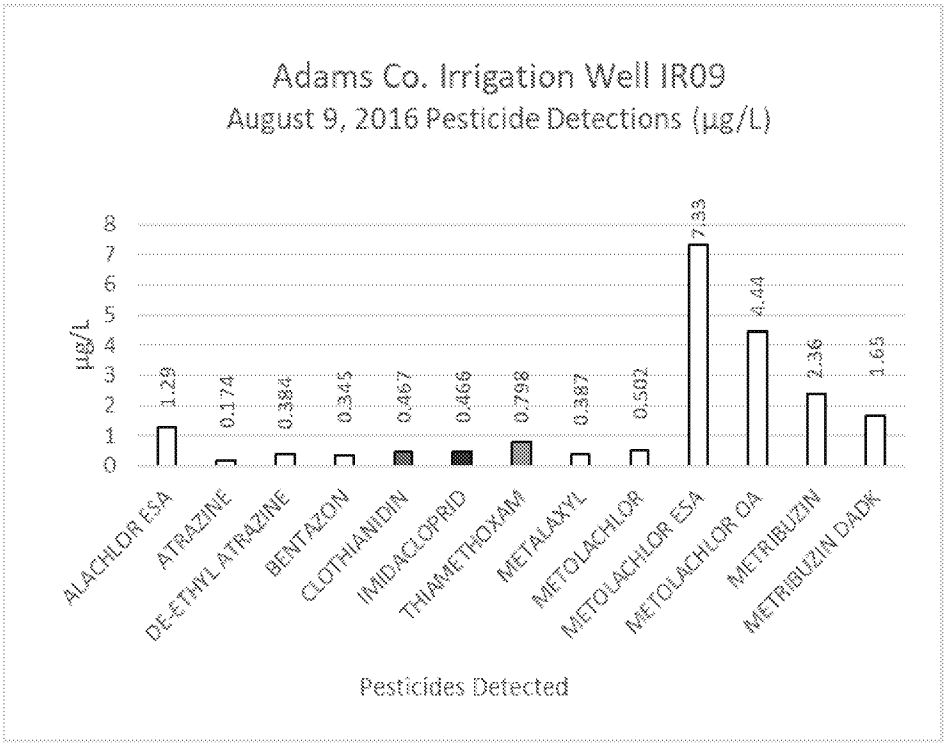
µg/L) in 69 percent of the 92 wells sampled. Six samples exceeded 1.0 µg/L. The concentration of thiamethoxam ranged from non-detect to 1.69 µg/L. The mean concentration detected was 0.291 µg/L thiamethoxam (Groves, Prince, & Bradford, 2017).

Neonicotinoids were not the only pesticide compounds detected in irrigation well samples collected by ARM. Numerous other pesticides were also detected. Figure 15 shows concentrations for thirteen pesticide compounds detected in the August 2016 sample collected from irrigation well IR09. Nitrate was also detected at a concentration of 29.1 milligrams per liter (mg/L) in this sample, but is not shown on the chart.

FIGURE 15 PESTICIDES DETECTED IN A SAMPLE FROM WELL IR09, ADAMS COUNTY



Left: Irrigation wells sampled by ARM typically detected multiple pesticides in addition to clothianidin, imidacloprid and thiamethoxam. The example to the left shows one sample from irrigation well IR09 collected in August 2016. A total of 13 pesticides and pesticide metabolites were detected at concentrations ranging from 0.174 (atrazine) to 7.33 µg/L (metolachlor ESA).

Like the neonicotinoids, many of the compounds detected have no established drinking water standard. For compounds with drinking water standards, the concentrations detected did not exceed their corresponding ES. The herbicidal metabolite metolachlor-ESA (7.33 µg/L) was present at a concentration nearly 10 times greater than the highest neonicotinoid concentration (thiamethoxam, 0.798 µg/L). In addition to pesticide concentrations, the number of pesticides detected in irrigation well samples is also a concern. The presence of numerous pesticide compounds in irrigation well samples indicates that agricultural contaminants are migrating to significant depths within the sand and gravel aquifer, increasing the likelihood that they can also enter nearby private drinking water wells.

Comparison of Groundwater Results across Well Types

Because most detections of neonicotinoids occurred at wells located in similar geologic conditions at areas with similar agricultural practices, the averaged concentrations of neonicotinoid detections for all well types

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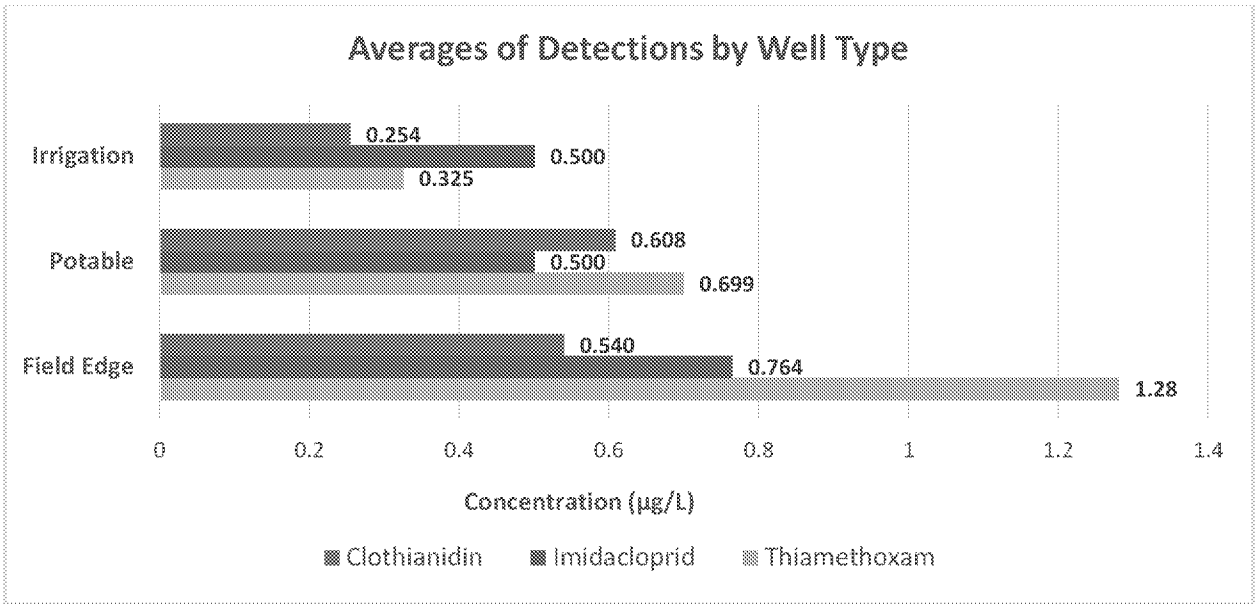
was evaluated. Figure 16 shows a comparison of the average concentrations of neonicotinoid compounds detected at field edge monitoring wells compared to irrigation wells and potable wells sampled. As shown in the figure, the average concentrations of thiamethoxam were significantly higher in field-edge monitoring well samples, intermediate in potable well samples, and lowest in irrigation well samples. Average imidacloprid concentrations were highest in field edge monitoring well samples, and about the same in potable and irrigation well samples. Average clothianidin concentrations were the greatest in potable well samples, followed by field edge monitoring well samples, and lowest in irrigation well samples.

FIGURE 16 AVERAGES OF NEONICOTINOID DETECTIONS BY WELL TYPE



Well location and design likely explains why monitoring wells have the highest average concentrations observed. Field edge monitoring wells are located within or immediately adjacent to fields where pesticides are applied. These test wells are designed to allow samples to be collected from the uppermost few feet of the aquifer. This is not the case with potable wells. Potable wells may be located near a cropped field, but they are not within a field of application. Potable wells may be shallow or deep, but are typically designed to draw water from deeper reaches of an aquifer. With consideration to these differences, it is reasonable to conclude that if a pesticide is present, it would likely be observed and at higher concentrations at a shallow field edge monitoring well, and at lower concentrations at deeper potable wells. Irrigation wells, like monitoring wells, are also often located within or adjacent to fields of application. To achieve sustained high volume water withdrawals, irrigation wells are constructed with large diameter casing and installed deeper with well screens open to longer intervals of an aquifer. Considering this design, pumping an irrigation well is likely to induce significant dilution in the well as clean water is drawn from depth and mixes with contaminants that are more likely to enter the well from shallower depths of the aquifer. This helps explain why irrigation wells had the lowest average concentrations observed.

It is unknown why a higher average clothianidin concentration was observed for deeper potable wells rather than at shallow field edge monitoring wells. Migration of pesticides to deeper portions of the aquifer is concerning, and deserves some discussion. Additional research and monitoring is likely needed to explain why. Based in limited data, two possible explanations are discussed below.

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First, the average clothianidin concentration detected in potable wells (0.608 µg/L) is only slightly higher than the average detected in field edge monitoring wells (0.54 µg/L). This could be the result of an outlier. If one sample has a concentration that greatly exceeded others in the dataset, the result could be questioned. One potable well (Well 07, 3.88 µg/L, Figure 5) did, in fact, have an observed detection of clothianidin that was significantly higher than other detects observed at the remaining potable wells. By removing this single high detection of clothianidin from the potable well dataset, the average clothianidin concentration drops to 0.486 µg/L, a concentration much closer to other detected concentrations. However, there is no valid reason for discarding this data point, as a second sample collected from the same well (two years later) revealed yet another high detection of clothianidin along with similar ratios of the other neonicotinoids previously observed. Furthermore, irrigation wells *and* monitoring wells in the area near potable Well 07 identified similar detections of all three neonicotinoids.

A second explanation for higher average clothianidin concentrations at potable wells rather than at shallow monitoring wells may involve chemical degradation. The physiochemical characteristics of these compounds suggests that, once in groundwater, these compounds degrade more slowly and can migrate significant distances in highly permeable sand and gravel aquifers, especially with repeated field use. The Environmental Protection Agency (EPA) reports that clothianidin was observed as a significant component of thiamethoxam degradation. From fate studies, they suggest that thiamethoxam degradation could result in as much as 13 percent of clothianidin observed in the subsurface (U.S. EPA, January 5, 2017). Therefore, it is possible that degradation of thiamethoxam that occurs along the groundwater flow path may result in elevated clothianidin concentrations at wells away from fields of application. This explanation seems reasonable because both clothianidin and thiamethoxam were detected in many shallow monitoring wells near fields of application. The average concentration of thiamethoxam in field edge monitoring wells was significantly higher than the clothianidin average, and the physiochemical characteristics of both compounds suggests they both migrate and persist in subsurface soils and groundwater. Additionally, thiamethoxam degradation has (in part) been shown to result in clothianidin formation; and thiamethoxam degradation is likely to occur with greater time and migration distance from the application site. The most likely explanation for the higher average clothianidin at potable wells is likely more complicated and would require a more detailed study to verify conditions observed.

Surface Water Monitoring Results

Between March 2011 and December 2016, ARM collaborated with DNR to obtain surface water samples from 34 streams that were being sampled under other DNR sampling programs. A total of 430 surface water samples were collected and analyzed for pesticides. Streams were generally sampled six or more times, with samples collected monthly from spring through fall. Some streams were sampled two or more years.

Figure 17 shows 34 streams sample locations as well as locations of neonicotinoids detections. The neonicotinoid compound imidacloprid was detected in samples from two streams, while thiamethoxam was detected in samples from four streams. Most detections occurred in samples collected from just two streams, Tenmile Creek in Portage County (two sampling stations) and Carter Creek in Adams County. Both of these streams are located within the Central Sands Region. A third stream located in the Central Sands, Little-Roche-Cri Creek was sampled eight times in 2011, but had no neonicotinoid detections.

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FIGURE 17 STREAM SAMPLING LOCATIONS WITH NEONICOTINOID DETECTIONS

Above: Between March 2011 and December 2016, DATCP analyzed a total of 430 surface water samples from 34 streams for pesticides. Each stream was sampled six or more times, and some streams were sampled at more than one location.

Two streams not located within the Central Sands, the Milwaukee River (33 samples) and the Neenah Slough (eight samples), each had one detection of the compound thiamethoxam. Figure 18 shows the dates and concentrations of neonicotinoid compounds detected in samples from Tenmile and Carter Creeks. The compound clothianidin was not detected in any stream samples.

Implications for Important Receptors

There are a number of important receptors that could receive inadvertent exposures to the neonicotinoids detected during DATCP groundwater and surface water sampling efforts. Below is a discussion of potential

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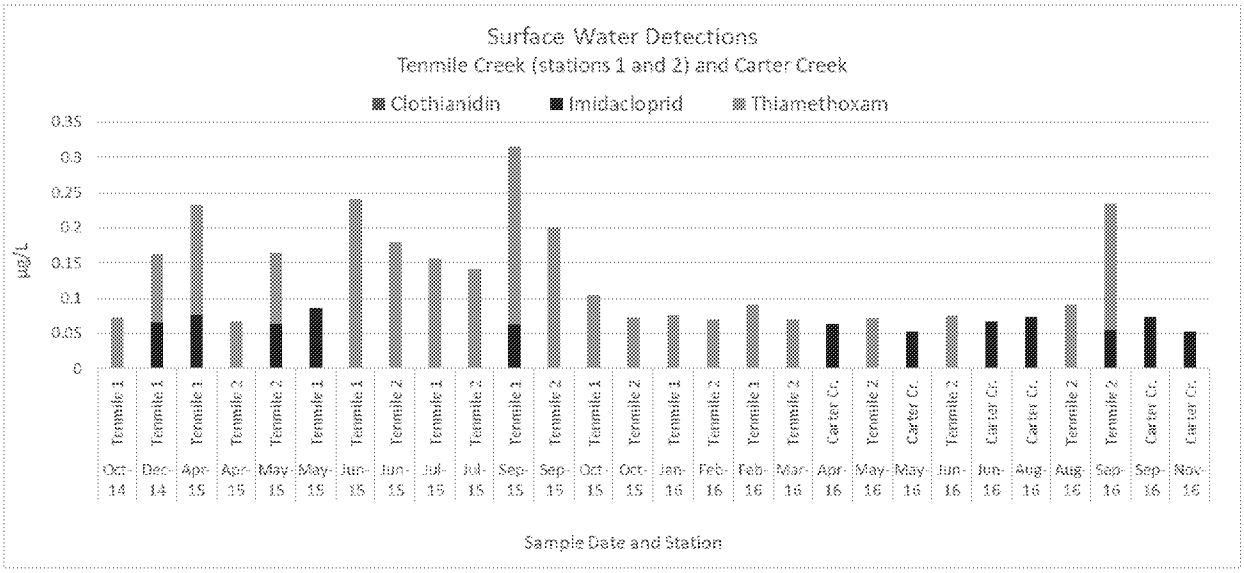
FIGURE 18 NEONICOTINOID DETECTIONS IN TWO STREAMS IN WISCONSIN'S CENTRAL SANDS REGION



exposure pathways for humans through drinking water, potential exposure pathways for target and non-target insects and pollinators through contaminated groundwater, and potential surface water exposure pathways for aquatic invertebrates and other organisms.

Risks for Human Consumption of Drinking Water

National Primary Drinking Water Standards (e.g., maximum contaminant levels-MCLs) or state drinking water standards have not been established for clothianidin, imidacloprid or thiamethoxam. Considering the number of detections observed by ARM in private potable water wells and the extensive use of neonicotinoids in agriculture, DATCP has requested the Department of Health Services (DHS) and DNR review these compounds for new standards development under chapter 160, Wis. Stats. In the event these agencies determine standards are warranted, DNR will draft administrative rules to promulgate new standards. Once the decision is made to set standards by administrative rule, it will take two to three years to develop and finalize the standards through Wisconsin's administrative rulemaking process.

In the absence of specific state drinking water standards or federal MCLs, EPA publishes a list of Human Health Benchmarks for Pesticides (HHBPs). HHBPs are not in themselves enforceable as numeric standards. They are developed based on an assumed lifetime exposure scenario to assist in considering whether the detection of a pesticide in drinking water may pose a significant human health risk (U.S. EPA, January 2017). Detected concentrations of neonicotinoids in potable water samples are below the chronic HHBP values derived by EPA for clothianidin (630 µg/L), imidacloprid (360 µg/L), and thiamethoxam (77 µg/L).

With regard to consumptive exposures to neonicotinoids and other pesticides in drinking water, there is added uncertainty related to possible combined effects that may result from exposures to multiple pesticide contaminants and elevated nitrate in drinking water. The DATCP data show that, in agricultural areas, wells often contain detectable concentrations of several pesticides and high nitrate. Figure 19 shows all pesticides detected in samples collected from two potable wells during ARM exceedance well sampling efforts.

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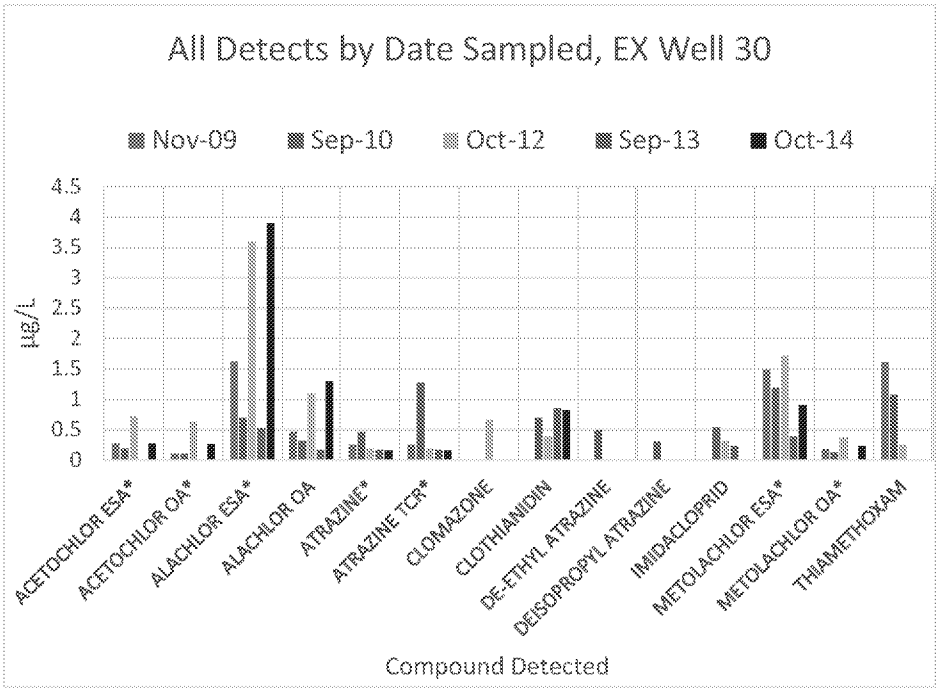
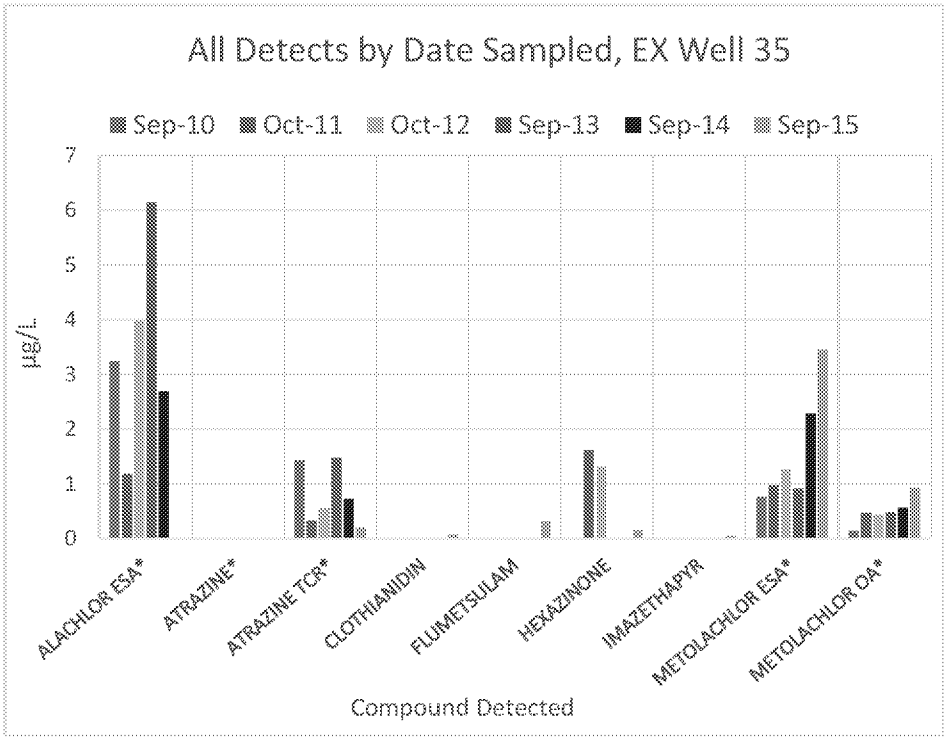
FIGURE 19 ALL PESTICIDE COMPOUNDS DETECTED ON MULTIPLE SAMPLE DATES -- POTABLE WELLS 30 AND 35



Left: Range of pesticide concentrations detected at Exceedance (EX) Well 30 (southern Dane County) between November 2009 and October 2014. Between 7 and 12 pesticides were detected in each sampling event. There were no ES exceedances for any pesticides detected. Only those compounds with an asterisk (*) have a published ES. In addition to pesticides detected, all samples exceeded the NR 140 ES of 10 mg/L for nitrate. Nitrate ranged from 15.4 mg/L to 38.2 mg/L, with an average concentration of 22.6 mg/L.

Right: Concentrations of eight pesticide compounds detected at EX Well 35 (Sauk County, LWRV) between September 2010 and September 2015. Four to seven pesticides were detected during each sampling event, plus nitrate. There were no ES exceedances for any pesticides detected. Only those compounds with an asterisk (*) have a published ES. Nitrate concentrations ranged from 6.76 to 16.1 mg/L, with an average concentration of 12.4 mg/L.



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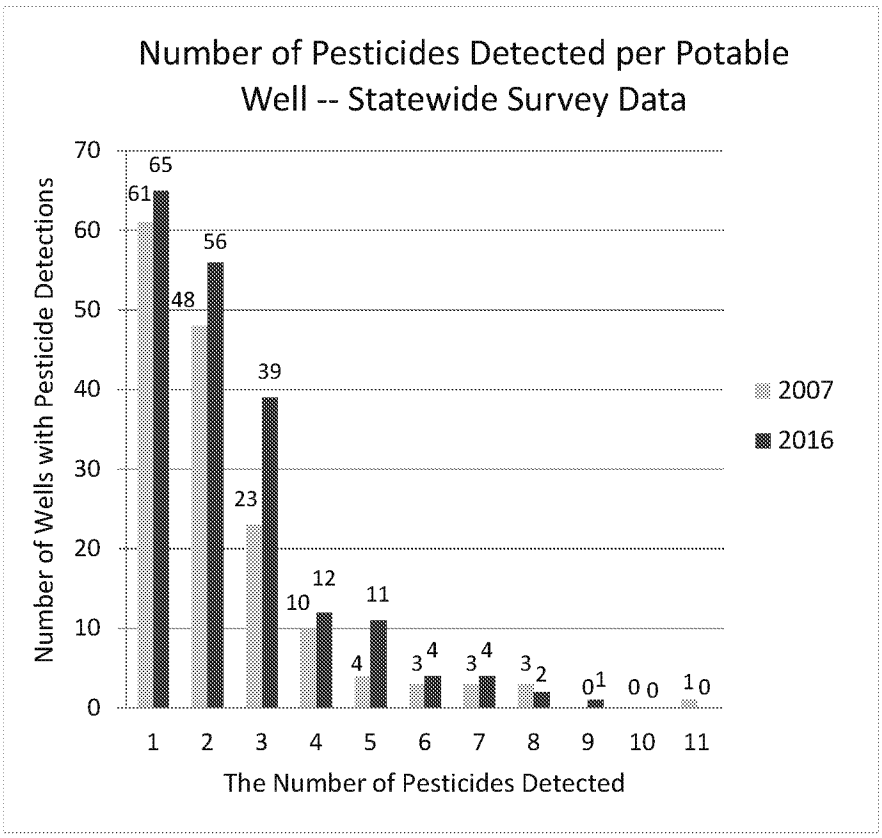
At a southern Dane County well, five samples revealed high nitrate and between seven to 12 pesticide compounds in each sample. At a Sauk County well that was sampled six times, high nitrate and between four to seven pesticides were detected in each sample. The concentrations of individual pesticides detected at these private wells is low, but these results suggest that multiple pesticides and nitrate are often present in a mixture or "cocktail". Standards and benchmarks may exist for individual compounds, but there is currently a lack of research on additive or synergistic effects when these conditions exist in drinking water.

The presence of multiple pesticides in groundwater detected at both potable private wells and irrigation wells is a significant concern. Unfortunately, random sampling surveys of private wells conducted by ARM suggests that the number of pesticides detected in potable wells is increasing. To help illustrate this, Figure 20 shows a comparison of the numbers of wells having multiple pesticide detections in the 2007 and 2016 Statewide Surveys. After adjusting for differences in laboratory detection limits and reporting limits, this figure shows that the number of wells with more than two, three, four, five, six and seven different pesticides increased between 2007 and 2016.

FIGURE 20 PESTICIDE DETECTIONS PER WELL IN STATEWIDE SURVEYS



Left: The number of pesticides detected per well generally increased between the 2007 and 2016 surveys.

DATCP identified all wells in the 2007 and 2016 surveys with a single pesticide detection. Next the number of wells with two pesticides were identified, then three, then four, *etc.*, until all wells with multiple detected pesticides are included. To eliminate bias in comparison, only pesticides that were included on the analytical list for both the 2007 and 2016 surveys were included for this evaluation. The data were also adjusted for differences in detection and reporting limits between the two surveys.

Risks for Target and Non-target Insects and Pollinators

Direct exposures to bees and other non-target insects can occur when neonicotinoids are applied as foliar sprays when bees are foraging. Significant bee kills resulting from exposures to pesticide dust abraded from treated seed during bulk transfers in the field have been documented (Pistorius J, Bischoff G, Heimback U,

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and Stahler M, 2009). In recent years, the number of studies that look at the effects of low-dose exposures to bees and non-target organisms has increased tremendously. It is estimated that more than half of all research published on neonicotinoids since 1995 has occurred from 2013 to 2016 (Wood & Goulson, 2017). The following paragraphs highlight recent research and data collected specifically within the Central Sands region. These studies help illustrate concerns over the presence of low level concentrations of neonicotinoids like those detected in samples collected during ARM groundwater and surface water sampling efforts.

A study performed by UW researchers in the Central Sands (Huseth, Lindholm, Groves, & Groves, 2014) determined that applications of low concentrations of neonicotinoids may be a significant concern for target insects that are repeatedly exposed to low concentrations. The study focused on insecticide resistance development in Colorado potato beetle (*Leptinotarsa decemlineata*) populations resulting from different types of soil-applied applications of imidacloprid and thiamethoxam on potatoes. Researchers measured insecticide concentrations in plant tissues over time and monitored beetle populations for two-years following applications. They found that regardless of soil application method or neonicotinoid used, the insecticides lost efficacy over time, but were still detectible in plant tissues at sub-lethal concentrations long after being applied. An important conclusion from the study was that chronic exposure to soil-applied systemic insecticides resulting from in-furrow, treated seed, and side dress application methods may accelerate selection for resistance in insect pests in potatoes.

Another study by UW researchers (Huseth & Groves, 2014) looked closely at leaching of soil-applied thiamethoxam on irrigated Central Sands potato plots over two years. Researchers used pan lysimeters to collect leachate beneath a control plot, three plots that received different soil-applied treatments, and one plot that received a foliar treatment of thiamethoxam. Samples were collected from lysimeters throughout the growing season each year, including after vine-kill and harvest. Samples were analyzed and leaching of thiamethoxam was documented at all plots ranging from trace concentrations within the control plots to samples containing as high as 20 µg/L (Figure 21). Interestingly, concentrations in lysimeter samples were highest in samples collected late in the season after the potato vines were killed to prepare the crop for harvest. To evaluate why there were low concentrations of thiamethoxam detected in the control plots, the researchers collected water samples directly from the operating irrigation systems. Testing revealed low concentrations of thiamethoxam being unintentionally applied to the crop through irrigation water. This study points to the need for additional research to help determine the effects related to exposures of non-target species of arthropods to low concentrations of thiamethoxam via contaminated groundwater used as irrigation water. It suggests that area-wide applications of low concentrations in irrigation water may have unanticipated effects on non-target organisms through repeated low-dose exposures to insecticides (Huseth & Groves, 2014). The study further highlights the potential for leaching of thiamethoxam residues from treated and decaying plant tissues and suggests the need for studies over the final disposition and use of residues of crops treated with thiamethoxam and other neonicotinoids.

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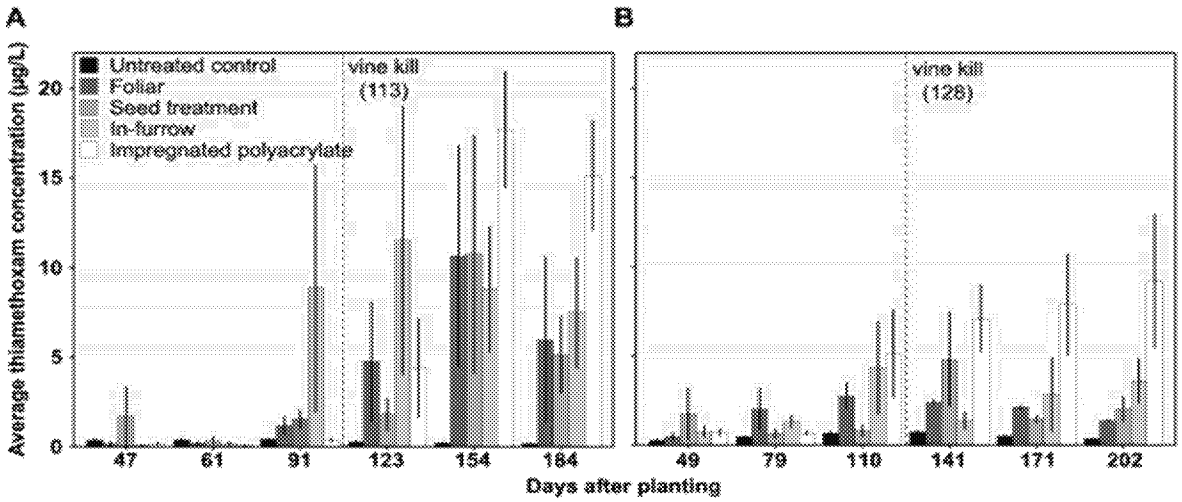
FIGURE 21 THIAMETHOXAM CONCENTRATIONS IN LYSIMETER SAMPLES BENEATH POTATO (HUSETH & GROVES, 2014)



Above: Huseth and Groves studied thiamethoxam concentrations in leachate from potato. Average thiamethoxam recovered from in-furrow and foliar treatments in (A) 2011 and (B) 2012 (error bars show range in +/- standard deviations). Dotted lines indicate the date that the producer applied vine desiccant prior to harvest. Lysimeter studies continued in undisturbed soil following vine kill. doi:10.1371/journal.pone.0097081.g002

Another study by UW researchers in the Central Sands region examined whether flowering vegetable crops in the Central Sands represented a significant foraging source for honeybees and wild bees, and looked to quantify thiamethoxam concentrations in tissues and floral structures of treated plants for comparison to a proposed EPA threshold of 5 parts per billion (ppb) for floral structures (Prince & Groves, 2016). Researchers identified captures of both honeybees (Apis mellifera) and wild bee species in fields planted to sweet corn, snap beans and green peas throughout the growing season. A total of 53 fields and 13 field edges were included during the peak bee foraging months of June through August, representing about 6,000 acres. Within cropped fields, 60 percent of bees captured were honeybees and 40 percent were wild bees. In field edges, 15 percent of captures were honeybees and 85 percent were wild bees, representing 39 different species. Lasioglossum was the most prevalent wild genus, comprising mostly solitary and soil-nesting species. The diversity of bee species was comparable between fields and field edges across all three crop types, though bee abundance was consistently higher in field edges. Thiamethoxam concentrations were measured in plant foliage and flowers over time, as a result of applications made via treated seed at time of planting. Thiamethoxam was detected in crop leaves at concentrations that effectively control and repel pest insects, but were not detected in floral structures above the 30 ppb detection limit. Preliminary conclusions from the study indicate that vegetable processing crops in Central Wisconsin may provide foraging habitat for both honey and wild bees, and that average concentrations of thiamethoxam in tested floral structures of treated crops were not detected at or above 30 ppb, the study detection limit. Unfortunately, due to the elevated detection level, researchers were unable to evaluate whether the 5 ppb proposed EPA threshold for floral structures might be exceeded (Prince & Groves, 2016). Regardless, the study showed that honeybees and wild bee species do forage on significant areas of vegetable crops and on adjacent field-edge areas in the Central Sands where neonicotinoids are used.

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Other than honeybees and wild bees, studies have identified endangered and threatened species of pollinators in Central Sands and/or LWRV areas where neonicotinoids are used and where irrigation water has been documented to have concentrations of neonicotinoids present. The application of irrigation water contaminated with neonicotinoids increases the potential for unintended exposures of these pollinators to these compounds. A list of pollinators whose populations are low enough to be classified as endangered, threatened or of special concern in Wisconsin is provided in Table 4. The list includes the likelihood that these pollinators could frequent agricultural areas based on their foraging habits and proximity of known habitat areas to agricultural fields (WDNR, January 5, 2017).

TABLE 4: ENDANGERED, THREATENED AND SPECIAL CONCERN POLLINATORS IN WISCONSIN

Scientific Name	Common Name	Species Group	Federal Status*	WI Status**	Could be found Adjacent to Agriculture
Bombus affinis	Rusty-patched Bumble Bee	Bee	LE	SC/FL	Yes (historically statewide)
Bombus terricola	Yellowbanded Bumble Bee	Bee	SOC	SC/N	Yes (historically central through north)
Calephelis muticum	Swamp Metalmark	Butterfly	SOC	END	No
Danaus plexippus	Monarch	Butterfly	SOC		Yes statewide
Hesperia ottoe	Ottoe Skipper	Butterfly		END	Probably not
Lycaeides idas	Northern Blue	Butterfly		END	No
Lycaeides melissa samuelis	Karner Blue	Butterfly	LE	SC/FL	Possibly
Oarisma poweshiek	Poweshiek Skipperling	Butterfly	LE	END	No
Speyeria idalia	Regal Fritillary	Butterfly	SOC	END	Possibly
Papaipema silphii	Silphium Borer Moth	Moth		END	Probably not
Schinia indiana	Phlox Moth	Moth		END	Probably not

* LE=Federal listed endangered. SOC=Federal listed species of concern.
**SC/FL=Federally protected as endangered or threatened, but not so designated by DNR. SC/N=Species of special concern, no laws regulating use, possession, or harvesting. END=State listed endangered.

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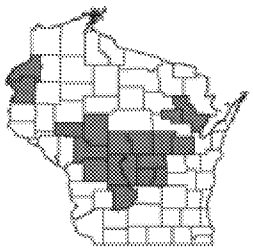
Figure 22 shows four species from Table 4 whose known habitat areas overlap or are adjacent to the Central Sands and LWRV growing areas. Maps for the Monarch butterfly and Yellow Banded Bumblebee are not

FIGURE 22 DOCUMENTED HABITAT AREAS OF FOUR ENDANGERED/SPECIAL CONCERN POLLINATOR SPECIES

Karner Blue Butterfly

Summary Information	
State Status	SC/FL
Federal Status in Wisconsin	LE

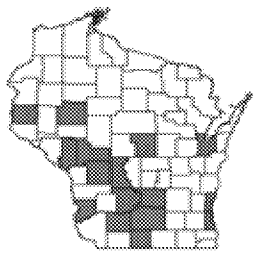


Left: DNR reports show the Karner Blue butterfly range in the central part of the State. The range overlaps the Central Sands region and includes the LWRV vegetable growing area in the State.

Regal Fritillary

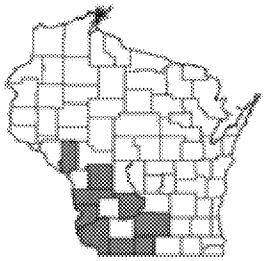
Right: The range of the Regal Fritillary overlaps the LWRV growing area.

Summary Information	
State Status	END
Federal Status in Wisconsin	SOC



Ottoe Skipper

Summary Information	
State Status	END
Federal Status in Wisconsin	none

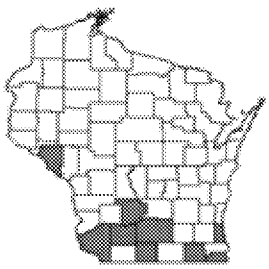


Left: The Ottoe Skipper has been identified in counties that include the LWRV vegetable growing area.

Rusty Patched Bumblebee

Right: The Rusty Patched Bumblebee is a special concern species in Wisconsin (federally endangered) with sitings near the LWRV vegetable growing area.

Summary Information	
State Status	SC/FL
Federal Status in Wisconsin	LE



Source: (WDNR, January 5, 2017) http://dnr.wi.gov/topic/EndangeredResources/ETList.html

LE-federal listed endangered. SOC-federally listed species of concern. SC/FL-federally protected as endangered or threatened, but not so designated by DNR. SC/N-species of special concern, no laws regulating use, possession, or harvesting. END-state listed endangered.

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included in Figure 20, but their historic ranges include central sands counties and other areas having significant cropland. The habitat areas for these species increases the likelihood for possible interactions with crops treated with neonicotinoids. Research is needed to document any such interaction, as well as the potential for exposures and any resulting effects. Neonicotinoids found in groundwater sprayed by irrigation wells creates an added potential for chronic exposures to pollinators foraging in areas where neonicotinoids are unintentionally applied through irrigation water.

Risks for Aquatic Invertebrates

The Central Sands region is a unique geologic area of the state. With nearly level topography and unconsolidated sandy deposits often greater than 30 meters thick, infiltration rates are high and there is very little runoff (Stites & Kraft, 2000). Most areas are extremely well drained and groundwater recharge is high (USDA-Soil Conservation Service, 1980). Groundwater is intimately connected to surface waters, with base flow representing upwards of 90 percent of annual streamflow of headwater streams (Weeks, Ericson, & Holt, 1965). Contaminants that persist and leach to groundwater and potable wells in the region are likely to also be detected in streams and other surface water bodies.

Out of 33 streams sampled by DNR and ARM, two streams, Tenmile Creek and Carter Creek are located within the Central Sands vegetable growing area. Samples from each stream had multiple detects of the neonicotinoid compounds imidacloprid and thiamethoxam. The highest detections occurred during the months of June through September, but detections were also noted during the winter months from November through March, when concentrations detected are more likely a result of contaminated groundwater that is discharging into the streams as base flow. Clothianidin was not detected in samples from either stream.

For imidacloprid, EPA lists the aquatic life benchmarks for invertebrates in freshwater at 0.385 µg/L (acute) and 0.01 µg/L (chronic). For thiamethoxam, EPA lists only an acute benchmark for invertebrates at 17.5 µg/L (U.S. EPA OPP, November 2017). With specific regard to total neonicotinoids present in surface water environments, a recent and growing body of research is forming on the acute and chronic effects of neonicotinoids on aquatic invertebrates. Several researchers recently reviewed some of the most recent aquatic toxicity studies performed in surface waters from 29 studies in nine countries worldwide along with published data on their acute and chronic toxicities to 49 species of aquatic insects and crustaceans, including 12 invertebrate orders (Morrissey, et al., 2015). These reviewers recommended that in order to avoid lasting effects on aquatic invertebrate communities, ecological thresholds for cumulative concentrations of neonicotinoids needs to remain below 0.2 µg/L for short-term acute, or 0.035 µg/L for long-term chronic exposures. Figure 23 shows these recommended cumulative thresholds relative to the results of samples from Tenmile and Carter Creeks.

The figure shows that the recommended acute threshold was reached or exceeded in five samples collected from Tenmile Creek. The recommended chronic threshold was exceeded in all samples for which there were detections, in both streams. It is important to note that the 0.035 µg/L recommended chronic threshold for cumulative neonicotinoids is less than the BLS reporting limits of 0.05 µg/L for imidacloprid and 0.067 µg/L for both clothianidin and thiamethoxam.

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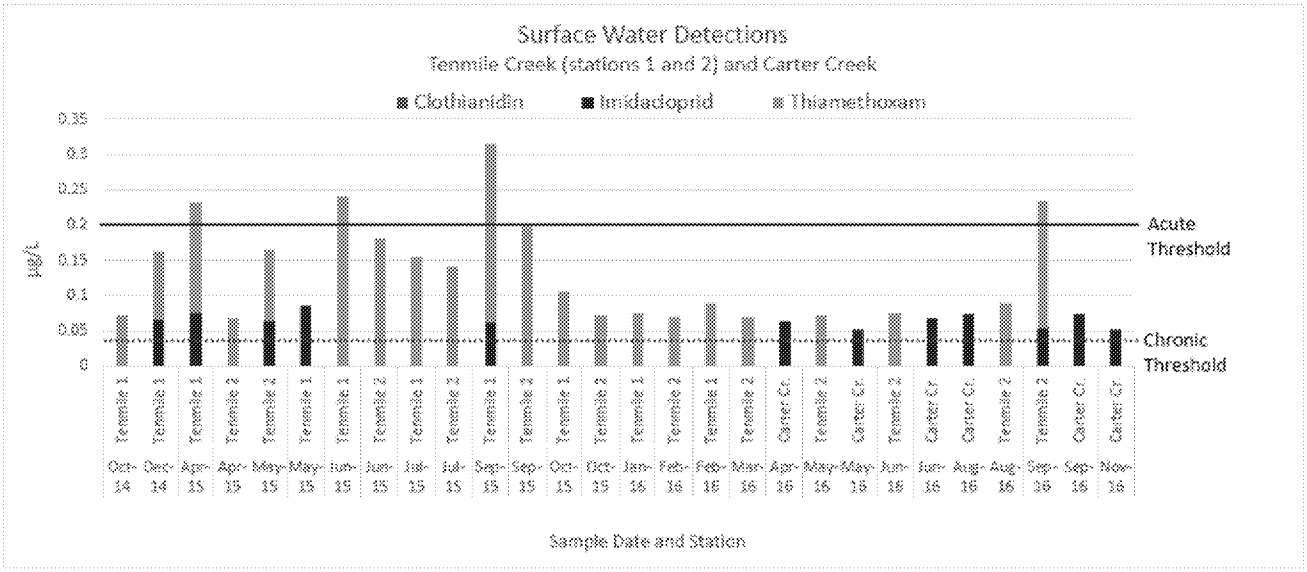
FIGURE 23 SURFACE WATER RESULTS WITH RECOMMENDED ACUTE AND CHRONIC THRESHOLDS, AFTER MORRISSEY ET AL.



As with the groundwater samples collected, the surface water samples collected from Tenmile and Carter Creeks also had multiple pesticides detected. Figures 24 and 25 illustrate the total number of pesticide compounds and frequency of detections of these compounds in these streams. Figure 24 shows concentrations of all pesticides detected in 10 samples collected during consecutive months in 2014 from Tenmile Creek. Figure 25 shows concentrations of all pesticides detected in 10 samples collected during consecutive months in 2016 from Carter Creek. Samples from Tenmile Creek had one to seven pesticides detected, with an average of about five pesticides per sample. Samples from Carter Creek had six to 15 compounds detected with an average of 13 pesticides detected per sample. Similar to the groundwater samples collected from wells in the Central Sands, most detects of individual compounds were less than 3 µg/L, and none of the pesticides detected in stream samples exceeded state ESs.

Although there is a growing body of research worldwide on the effects of neonicotinoids in aquatic communities, additional research is needed locally on these and other streams in the Central Sands where neonicotinoids are being detected in shallow groundwater that makes up a large percentage of stream base flow. ARM and DNR sampling to date has been primarily opportunistic and exploratory in nature, limited to monthly grab sampling, which tends to miss acute episodic concentrations of chemicals associated with timing of pesticide applications and flushes of agricultural chemicals via precipitation and runoff. ARM plans to continue providing DNR with analytical services for surface water bodies that they sample, but more comprehensive studies are needed, and on more streams in the Central Sands and in other areas of the state. Studies need to not only look at the presence of neonicotinoids, but also at the effects they have on aquatic life in these and other streams in the Central Sands area, including possible combined effects that may result from the numerous pesticide compounds present.

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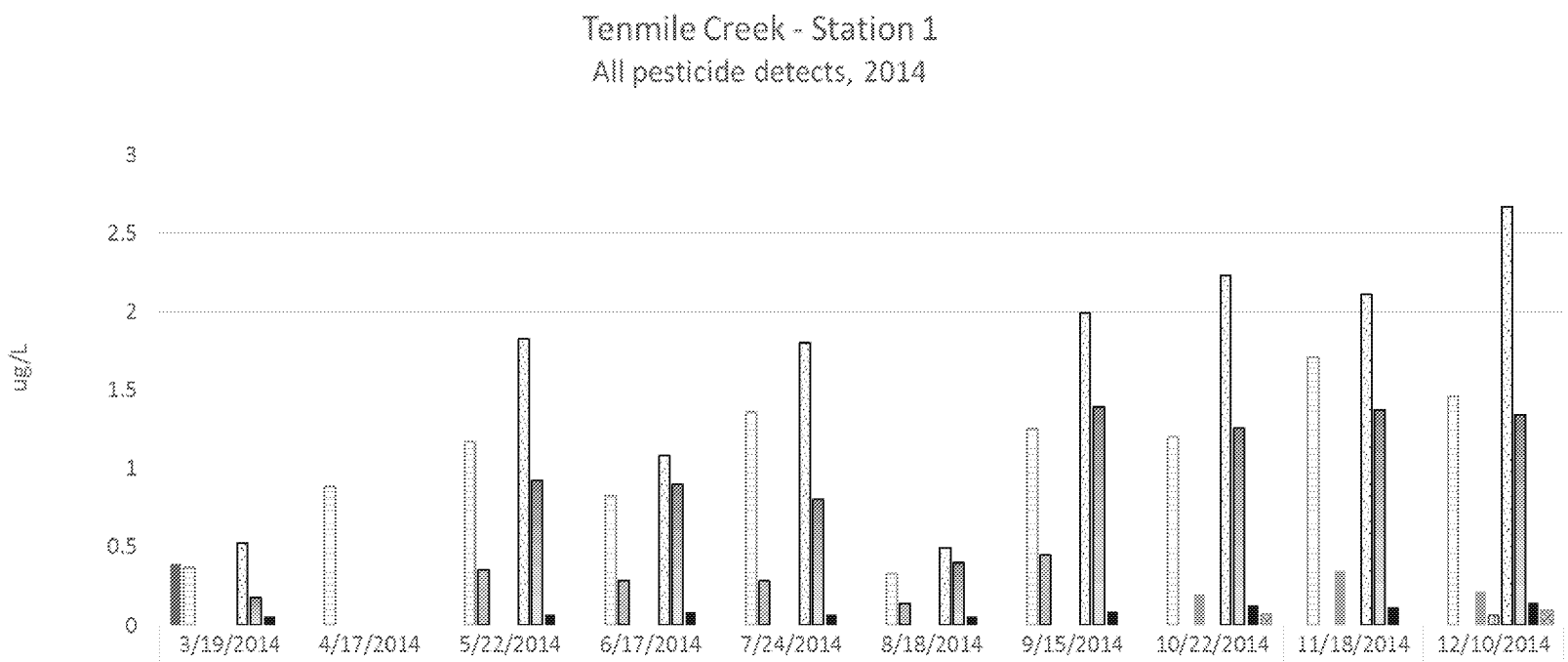
FIGURE 24 ALL PESTICIDE DETECTIONS IN TENMILE CREEK AT STATION 1 IN 2014



	3/19/2014	4/17/2014	5/22/2014	6/17/2014	7/24/2014	8/18/2014	9/15/2014	10/22/2014	11/18/2014	12/10/2014
Acetochlor ESA	0.396									
Alachlor ESA	0.367	0.885	1.17	0.829	1.36	0.331	1.25	1.2	1.71	1.46
Alachlor OA			0.354	0.283	0.284	0.141	0.452			
Bentazon								0.195	0.347	0.214
Imidacloprid										0.0656
Metolachlor ESA	0.525		1.83	1.08	1.8	0.494	1.99	2.23	2.11	2.67
Metolachlor OA	0.177		0.924	0.897	0.803	0.4	1.39	1.26	1.37	1.34
Metribuzin	0.0615		0.0706	0.0841	0.0698	0.0559	0.0924	0.127	0.116	0.148
Thiamethoxam								0.0726		0.0969

Combined sum of neonicotinoids exceeds 0.035 µg/L chronic threshold recommended by Morrissey, et al.

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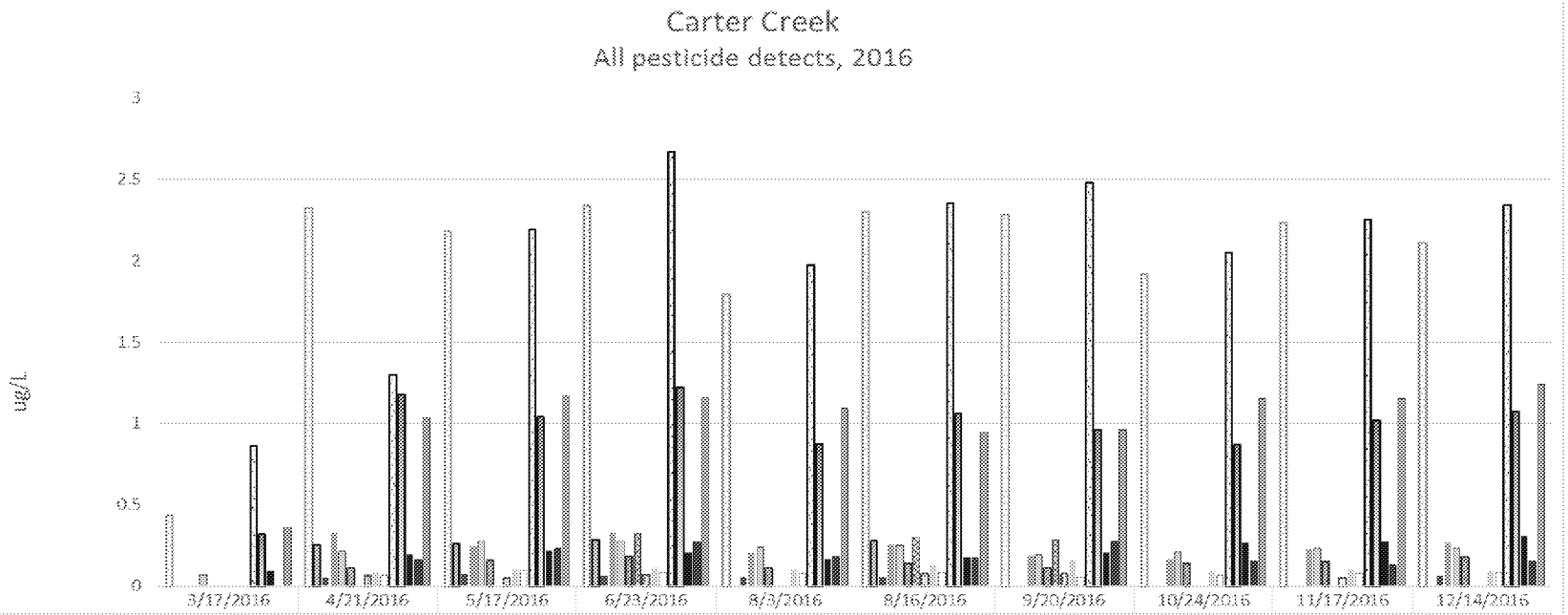
FIGURE 25 ALL PESTICIDE DETECTIONS IN CARTER CREEK, 2016



	3/17/2016	4/21/2016	5/17/2016	6/23/2016	8/3/2016	8/16/2016	9/20/2016	10/24/2016	11/17/2016	12/14/2016
Alachlor ESA	0.434	2.32	2.18	2.34	1.79	2.3	2.28	1.92	2.23	2.11
Alachlor OA		0.255	0.26	0.284		0.28				
Atrazine		0.0508	0.0737	0.0648	0.0581	0.0575				0.0622
Bentazon		0.328	0.243	0.336	0.209	0.256	0.187	0.165	0.233	0.264
De-ethyl Atrazine	0.0644	0.213	0.278	0.274	0.242	0.249	0.192	0.207	0.232	0.236
Deisopropyl Atrazine		0.111	0.158	0.183	0.114	0.139	0.11	0.14	0.152	0.176
Diamino Atrazine				0.326		0.298	0.281			
Imidacloprid		0.0644	0.0529	0.0679		0.0745	0.0746		0.0528	
Metalaxyl		0.0872	0.102	0.106	0.102	0.124	0.102	0.0964	0.101	0.0941
Metolachlor		0.0678	0.0949	0.0835	0.0774	0.0846	0.0556	0.0695	0.0775	0.0861
Metolachlor ESA	0.865	1.3	2.19	2.67	1.97	2.35	2.48	2.05	2.25	2.34
Metolachlor OA	0.323	1.18	1.04	1.22	0.875	1.06	0.96	0.871	1.02	1.07
Metribuzin	0.092	0.193	0.22	0.205	0.165	0.176	0.209	0.269	0.27	0.311
Metribuzin DA		0.16	0.231	0.271	0.18	0.174	0.278	0.155	0.132	0.156
Metribuzin DADK	0.359	1.03	1.17	1.16	1.09	0.943	0.962	1.15	1.15	1.24

Combined sum of neonicotinoids exceeds 0.035 µg/L chronic threshold recommended by Morrissey, et al.

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Conclusions

An evaluation of DATCP groundwater and surface water sample results for neonicotinoids in Wisconsin between 2008 and 2016 has revealed the following:

- Statewide randomized sampling of 401 private potable wells in 2016 revealed one detection of the neonicotinoid imidacloprid (0.25 percent of wells). Targeted sampling of 511 private potable wells in agricultural areas from June 2008 to October 2016 revealed 29 wells with detections of one or more of the neonicotinoid compounds clothianidin, imidacloprid and thiamethoxam (5.7 percent of wells).

- No drinking water standards have been established for neonicotinoid compounds. However, the concentrations detected in drinking water samples are below advisory human health benchmarks for these pesticides established by EPA (HHBPs).

- Testing of water table monitoring wells installed near agricultural fields identified detectable concentrations of one or more neonicotinoid compounds at 53 percent of sites tested (17 of 32 field locations).

- Irrigation well sampling was limited, but neonicotinoid compounds were detected in 18 of 22 high-capacity irrigation wells sampled (82 percent).

- Most detections in groundwater occurred in the Central Sands Region (Portage, Waushara and Adams counties) and other agricultural areas with sandy soils and a shallow water table. Other commonalities included crop rotations of corn or soybeans with vegetable crops like potatoes or sweet corn, and irrigation.

- Between 2011 and 2016, a total of 430 samples were collected from 34 streams. Neonicotinoids detected in stream samples included imidacloprid and thiamethoxam. Imidacloprid was detected in two streams, and thiamethoxam was detected in four streams.

- The majority of surface water detections occurred in samples collected from just two streams, Tenmile and Carter Creeks, whose drainage basins both lie within the Central Sands growing area.

- Imidacloprid and thiamethoxam detections in Tenmile and Carter Creeks exceeded newly recommended chronic toxicity values of 0.035 µg/L for total neonicotinoids (Morrissey, et. al) in every sample where a detection occurred (28 samples).

- Imidacloprid and thiamethoxam detections in Tenmile Creek exceeded newly recommended acute toxicity value of 0.2 µg/L for total neonicotinoids (Morrissey, et. al) in five samples.

- Neonicotinoid detections at field edge monitoring wells suggest leaching of these compounds to shallow groundwater occurs in sandy settings following routine pesticide applications.

- Neonicotinoid detections in high capacity irrigation wells and private potable wells shows that these compounds can migrate significant distances vertically and horizontally away from application sites.

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- Numerous pesticides and nitrate detected over time in two streams within the Central Sands indicates that surface water quality can be influenced by contaminated groundwater that discharges to streams draining the Central Sands grower region.

- In addition to clothianidin, imidacloprid and thiamethoxam, numerous herbicide compounds and nitrate were also detected in groundwater samples collected from potable, water table monitoring and irrigation wells, and in stream samples collected.

Recommendations

Considering recent reductions in EPA acute and chronic thresholds for aquatic invertebrates, DATCP BLS should work to achieve lower laboratory reporting limits for clothianidin, imidacloprid and thiamethoxam. In addition, DATCP suggests numerous areas for further study, including:

- Studies of Tenmile and Carter Creeks are needed to better understand the chronic effects of neonicotinoids on aquatic invertebrates and other organisms in both creeks. Studies should employ the use of auto samplers to more fully characterize temporal changes in neonicotinoid concentrations and to allow for better evaluations of acute and chronic effects on aquatic organisms.

- Pesticide sampling studies are warranted for streams within the Central Sands region and within other agricultural areas where similar pesticide use patterns occur and where contaminated groundwater discharge is likely to contribute to a significant percentage of total stream flow.

- Studies are needed to help determine the effects that repetitive low-dose exposures have on pollinators and non-target insects through recurring applications of contaminated irrigation water.

- Studies are needed to better understand whether accelerated resistance development occurs in target pest species through unintended recurring exposures to neonicotinoid insecticides at less than acute concentrations through contaminated irrigation water.

- Studies are needed to better define the risks posed by the observed complex mixtures of pesticides and high nitrate to humans through contaminated drinking water; to pollinators and non-target insects exposed to contaminated irrigation water; and to aquatic invertebrates and other aquatic life exposed to contaminated surface water.

DATCP shares the data it collects with the public, University of WI-Extension, local government officials, and other agencies like the Department of Natural Resources (DNR), the Department of Health Services (DHS) and the U.S. Environmental Protection Agency (EPA). It is hoped that this report and accompanying data will aide EPA's current efforts to evaluate neonicotinoid compounds for effects on pollinators and non-target organisms. DATCP remains dedicated to helping the citizens of Wisconsin grow quality food and healthy plants and animals through the sound use of land and water resources.

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Appendix 1 Physical, Chemical and Fate Properties of Clothianidin, Imidacloprid and Thiamethoxam

Parameter (units)	Clothianidin*	Imidacloprid**	Thiamethoxam*
Formula	C6H8ClN5O2S	C9H10ClN5O2	C8H10ClN5O3S
Molecular weight (g/mol)	249.7	255.7	291.7
Water solubility (mg/L)	327 (@20oC)	580 (@ 20oC)	4100 (@25oC)
Vapor pressure (mm Hg)	2.9x10-13 (@20oC)	1.5 x 10-9 torr (@ 20 oC)	4.95 x10-11(@25oC)
Henry's law constant (atm m3/mol)	2.9x10-16 (calculated)	9.9 x 10-13	4.62x10-15 (calculated)
Octanol-water partition coefficient (Kow)	13	3.7	0.74
Soil partition coefficient (Koc; L/kgoc)	84 (sandy loam) 119 (sand) 123 (clay loam) 129 (loamy sand) 345 (sandy loam)	Average= 318 (n=5) with a range from 277 to 411 L Kg-1 in soils differing in texture (sand, loamy sand, silt loam "replicated" and loam), cation exchange capacity (4-16 meq/100 g), organic carbon content (0.4-2.6%) and pH (4.5-6.5); Found no relation with O.C., clay or pH	33.1 (silty clay loam) 38.3 (loam) 43.0 (sand) 53.1 (loam) 77.2 (sandy clay loam) 176.7 (sandy loam)
Hydrolysis half-life (t½) (days)	Stable (at pH 5,7,9)	Stable (at pH 5 and 7) Hydrolyzed slowly (extrapolated t ½= 355 d) in sterile alkaline solution pH 9	Stable (at pH 5 and 7) 4.2, 8.4 (at pH 9)
Aqueous Photolysis t½ (days)	14.4	---	2.3, 3.1
Soil Photolysis t½ (days)	34	171	79, 97
Aerobic soil metabolism t½ (days)	148 – 1155	305 -- 1669	101 – 353
Anaerobic aquatic metabolism t½ (days)	27	---	25.3, 28.6
Terrestrial field dissipation t½ (days)	277 – 1386	---	1.1 - 111
GUS leaching potential index (calculated)***	4.91 (high)	3.74 (high)	4.69 (high)

Sources: *Clothianidin and Thiamethoxam (USEPA, January 5, 2017)
**Imidacloprid (USEPA, January 4, 2016)
***Groundwater Ubiquity Score index (Lewis, 2016)

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Appendix 2 DATCP BLS Groundwater Sample Analytes and Reporting Limits (RL)

Analyte	RL (µg/L)	Analyte	RL (µg/L)	Analyte	RL (µg/L)
2,4-D	0.050	lambda-Cyhalothrin	0.050	MCPA	0.050
2,4-DB	0.57	Cypermethrin	0.15	MCPB	0.21
2,4-DP	0.058	Cyprosulfamide	0.074	MCPP	0.055
2,4,5-T	0.050	De-ethyl atrazine	0.050	Malathion	0.050
2,4,5-TP	0.050	De-isopropyl atrazine	0.050	Mesotrione	0.18
Acetamiprid	0.050	Dacthal	0.050	Metalaxyl	0.050
Acetochlor	0.050	Di-amino atrazine	0.28	Methyl Parathion	0.078
Acetochlor ESA	0.050	Diazinon	0.050	Metolachlor	0.050
Acetochlor OA	0.30	Diazinon oxon	0.050	Metolachlor ESA	0.050
Acifluorfen	0.056	Dicamba	0.89	Metolachlor OA	0.27
Alachlor	0.050	Dichlobenil	0.050	Metribuzin	0.050
Alachlor ESA	0.050	Dichlorvos	0.076	Metribuzin DA	0.10
Alachlor OA	0.25	Dimethenamid	0.050	Metribuzin DADK	0.12
Aldicarb Sulfone	0.059	Dimethenamid ESA	0.050	Metsulfuron methyl	0.094
Aldicarb Sulfoxide	0.13	Dimethenamid OA	0.054	Nicosulfuron	0.050
Aminopyralid	0.050	Dimethoate	0.050	Norflurazon	0.058
Atrazine	0.050	Dinotefuran	0.050	Oxadiazon	0.050
Azoxystrobin	0.050	Diuron	0.18	Pendimethalin	0.050
Benfluralin	0.050	EPTC	0.050	Picloram	0.050
Bentazon	0.050	Esfenvalerate	0.050	Prometone	0.050
Bicyclopyrone	0.050	Ethalfluralin	0.074	Prometryn	0.050
Bromacil	0.084	Ethofumesate	0.050	Propiconazole	0.055
Carbaryl	0.067	Flumetsulam	0.17	Saflufenacil	0.20
Carbofuran	0.051	Flupyradifurone	0.050	Simazine	0.050
Chloramben	0.57	Fluroxypyr	0.32	Sulfentrazone	0.75
Chlorantraniliprole	0.20	Fomesafen	0.050	Sulfometuron methyl	0.050
Chlorothalonil	0.16	Halosulfuron methyl	0.080	Tebupirimphos	0.050
Chlorpyrifos	0.050	Hexazinone	0.050	Tembotrione	0.21
Chlorpyrifos Oxon	0.050	Imazapyr	0.050	Thiacloprid	0.067
Clomazone	0.050	Imazethapyr	0.050	Thiamethoxam	0.067
Clopyralid	0.078	Imidacloprid	0.050	Thiencarbazone methyl	0.38
Clothianidin	0.067	Isoxaflutole	0.32	Triclopyr	0.10
Cyclaniliprole	2.0	Isoxaflutole DKN	0.47	Trifluralin	0.050
Cyfluthrin	0.10	Linuron	0.087	N-Nitrate/Nitrite	0.50 mg/L

BLS groundwater analyses performed in conformance with ISO 17025 accreditation standards.

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Appendix 3 List of Abbreviations

ARM	Agricultural Resource Management Division
Bgal	Billion gallons
BLS	Bureau of Laboratory Services
DATCP	Wisconsin Department of Agriculture, Trade and Consumer Protection
DHS	Wisconsin Department of Health Services
DNR	Wisconsin Department of Natural Resources
END	State listed endangered species
EPA	U.S. Environmental Protection Agency
ESs	Chapter NR 140, Wis. Administrative Code, Enforcement Standards
Ex	Exceedance Well
GIS	Geographic Information Systems
HHBP	Human Health Benchmark for Pesticides
ISO	International Organization for Standardization
Koc	Soil Organic Carbon Partition Coefficient
Kow	Octanol-Water Partition Coefficient
LE	Federally listed endangered species
LWRV	Lower Wisconsin River Valley
MCLs	National Primary Drinking Water Standards Maximum Contaminant Levels
mg/L	Milligrams per Liter
nAChRs	Nicotinic Acetylcholine Receptors
NASS	National Agricultural Statistics Service
PALs	Chapter NR 140, Wis. Administrative Code, Preventive Action Limits
ppb	Parts per Billion
RL	Reporting Limit
SC/F	Species federally protected as endangered or threatened, but not so designated by DNR
SOC	Federally listed species of concern
SC/N	Species of special concern, no laws regulating use, possession or harvest
µg/L	Micrograms per Liter
USDA	U.S. Department of Agriculture
UW	University of Wisconsin-Madison

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Message

From: Randy Bruins [Personal Email / Ex. 6
Sent: 7/26/2018 7:37:28 PM
To: Jager, Henriette [jagerhi@ornl.gov]
CC: Galic Nika USGR [Nika.Galic@syngenta.com]; Garber, Kristina [Garber.Kristina@epa.gov]; Valery Forbes [veforbes@umn.edu]; Salice, Christopher J. [csalice@towson.edu]
Subject: Re: Nimbios AQUATOX ms
Attachments: Nimbios_Aquatox case study Jun 11-yj, rb.docx

Nika et al.,
I heartily agree, the paper is looking very good! I've made a number of suggestions which should not be very difficult to implement. The main one is the need for a fuller description of the beneficiaries and WTP estimation methods in the Maelstrom and Egan papers, so that it is more clear to which Coralville beneficiaries the WTP estimates should apply.
Randy

On Jun 26, 2018, at 11:23 PM, Jager, Henriette <jagerhi@ornl.gov> wrote:

Hi Nika,

Its really looking pretty good, not reflected in the track-changes red. I only got to the Intro and figures this evening, but I can look at the rest later. Also, my endnote stopped working, so I can fix that later, hopefully (added some refs to the intro). Feel free to ignore.

Yetta

From: Galic Nika USGR <Nika.Galic@syngenta.com>
Sent: Tuesday, June 26, 2018 5:27 PM
To: Jager, Henriette <jagerhi@ornl.gov>; randybruins@gmail.com; Garber, Kristina <Garber.Kristina@epa.gov>
Cc: Valery E Forbes-1 (veforbes@umn.edu) (veforbes@umn.edu) <veforbes@umn.edu>; Salice, Christopher J. <csalice@towson.edu>
Subject: Nimbios AQUATOX ms

This message may contain confidential information. If you are not the designated recipient, please notify the sender immediately, and delete the original and any copies. Any use of the message by you is prohibited.
<Nimbios_Aquatox case study Jun 11-yj.docx>

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Environmental Toxicology

Acute Toxicity of 6 Neonicotinoid Insecticides to Freshwater Invertebrates

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Abstract: Neonicotinoids are a group of insecticides commonly used in agriculture. Due to their high water solubility, neonicotinoids can be transported to surface waters and have the potential to be toxic to aquatic life. The present study assessed and compared the acute (48- or 96-h) toxicity of 6 neonicotinoids (acetamiprid, clothianidin, dinotefuran, imidacloprid, thiacloprid, and thiamethoxam) to 21 laboratory-cultured and field-collected aquatic invertebrates spanning 10 aquatic arthropod orders. Test conditions mimicked species' habitat, with lentic taxa exposed under static conditions, and lotic taxa exposed under recirculating systems. Median lethal concentrations (LC50s) and median effect concentrations (EC50s; immobility) were calculated and used to construct separate lethal- and immobilization-derived species sensitivity distributions for each neonicotinoid, from which 5th percentile hazard concentrations (HC5s) were calculated. The results showed that the most sensitive invertebrates were insects from the orders Ephemeroptera (*Neocloeon triangulifer*) and Diptera (*Chironomus dilutus*), whereas cladocerans (*Daphnia magna*, *Ceriodaphnia dubia*) were the least sensitive. The HC5s were compared with neonicotinoid environmental concentrations from Ontario (Canada) monitoring studies. For all neonicotinoids except imidacloprid, the resulting hazard quotients indicated little to no hazard in terms of acute toxicity to aquatic communities in Ontario freshwater streams. For the neonicotinoid imidacloprid, a moderate hazard was found when only invertebrate immobilization, and not lethality, data were considered. *Environ Toxicol Chem* 2018;37:1430–1445. © 2018 SETAC

Keywords: Insecticides; Pesticides; Aquatic invertebrates; Species sensitivity distributions; Toxic effects

INTRODUCTION

Neonicotinoids are a class of pesticides widely used in agriculture as plant protectants. All are water soluble, can be applied as seed or spray treatments, and act systemically to protect the entire plant (Jeschke et al. 2011). They bind strongly to the nicotinic acetylcholine receptor (*n*AChR) found in the central nervous system of insects, causing constant nerve stimulation that eventually leads to death or other toxic effects (Morrissey et al. 2015).

The first neonicotinoid brought to market was imidacloprid in 1991, and 6 others followed in the next decade: acetamiprid (1995), nitenpyram (1995), thiamethoxam (1998), thiacloprid (2000), clothianidin (2002), and dinotefuran (2002; Jeschke and Nauen 2008). Their systemic actions, combined with low mammalian toxicity, effectiveness against pests, and low application volumes, have made neonicotinoids the most popular class of insecticides worldwide (Jeschke et al. 2011; Simon-Delso et al. 2015). Due to their widespread use, high water solubility, and persistence, neonicotinoid residues are therefore frequently detected in surface waters that drain agricultural lands (Morrissey et al. 2015).

Transport of neonicotinoids to aquatic environments is typically driven by precipitation events (Hladik and Kolpin 2016; Struger et al. 2017), snowmelt (Main et al. 2016), and contaminated dust (Limay-Rios et al. 2016). Median neonicotinoid surface water concentrations are on the order of ng L^{-1}, with peak concentrations rising to low $\mu g\ L^{-1}$. Struger et al. (2017) detected the 3 most common neonicotinoids—imidacloprid, thiamethoxam, and clothianidin—in >90% of samples from more than half of their 15 Ontario (Canada) surface water sites. Median concentrations in that study ranged from <0.00128 to 0.585 $\mu g\ L^{-1}$, and the maximum detection was 10.4 $\mu g\ L^{-1}$ imidacloprid. Detections were correlated with registered uses; thiamethoxam and clothianidin were correlated

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with row crops, imidacloprid and acetamiprid with vegetable crops, greenhouses, and other agriculture, and thiacloprid with fruit production (Struger et al. 2017). In addition, neonicotinoid detections showed seasonality, with maximum concentrations occurring in the late spring and fall (Struger et al. 2017). Monitoring by the Ontario Ministry of the Environment and Climate Change in 2015 also showed the most common detections to be imidacloprid, thiamethoxam, and clothianidin, with median concentrations from 0.018 to 0.050 $\mu g\ L^{-1}$ and maximum concentrations of 2.3 $\mu g\ L^{-1}$ imidacloprid and 2.7 $\mu g\ L^{-1}$ thiamethoxam (Ontario Ministry of the Environment and Climate Change 2016). Environmental concentrations are similar on a global scale; Morrissey et al. (2015) surveyed 29 surface water neonicotinoid monitoring studies from 9 countries and reported mean surface water concentrations of 0.13 $\mu g\ L^{-1}$, with a geometric mean for peak concentrations of 0.63 $\mu g\ L^{-1}$.

Neonicotinoids are acutely toxic to aquatic macroinvertebrates at concentrations that span 6 orders of magnitude, with insects generally showing greater sensitivity than crustaceans (Morrissey et al. 2015). However, within the Insecta, sensitivity varies widely. Roessink et al. (2013) reported 96-h median lethal concentrations (LC50s) for imidacloprid of between 6.68 $\mu g\ L^{-1}$ for *Caenis horaria* (Ephemeroptera) and >10 000 $\mu g\ L^{-1}$ for *Notonecta* spp. (Hemiptera) and *Sialis lutaria* (Megaloptera). Reported 96-h LC50s for mayflies vary from 0.65 $\mu g\ L^{-1}$ for *Epeorus longimanus* (Alexander et al. 2007) to 154 $\mu g\ L^{-1}$ for *Cloeon dipterum* (Van den Brink et al. 2016). Such variability in toxicity also exists in the Crustacea, with 48-h LC50s and EC50s (median effect concentration) >10 000 $\mu g\ L^{-1}$ reported for *Daphnia magna* (Song et al. 1997; Hayasaka et al. 2012), compared with 48-h LC50 of 2.07 $\mu g\ L^{-1}$ (Chen et al. 2010) and 48-h EC50 of 571.62 $\mu g\ L^{-1}$ (Hayasaka et al. 2012) for *Ceriodaphnia dubia*. This wide range in sensitivity within and between groups highlights the need for testing across different aquatic invertebrate orders, including those outside the typical model species, to better understand the range of neonicotinoid toxicity and hence potential risks in aquatic ecosystems.

A few government regulatory bodies around the world have derived water quality guidelines for neonicotinoids, as summarized by Morrissey et al. (2015). In North America, the US Environmental Protection Agency has published acute aquatic life benchmarks for 6 neonicotinoids, ranging from 10.5 $\mu g\ L^{-1}$ for acetamiprid to >484 150 $\mu g\ L^{-1}$ for dinotefuran (US Environmental Protection Agency 2017). The Canadian Council of Ministers of the Environment (CCME) has an interim water quality guideline for imidacloprid of 0.23 $\mu g\ L^{-1}$. This guideline is based on a 28-d lowest-observed-effect concentration (LOEC) for *Chironomus riparius* of 2.25 $\mu g\ L^{-1}$ imidacloprid, with a 0.1 safety factor applied (Canadian Council of Ministers of the Environment 2007a). Individual water quality guidelines for other neonicotinoids do not exist in Canada, partly because of the lack of toxicity data and minimum data requirements (minimum of 3 aquatic invertebrates, ideally with 1 being a mayfly, caddisfly, or stonefly) stipulated by the CCME for constructing species sensitivity distributions (SSDs; Canadian Council of Ministers of the Environment 2007b). In 2015, Morrissey et al. constructed an acute SSD using a combined dataset whereby toxicity data for all neonicotinoids were standardized and weighted by molecular mass to imidacloprid to establish a recommended short-term acute guideline of 0.2 $\mu g\ L^{-1}$. However, although this is a useful interim approach, it is based on the assumption that the most toxic neonicotinoid across all aquatic species is imidacloprid, which, as we show in the present study, is not always the case.

The objective of the present study was to evaluate the acute toxicity of 6 neonicotinoid insecticides (imidacloprid, thiamethoxam, clothianidin, acetamiprid, thiacloprid, and dinotefuran) to a suite of aquatic invertebrate species ($n = 21$), with the goal of better defining the range of sensitivity, creating a comprehensive dataset to characterize the potential risk of the 6 neonicotinoids, and developing rigorous short-term acute water quality guidelines. The invertebrates tested in the present study included both laboratory-cultured model aquatic invertebrates and field-collected species; they represent both lotic and lentic habitats, have broad representation within the Arthropoda, and include several functional feeding groups (Merritt and Cummings 1996). All species tested are relevant to freshwaters near agricultural areas where neonicotinoids could be applied.

MATERIALS AND METHODS

Toxicity tests: Test solution preparation

Toxicity tests were conducted with 6 technical-grade neonicotinoids purchased from Sigma-Aldrich: acetamiprid (99.9%, CAS 135410-20-7), clothianidin (99.9%, CAS 210880-92-5), dinotefuran (98.6–98.8%, CAS 165252-70-0), imidacloprid (99.9%, CAS 138261-41-3), thiacloprid (99.9%, CAS 111988-49-9), and thiamethoxam (99.6%, CAS 153719-23-4). Imidacloprid (99.8%) was also obtained from Bayer CropScience and thiamethoxam (technical grade, purity not stated) from Syngenta Crop Protection. Stock solutions were prepared by dissolving up to 100 mg of chemical/1 L of dechlorinated municipal tap water (physicochemical properties provided in the Supplemental Data, Table S2) in class A glassware. The solutions were covered with tin foil to limit photodegradation stirred overnight, and then stored in 1-L amber glass bottles at 4 ± 2 °C. Test solutions were prepared by either spiking the appropriate volume of stock into a known volume of dechlorinated water or by serial dilutions in which 5 to 7 test solutions were prepared for each test in a 50 or 70% geometric series. Each test included a negative control of dechlorinated water. Conductivity, pH, dissolved oxygen, and temperature were measured on at least the negative control and the highest test concentration before and after each toxicity test. Temperature was monitored throughout the test.

Test species

Laboratory-cultured species. Acute toxicity tests were performed with 7 laboratory-cultured species: *Ceriodaphnia dubia*, *Chironomus dilutus*, *Daphnia magna*, *Hexagenia* spp., *Hyalella azteca*, *Lumbriculus variegatus*, and *Neocloeon triangulifer*. Culturing methods are outlined in the Supplemental Data, Table S1. Test methods were based on standard Ontario

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Ministry of the Environment and Climate Change and literature-derived methods (Rodrigues and Kaushik 1984; Environment Canada 2007a, 2007b; Soucek and Dickinson 2015; Ontario Ministry of the Environment and Climate Change Aquatic Toxicology Unit 2016). Tests were conducted with 5 or 10 organisms/replicate, with 3 to 5 replicates/treatment using a regression design. Test parameters are described in Table 1. Endpoints of immobility and mortality were observed at 96 ± 4 h; obvious mortalities (i.e., individuals starting to decompose) during the tests were counted, and the dead organisms were removed daily. Mortality was classified as complete cessation of organism movement even after gentle prodding with a pipette and was confirmed under a dissecting microscope (×2–4 magnification). Immobilization responses included lethargy, limited swimming behaviors, and muscle spasms, as described by Camp and Buchwalter (2016). In preliminary tests, we had noted that immobile individuals that did not respond to prodding were alive when observed under a microscope, often with mouthparts, legs, and/or gills moving. We therefore confirmed all mortality under a microscope, and only scored individuals as dead when there was complete cessation of all organism movement. Other studies noting the effects of neonicotinoids on aquatic invertebrates have generally counted what we considered to be immobility as death, and this should be kept in mind when the results of the present study are compared with those of other studies. For example, it is possible that the LC50s reported in other literature in which immobility was taken to mean death are equivalent to the median EC50s reported in the present study. Organisms were considered immobile if the customary swimming response or movement was not observed over a period of ≥15 s after gentle agitation of the solution or gentle stimulation with a probe. Tests with laboratory-cultured species were considered invalid if control mortality exceeded 10%, except for *Hexagenia* spp., for which the acceptability criterion was 20% mortality due to inherent increased variability when these sediment-burrowing mayflies were tested in water-only exposures.

Field-collected species. Test organisms were collected from ponds and streams around Guelph (ON, Canada) and Ottawa (ON, Canada; Table 1). Grab samples taken from one of the ponds and the Eramosa River (Guelph) during one of the organism collecting events showed that all neonicotinoid levels were less than the method detection limit (ranging from 5–10 ng L^{-1}, as shown in Table 2).

Toxicity tests were conducted with 14 field-collected taxa from the orders Ephemeroptera (*Caenis* sp., *Cloeon* sp., *Ephemerella* sp., *Isonychia bicolor*, *McCaffertium* sp.), Trichoptera (*Cheumatopsyche* sp., *Micrasema* sp.), Coleoptera (*Stenelmis* sp., *Gyrinus* sp.), Isopoda (*Caecidotea* sp.), Hemiptera (*Trichocorixa* sp.), Odonata (*Coenagrion* sp.), Diptera (*Aedes* sp.), and Plecoptera (*Agnetina* sp. and *Paragnetina* sp.). *Agnetina* sp. and *Paragnetina* sp. were tested together and treated as a single test unit due to low collection numbers for the individual genera. All field-collected taxa were nymphs/juveniles, with the exception of *Caecidotea* sp., *Trichocorixa* sp., *Gyrinus* sp., and *Stenelmis* sp., which were tested as adults. An attempt was made to collect and test species within a similar visual size range. Animals were collected using a kick-sampling technique and a 500-μm D-net, or careful removal from the underside of stream rocks. After collection, organisms were transferred to aerating dechlorinated water and maintained at 12 to 20 °C (depending on the water temperature at the collection site) for 1 to 5 d prior to testing. A selection of aquatic vegetation and/or leaf litter was provided for food and substrate during the holding period. Species were chosen to represent a diverse set of lentic and lotic invertebrate taxa known to occur in agricultural landscapes in Ontario.

Tests with pond (lentic)-dwelling species were conducted under the static conditions just described in the *Laboratory-cultured species* section. Tests were conducted with 7 to 10 organisms/treatment, with 1 replicate/treatment, using a regression design (see *Concentration–response models* section). Tests with stream (lotic)-dwelling species were conducted in recirculating exposure systems that mimic stream flow (Rodrigues and Kaushik 1984). Details concerning the construction of the flow-through exposure system are provided in the Supplemental Data, S7. Tests were conducted with 7 to 20 organisms/treatment in each simulated stream (flow rate 25 ± 6 mL/s), with 1 replicate/treatment, using a regression design (see *Concentration–response models* section). Water depth in the artificial streams was maintained at approximately 2 cm using a glass partial wall and 280-μm Nitex screen on the lower end of the ramp. Test temperatures were 12 to 15 (±2) °C to mimic collection conditions and reduce thermal stress. In all tests, endpoints of immobility and mortality were observed and recorded as described in the *Laboratory-cultured species* section. Organisms were removed from the stream apparatus and examined under microscopy to confirm mortality at test completion, or when mortality was obvious (i.e., decomposition).

Test acceptability criteria was set at 20% mortality due to higher inherent variability in field-collected species. Any tests with >20% mortality in the controls were rejected except for clothianidin toxicity to *Aedes* sp., for which the control mortality was 2/9 = 22%.

Chemical analysis

Samples from the exposure treatments were collected and analyzed to confirm nominal concentrations using liquid chromatography–tandem mass spectrometry. Samples (40 mL) of 1 to 4 exposure treatments/test were collected at the time of initial organism exposure and stored in amber glass bottles at 4 ± 2 °C prior to analysis. Across all toxicity tests, a total of 149 samples were collected to measure actual concentrations. Complete details of the chemical analyses are provided in the Supplemental Data, S3.

Statistical analysis

Concentration–response models. Endpoints were tallied as percentage of total organisms in each treatment exhibiting

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TABLE 1: Summary of tested species and associated test conditions used to assess the acute toxicity of 6 neonicotinoid insecticides

Species	*Ceriodaphnia dubia*	*Daphnia magna*	*Chironomus dilutus* *Hexagenia* spp. *Hyalella azteca* *Lumbriculus variegatus*	*Neocloeon triangulifer*	*Caecidotea* sp. *Gyrinus* sp. *Caenis* sp. *Cloeon* sp.	*Trichocorixa* sp. *Aedes* sp.	*Coenagrion* sp.	*McCaffertium* sp. *Ephemerella* sp. *Isonychia bicolor* *Cheumatopsyche* sp. *Micrasema* sp. *Stenelmis* sp. *Agnetina/Paragnetina* sp.
Origin	MOECC laboratory culture	MOECC laboratory culture	MOECC laboratory culture	MOECC laboratory culture	Field-collected in ponds in Guelph, ON, Canada; *Gyrinus* sp. from Lac Gerard, QC, Canada	Field-collected in quiet eddies of Speed River and ponds in Guelph, ON	Field-collected in pond in Guelph, ON	Field-collected in Speed River, Eramosa River, Guelph, ON
Test protocol	Environment Canada (2007a)	Environment Canada (2007b)	Ontario Ministry of the Environment and Climate Change Aquatic Toxicology Unit (2016)	Soucek and Dickinson (2015)	Adapted from Ontario Ministry of the Environment and Climate Change Aquatic Toxicology Unit (2016)	Adapted from Ontario Ministry of the Environment and Climate Change Aquatic Toxicology Unit (2016)	Adapted from Ontario Ministry of the Environment and Climate Change Aquatic Toxicology Unit (2016)	Adapted from Rodrigues and Kaushik (1984)
Life stage	<24 h old	<24 h old	*Chironomus*: 3rd instar, as measured by mean head capsule width (0.33–0.45 mm) *Hexagenia*: mean wet wt ~4–6 mg *Hyalella*: 2–9 d old, within 2 d of each other *Lumbriculus*: age-synchronized to 7 d	<24 h old	*Caecidotea* sp.: adults *Gyrinus* sp.: adults *Caenis* sp.: nymphs *Cloeon* sp.: nymphs	*Trichocorixa* sp.: adults *Aedes* sp.: larvae	Nymphs	All nymphs, except *Stenelmis* sp.
Exposure duration (h)	48 ± 2	48 ± 2	96 ± 4	96 ± 4	96 ± 4	48 ± 2	96 ± 4	96 ± 4
Exposure temp. (°C)	25 ± 2	20 ± 2	23 ± 2	25 ± 2	15 ± 2	14 ± 2, except thiamethoxam to *Trichocorixa* sp., 18 ± 2	13 ± 3	15 ± 2
Light intensity (lux)	All tests conducted at 500–1000 lux, 16:8-h light:dark photoperiod							
Test vessel	15 mL solution in 30-mL flat-bottom glass tube	50 mL solution in 50-mL glass tube	200 mL solution in 250-mL glass beaker	200 mL solution in 250-mL glass beaker	200 mL solution in 250 mL glass beaker	200 mL solution in 250-mL glass beaker	20 mL solution in 30-mL flat-bottom glass tube	Recirculating apparatus
Minimum no. of treatments	8	8	8	8	8	8	6	6
No. of test replicates	10	10	3	4	1	1	10	1
No. of animals/vessel/replicate	1	1	10	5	10	10	1	10

(*Continued*)

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TABLE 1: *(Continued)*

Species	*Ceriodaphnia dubia*	*Daphnia magna*	*Chironomus dilutus* *Hexagenia* spp. *Hyalella azteca* *Lumbriculus variegatus*	*Neocloeon triangulifer*	*Caecidotea* sp. *Gyrinus* sp. *Caenis* sp. *Cloeon* sp.	*Trichocorixa* sp. *Aedes* sp.	*Coenagrion* sp.	*McCaffertium* sp. *Ephemerella* sp. *Isonychia bicolor* *Cheumatopsyche* sp. *Micrasema* sp. *Stenelmis* sp. *Agnetina/Paragnetina* sp.
Feeding during test	0.5 mL *Pseudokirchneriella subcapitata*, 0.1 mL YCT	0.5 mL *P. subcapitata*, 0.5 mL *Chlorella* sp.	*Chironomus*: 1.25 mL 3:2 ratio cereal grass: ground Nutrafin *Hexagenia*: None *Hyalella*: 2 mg ground Nutrafin *Lumbriculus*: None	0.3 mL *Navicula* sp.: free diatoms	None	None	None	None
Test substrate	None	None	*Chironomus*: 20 mL silica sand *Hexagenia*: glass tubes *Hyalella*: 5 × 5 cm cotton gauze *Lumbriculus*: None	None	None	None	3 × 1 cm 500 µm Nitex screen	280 µm Nitex screen

MOECC = Ontario Ministry of the Environment and Climate Change; YCT = yeast, cereal leaves, and Tetramin.

mortality or immobilization. The number of treatment replicates for each test varied depending on organism availability; tests with laboratory-cultured organisms used a minimum of 3 replicates, whereas tests with field-collected organisms used 1 replicate due to the logistical constraint of collecting large numbers of organisms in the field. All tests were conducted using a regression design, with 5 to 7 treatments/test in a dilution series. Nominal concentrations were corrected using the mean percentage difference between the nominal and measured concentrations taken from each test and the corrected concentrations used to model the concentration–response relationships.

Concentration–response relationships were modeled for datasets containing one or more partial values using a 4-parameter log-logistic regression model (Equation 1) in R Ver 3.3.3 with the *drc* package (Ritz and Streibig 2005). The lower limit was fixed at 0, and the upper limit at 100, representing the minimum and maximum possible effects, respectively. The variables *b*, *c*, *d*, and *e* represent the relative slope around the LC/EC value, the lower and upper limits, and the LC/EC value, respectively.

$$\text{Mortality or immobilization} = c + \frac{d - c}{1 + \exp\left\{b\left(\log(concentration) - \log(e)\right)\right\}} \quad (1)$$

Datasets containing no partial values were analyzed using the binomial method (Environment Canada 2007c). Lethal and effective concentrations for the 10th and 50th percentiles (LC10/LC50 and EC10/EC50, respectively) and associated 95% confidence intervals were calculated from regression models. The LC/EC10s were not calculated for binomial distributions.

SSDs and hazard assessment. The dataset of 112 neonicotinoid/species combinations was used to construct short-term SSDs for each neonicotinoid. These SSDs allow for comparison of toxicity among aquatic taxa and among the 6 neonicotinoids. The SSDs were constructed according to CCME type A guidelines (Canadian Council of Minister of the Environment 2007b). Data from the present study were used to fulfill the dataset requirement for aquatic invertebrate species, and data from the ECOTOX database were used to fulfill requirements for fish (3 species, at least 1 salmonid and 1 nonsalmonid), suggested plant and amphibian species requirements, and to strengthen the invertebrate dataset from the present study. To be included in the SSDs, external data from the ECOTOX database had to be derived from tests using the active ingredient and not a formulated product and had to have an acute exposure duration (≤4 d for invertebrates, and ≤5 d for plants). If multiple entries were present for the same species, the lowest value was included. If multiple exposure durations were present for the same species from the same study, the duration closest to 4 d was chosen to maintain consistency with the present study exposure duration. Fish, plant, and invertebrate data were available for all neonicotinoids, but amphibian data were only available for imidacloprid. The SSDs were generated

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TABLE 2: Summary of percentage difference between nominal and measured neonicotinoid concentrations

	Method performance[a]	Sample analysis		
Neonicotinoid	Method detection limit ($\mu g\ L^{-1}$)	Mean % difference	SD	No.
Acetamiprid	0.010	11.0	7.1	20
Clothianidin	0.010	9.6	6.5	32
Dinotefuran	0.010	1.5	15.8	16
Imidacloprid	0.010	8.5	5.8	32
Thiacloprid	0.005	12.9	8.4	20
Thiamethoxam	0.010	16.0	7.5	29

[a]Estimated uncertainty for neonicotinoid analysis method is 9 to 13%.
SD = standard deviation.

using CCME SSD Ver 3 (2010) software (Rodney et al. 2013). Two short-term SSDs were generated for each neonicotinoid, one with invertebrate LC50 values (lethality derived), and one with invertebrate EC50 values (immobilization derived). Both SSDs used external, ECOTOX database EC50/median inhibitory concentration values for plant data and LC50s for fish and amphibian data. The LC50 or EC50 values that fell below the smallest concentration in a test (e.g., $<600\ \mu g\ L^{-1}$) were not used in the dataset. The LC50 or EC50 values that were greater than the largest concentration in a test (e.g., $>9600\ \mu g\ L^{-1}$) were included as-is (e.g., $9600\ \mu g\ L^{-1}$), in a conservative approach. Any species with LC50 or EC50 values $\geq 100\,000\ \mu g\ L^{-1}$ were considered tolerant, and were used to calculate plotting positions but were not fit to the model. Although they are less environmentally relevant, LC50s and EC50s were chosen due to the inherent increased statistical robustness and the greater abundance of LC50s and EC50s compared with the application of LOEC, no-observed-effect concentration, or L(E)Cx (where x = value other than 50) values.

To construct the SSDs, the datasets were fit to 4 models: normal, logistic, extreme value, and Gumbel. Models were assessed for goodness-of-fit using mean squared error for the entire dataset and for the lower tail using the Anderson–Darling (A^2 statistic) test and visual inspection. The model with the lowest mean squared error for the lower tail (the A^2 statistic) and best visual inspection was chosen. If a model failed a visual inspection and the Anderson–Darling test for goodness-of-fit, it was not chosen; if all models failed both tests, that SSD was not included in the hazard assessment. The chosen model was plotted in R with the ggplot2 (Ver 2.2.1) package. The 5th percentile of the distribution, termed the HC5 (hazardous concentration, 5%), and its associated 95% confidence intervals were calculated from the model.

The HC5s for each neonicotinoid were compared with environmental concentrations of associated neonicotinoids in Ontario surface waters in a hazard assessment. To assess environmental concentrations, monitoring data from 3 Ontario studies spanning the years 2012 to 2016 (see Supplemental Data, S14, for details on each study) with a total of 2790 observations on the 6 neonicotinoids were compiled. Due to the highly left-censored nature of the data (i.e., values below method detection or quantitation limits, or nondetects) coupled with multiple detection limits, the nonparametric Kaplan–Meier method was used to model the data as empirical cumulative distribution functions (ECDFs) using the NADA package (Ver 1.6-1) in R (Ver 3.3.2; Helsel 2012). The ECDFs were estimated for environmental monitoring data for clothianidin, imidacloprid, and thiamethoxam, but were not estimated for acetamiprid, dinotefuran, and thiacloprid due to low numbers of detections (>98% of observations were censored). The ECDF summary statistics are shown in Table 3, and ECDF plots are presented in the Supplemental Data, Figure S15.

Hazard quotients (HQs) were calculated for each neonicotinoid where HQ equals exposure divided by effects. Exposure was either the maximum environmental concentration (acetamiprid, dinotefuran, and thiacloprid, where ECDFs could not be calculated because there were too few detections) or the 99th percentile of the ECDF (clothianidin, imidacloprid, and thiamethoxam). Effects was the associated HC5 calculated for each compound from SSDs.

RESULTS AND DISCUSSION

Solution chemistry

Water chemistry parameters in exposure solutions were not affected by addition of neonicotinoids. For all tests at test initiation, measured pH values ranged from 7.9 to 8.4, conductivity ranged from 240 to 360 μS/cm, and dissolved oxygen ranged from 8.1 to 10.5 mg/L, or within 90 to 110% saturation at test temperatures varying by $<2\,^{\circ}C$ ($<3\,^{\circ}C$ for *Trichocorixa* sp.) from the intended temperatures (range 11–25 °C, depending on the test). For all tests at test completion, pH ranged from 7.5 to 8.8, conductivity ranged from 260 to 450 μS/cm, and dissolved oxygen ranged from 4.5 to 11.2 mg/L, or within 60 to 110% saturation at the same temperatures as test initiation. Summarized water chemistry parameters for all tests at test termination are available in the Supplemental Data, Table S8.

Mean percentage differences between measured and nominal neonicotinoid concentrations for the 149 water samples are summarized in Table 2. Measured concentrations were generally within 20% of nominal concentrations.

Toxicity

Many studies have assessed the acute toxicity of neonicotinoid insecticides, but most have focused on only 1 or 2 neonicotinoids and generally involved single or a small number of species. The novel contributions of the present study are that

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TABLE 3: Estimated Kaplan–Meier ECDF summary statistics, and hazard assessment for neonicotinoid concentrations in Ontario (Canada) surface waters from select monitoring studies

								Endpoint: immobilization			Endpoint: lethality		
Neonicotinoid	No.	% Censored	Maximum ($\mu g\,L^{-1}$)	ECDF median ($\mu g\,L^{-1}$)	ECDF mean (95% CI) ($\mu g\,L^{-1}$)	ECDF SD ($\mu g\,L^{-1}$)	99th Percentile	HC5 (95% CI) ($\mu g\,L^{-1}$)	HQ	Hazard	HC5 (95% CI) ($\mu g\,L^{-1}$)	HQ	Hazard
Acetamiprid	348	98.3	0.100		Not calculated			0.47 (0.16–1.41)	0.21	Low	0.48 (0.21–1.07)	0.21	Low
Clothianidin	544	60.3	0.970	0.020	0.052 (0.044–0.060)	0.093	0.44	0.14 (0.01–2.93)	No model passed goodness-of-fit test or visual inspection		4.13 (3.32–5.15)	0.11	Low
Dinotefuran	348	99.4	0.019		Not calculated			0.86 (0.17–4.43)	0.020	Low	7.81 (4.67–13.06)	<0.005	None
Imidacloprid	544	79.8	11.000	N/A[a]	0.091 (0.037–0.145)	0.645	2.70	1.08 (0.82–1.43)	1.92	Moderate	3.71 (2.82–4.87)	0.73	Low
Thiacloprid	462	99.8	0.002		Not calculated			0.30 (0.07–1.25)	0.0070	Low	0.90 (0.60–1.34)	<0.005	None
Thiamethoxam	544	63.4	2.700	0.012	0.077 (0.058–0.097)	0.230	1.20	6.09 (3.07–12.08)	0.20	Low	12.29 (9.90–15.25)	0.010	None

[a]Empirical cumulative distribution function median is not applicable for imidacloprid due to probability of detection > 0.5.
ECDF = empirical cumulative distribution function; CI = confidence interval; SD = standard deviation; HC5 = 5th percentile hazard concentration; HQ = hazard quotient.

we assessed and compared the acute toxicity of 6 neonicotinoid insecticides to aquatic invertebrates; that we included species from 10 aquatic arthropod orders, many of which have not previously been tested; and that we constructed individual SSDs for each compound. A total of 112 neonicotinoid/species combinations were tested, and the derived LC and EC values and control mortality are presented in Table 4.

The cladocerans *D. magna* and *C. dubia* were the least sensitive taxa, with effects only observed at concentrations >40 000 $\mu g\,L^{-1}$. Insects were among the most sensitive taxa tested, with 63% of the calculated LC50s, and 91% of the calculated or threshold estimated EC50s ≤1000 $\mu g\,L^{-1}$. The most sensitive species for both lethality and immobilization endpoints were the midge *C. dilutus* and the mayfly *N. triangulifer*, with LC50s < 65 $\mu g\,L^{-1}$ and as low as 1.7 $\mu g\,L^{-1}$, and EC50s < 40 $\mu g\,L^{-1}$ and as low as 0.8 $\mu g\,L^{-1}$, respectively. The amphipod *Hyalella azteca* also showed high sensitivity to clothianidin and acetamiprid, with LC50s and EC50s of approximately 5 $\mu g\,L^{-1}$. These results are consistent with published studies in which Diptera, especially *Chironomus* spp., and Ephemeroptera have been reported to be the most sensitive insect taxa to neonicotinoids (Roessink et al. 2013; Morrissey et al. 2015; Smit et al. 2015; Camp and Buchwalter 2016; Cavallaro et al. 2017). The greater sensitivity of insect taxa relative to noninsect taxa was not unexpected because neonicotinoids are designed to target and bind strongly with the insect *n*AChR.

Although on average the insects were most sensitive, toxicity values spanned 4 orders of magnitude, indicating wide variation in relative sensitivity within this group. Variation in the sensitivity of aquatic organisms to pesticides is a function of the toxicokinetic and toxicodynamic relationship between the organism and the pesticide (Rico and Van den Brink 2015). Interspecies differences in uptake rate and transport to the site of action, structure of the *n*AChR, and metabolism or detoxification mechanisms all have the potential to introduce variation in the expression of toxic effects. For example, uptake of the pesticide chlorpyrifos has been shown to be a function of insect body size and respiratory strategy, with small insects that rely on aqueous gas exchange to respire (such as Ephemeroptera, Trichoptera, and some Diptera) typically showing greater uptake (Buchwalter et al. 2002). Furthermore, and specifically for neonicotinoids, highly sensitive mayfly species such as *N. triangulifer* have been shown to bioconcentrate more imidacloprid per mass than less sensitive stoneflies or gastropods; uptake rates also increased with temperature (Camp and Buchwalter 2016). In the present study, species that were small at test initiation and that were tested at higher temperatures (23–25 °C), such as *N. triangulifer* and *H. azteca*, showed greater sensitivity relative to similar taxa (e.g., *Cloeon* sp., and *Caecidotea* sp., respectively). Many of these small, sensitive species were laboratory cultured and showed greater sensitivity than field-collected species, which were likely collected at later developmental stages (instars were not determined), which could be more resilient. As a result of differences in developmental stage and testing temperature, there is the potential in the present study for toxicity to field-collected species to be underestimated, and toxicity to

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TABLE 4: Summary of acute toxicity values for 6 neonicotinoids[a]

Species	LC10 ($\mu g\,L^{-1}$)	LC50 ($\mu g\,L^{-1}$)	Control mortality (%)[b]	EC10 ($\mu g\,L^{-1}$)	EC50 ($\mu g\,L^{-1}$)	Control immobilization (%)[b]
Acetamiprid						
Insecta						
Ephemeroptera						
Caenis sp.[c]	270.2 (106.6–433.8)	782.8 (499.3–1066.2)	0	NC	<138.8	0
Cloeon sp.[c]	201.0 (–0.5–402.5)	2369.7 (805.0–3934.4)	0	<16.6	<16.6	0
Ephemerella sp.[c]	29.1 (–6.3–64.5)	158.2 (76.6–239.8)	0	NC	<56.1	0
Hexagenia spp.	NC	>35600.0	0	NC	1.8 (1.3–2.6)	0
Isonychia bicolor[c]	NC	>9600.0	10	NC	<600.0	10
McCaffertium sp.[c]	NC	>890.0	0	NC	<56.1	0
Neocloeon triangulifer	NC	1.7 (1.2–2.2)	0	NC	1.6 (1.2–2.2)	0
Odonata						
Coenagrion sp.	5439.1 (345.8–10532.3)	24 392.9 (13151.1–35634.7)	10	<5625.0	<5625.0	10
Hemiptera						
Trichocorixa sp.[c,d]	359.9 (97.0–622.8)	1515.2 (758.6–2271.8)	0	NC	63.5 (44.9–89.9)	0
Trichoptera						
Cheumatopsyche sp.[c]	134.8 (47.5–222.1)	403.8 (245.9–561.6)	10	NC	<56.1	10
Coleoptera						
Gyrinus sp.[c]	165.3 (43.2–287.3)	686.5 (311.9–1061.1)	0	NC	88.2 (–35.0–248.7)	0
Stenelmis sp.[c]	152.8 (100.0–205.6)	238.3 (182.2–294.4)	10	63.6 (38.2–88.9)	104.6 (79.3–129.8)	10
Diptera						
Chironomus dilutus	1.6 (1.2–2.0)	2.8 (2.4–3.2)	10	1.4 (1.1–1.8)	2.7 (2.3–3.2)	10
Aedes sp.[c,d]	64.5 (30.0–99.0)	159.6 (106.5–212.8)	11	NC	NC	NC
Crustacea						
Caecidotea sp.[c]	146.7 (–118.3–411.7)	2129.6 (634.0–3625.1)	0	NC	<556.0	0
Ceriodaphnia dubia[d]	NC	>33 500	0	NC	NC	NC
Hyalella azteca	2.3 (1.6–2.9)	4.8 (4.0–5.7)	10	2.0 (1.4–2.6)	4.4 (3.6–5.2)	10
Oligochaeta						
Lumbriculus variegatus	21.6 (19.4–23.8)	26.5 (24.7–28.3)	0	14.6 (11.9–17.4)	15.6 (10.7–20.6)	0
Clothianidin						
Insecta						
Ephemeroptera						
Caenis sp.[c]	52.3 (25.2–79.3)	122.1 (82.9–161.3)	10	NC	NC	40
Cloeon sp.[c]	357.0 (9.1–704.9)	3939.2 (1044.9–6833.5)	0	NC	<16.4	0
Ephemerella sp.[c]	NC	586.9 (415.0–830.0)	0	NC	18.5 (13.3–25.7)	0
Hexagenia spp.	NC	>17 400.0	10	0.9 (0.4–1.5)	5.5 (3.9–7.0)	13
Isonychia bicolor[c]	NC	>17 40.0	0	NC	<108.8	0
McCaffertium sp.[c]	396.0 (120.7–671.3)	1328.3 (653.9–2002.7)	10	NC	<108.8	10
Neocloeon triangulifer	NC	3.5 (2.5–5.0)	0	NC	3.5 (2.5–5)	0
Odonata						
Coenagrion sp.	3336.9 (–197.5–6871.4)	145 56.3 (7632.8–21479.9)	10	<5918.8	<5918.8	10
Plecoptera						
Agnetina, Paragnetina sp.[c]	697.0 (287.0–1107.0)	1714.8 (1105.3–2324.2)	11	NC	<300.5	11
Hemiptera						
Trichocorixa sp.[c,d]	5.5 (–0.3–11.4)	34.8 (17.1–52.5)	0	5.1 (0.5–9.7)	21.3 (11.7–30.9)	0
Trichoptera						
Cheumatopsyche sp.[c]	235.8 (13.0–458.6)	1281.0 (423.1–2138.8)	0	NC	<108.8	0
Coleoptera						
Gyrinus sp.[c]	33.4 (18.8–48.1)	62.6 (45.4–79.8)	0	23.0 (13.6–32.5)	41.2 (30.2–52.1)	0

(*Continued*)

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TABLE 4: *(Continued)*

Species	LC10 ($\mu g\ L^{-1}$)	LC50 ($\mu g\ L^{-1}$)	Control mortality (%)[b]	EC10 ($\mu g\ L^{-1}$)	EC50 ($\mu g\ L^{-1}$)	Control immobilization (%)[b]
Stenelmis sp.[c]	81.2 (32.6–129.7)	208.0 (136.5–279.4)	0	NC	84.9 (60.0–120.0)	0
Diptera						
Chironomus dilutus	0.6 (–0.1–1.2)	11.6 (6.5–16.8)	0	NC	3.4 (2.7–5.5)	0
Aedes sp.[c,d]	10.3 (3.6–17.1)	28.5 (17.5–39.6)	22	NC	NC	NC
Crustacea						
Caecidotea sp.[c]	2368.7 (260.7–4476.8)	16085.8 (2636.6–29534.9)	0	108.5 (–17.8–234.8)	537.2 (248.0–826.3)	0
Ceriodaphnia dubia[d]	NC	>100 000.0	0	NC	NC	NC
Hyalella azteca	3.1 (2.4–3.8)	5.2 (4.4–5.9)	0	2.7 (2.0–3.3)	4.8 (4.1–5.6)	0
Oligochaeta						
Lumbriculus variegatus	NC	177.1 (145.3–207.5)	0	NC	41.7 (34.9–49.8)	0
Dinotefuran						
Insecta						
Ephemeroptera						
Caenis sp.[c]	1524.2 (500.3–2548.1)	5466.5 (3134.4–7798.6)	0	NC	<212.2	0
Cloeon sp.[c]	NC	>13 250.0	0	NC	<25.2	0
Ephemerella sp.[c]	82.0 (–53–217.1)	395.5 (119.4–671.6)	0	NC	<344.3	0
Hexagenia spp.	NC	>27 400.0	20	8.4 (6.0–10.9)	19.0 (15.6–22.5)	0
McCaffertium sp.[c]	NC	>5500.0	0	NC	<344.3	0
Neocloeon triangulifer	3.9 (2.4–5.3)	9.4 (7.1–11.6)	0	3.2 (2.2–4.1)	6.0 (4.8–7.2)	0
Odonata						
Coenagrion sp.	1193.8 (–2284.1–4671.7)	12 347.9 (29.3–24666.5)	20	NC	<5187.5	20
Hemiptera						
Trichocorixa sp.[c,d]	15.8 (7.6–24)	36.9 (25.1–48.8)	20	2.0 (–0.5–4.6)	10.1 (4.3–15.9)	20
Trichoptera						
Cheumatopsyche sp.[c]	NC	>5500.0	10	NC	419.5 (344.3–687.5)	10
Coleoptera						
Gyrinus sp.[c]	54.7 (–66.1–175.5)	1790.6 (306.6–3274.5)	0	NC	<163.0	0
Stenelmis sp.[c]	123.8 (7.8–239.8)	555.6 (299.5–811.8)	10	153.1 (71.1–235.1)	335.6 (230.6–440.7)	10
Diptera						
Chironomus dilutus	6.4 (3.9–8.9)	23.5 (18.0–29.0)	0	5.8 (4.2–7.4)	12.4 (10.2–14.5)	0
Aedes sp.[c,d]	134.8 (21.3–248.3)	741.5 (137.9–1345.1)	0	NC	NC	NC
Crustacea						
Caecidotea sp.[c]	NC	>27 300.0	0	NC	580.5 (426.6–853.1)	0
Ceriodaphnia dubia[d]	NC	>87000.0	0	NC	NC	NC
Hyalella azteca	36.2 (29.9–42.5)	57.8 (52.0–63.7)	0	32.8 (28.1–37.5)	44.5 (40.9–48.2)	0
Oligochaeta						
Lumbriculus variegatus	NC	2404.2 (1700.0–3400.0)	0	NC	601.0 (425.0–850.0)	0
Imidacloprid						
Insecta						
Ephemeroptera						
Caenis sp.[c]	NC	<21.8	0	NC	<21.8	0
Cloeon sp.[c]	126.3 (9.5–243.1)	1152.0 (513.1–1790.8)	0	NC	23.1 (16.2–33.2)	0
Ephemerella sp.[c]	16.1 (2.9–29.4)	68.2 (33.1–103.3)	10	NC	10.6 (7.5–15.0)	10
Hexagenia spp.	427.7 (47.7–807.6)	9320.5 (3757.2–14883.8)	0	NC	NC	20
Isonychia bicolor[c]	113.2 (–18.1–244.4)	715.2 (319.3–1111.0)	0	31.4 (16.9–45.8)	60.4 (43.2–77.7)	0
McCaffertium sp.[c]	738.7 (314.9–1162.5)	1810.2 (1018.2–2602.3)	10	NC	10.6 (7.5–15.0)	0
Neocloeon triangulifer	2.9 (2.0–3.7)	5.2 (4.2–6.2)	5	1.9 (1.4–2.4)	3.1 (2.6–3.7)	5

(Continued)

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TABLE 4: (*Continued*)

Species	LC10 ($\mu g\,L^{-1}$)	LC50 ($\mu g\,L^{-1}$)	Control mortality (%)[b]	EC10 ($\mu g\,L^{-1}$)	EC50 ($\mu g\,L^{-1}$)	Control immobilization (%)[b]
Odonata						
Coenagrion sp.	762.7 (–1835.1–3360.6)	3462.7 (–2046.6–8972.0)	20	NC	<5437.5	20
Hemiptera						
Trichocorixa sp.[c,d]	139.4 (51.4–227.4)	450.4 (274.0–626.7)	0	NC	63.1 (44.6–89.2)	0
Trichoptera						
Cheumatopsyche sp.[c]	48.9 (–3.2–101.0)	324.5 (72.1–576.8)	20	48.6 (12.2–85.1)	176.4 (99.7–253.1)	20
Micrasema sp.[c]	7.0 (3.8–10.1)	14.6 (11.0–18.2)	5	NC	<6.4	5
Coleoptera						
Gyrinus sp.[c]	79.6 (47.4–111.8)	132.2 (99.9–164.5)	0	28.0 (14.9–41.2)	57.5 (40.5–74.5)	0
Stenelmis sp.[c]	35.1 (–20.8–91.1)	365.7 (107.1–624.2)	10	44.6 (17.6–71.7)	99.2 (66.9–131.6)	10
Diptera						
Chironomus dilutes	1.7 (0.7–2.7)	11.8 (8.3–15.4)	0	1.4 (1.1–1.8)	2.5 (2.1–2.8)	0
Aedes sp.[c,d]	18.9 (99.4–28.5)	40.8 (27.9–53.6)	0	NC	NC	NC
Crustacea						
Caecidotea sp.[c]	NC	>15 600.0	0	98.0 (–6.1–202.2)	320.8 (162.0–479.6)	0
Ceriodaphnia dubia[d]	NC	72 124.9 (51 000.0–102 000.0)	0	NC	NC	NC
Daphnia magna[d]	NC	>102 000.0	0	NC	NC	NC
Hyalella azteca	114.0 (73.1–155.0)	363.2 (301.3–425.1)	0	77.2 (51.4–102.9)	176.9 (149.4–204.4)	0
Oligochaeta						
Lumbriculus variegatus	42.6 (9.9–75.4)	45.4 (30.6–60.1)	0	30.4 (8.5–52.3)	32.4 (26.7–38.0)	0
Thiacloprid						
Insecta						
Ephemeroptera						
Caenis sp.[c]	46.4 (1.6–91.3)	231.4 (120.7–342.1)	0	NC	<66.3	10
Cloeon sp.[c]	856.3 (232.7–1479.9)	3882.6 (1948.5–5816.7)	0	NC	23.1 (16.2–33.2)	0
Ephemerella sp.[c]	24.4 (–13.1–61.9)	190.6 (78.3–302.8)	0	NC	<58.0	0
Hexagenia spp.	NC	>9300.0	0	NC	<1.3	0
McCaffertium sp.[c]	NC	>920.0	0	NC	10.6 (7.5–15.0)	0
Neocloeon triangulifer	NC	1.9 (1.4–2.6)	0	NC	1.9 (1.4–2.6)	0
Odonata						
Coenagrion sp.	2377.4 (939.0–3815.9)	5647.2 (3762.42–7531.96)	0	NC	<2500.0	0
Hemiptera						
Trichocorixa sp.[c,d]	45.1 (15.1–75.1)	135.3 (84.7–185.9)	0	NC	<39.7	0
Trichoptera						
Cheumatopsyche sp.[c]	NC	>920.0	0	NC	162.6 (115.0–230.0)	0
Coleoptera						
Gyrinus sp.[c]	NC	180.9 (139.5–279.0)	0	40.8 (23.4–58.3)	72.3 (51.5–93.1)	0
Stenelmis sp.[c]	99.4 (55.5–143.2)	183.6 (133.1–234.1)	0	67.0 (33.8–100.2)	129.0 (92.2–165.9)	0
Diptera						
Chironomus dilutus	0.3 (0.2–0.5)	1.6 (1.2–2.0)	3	0.4 (0.3–0.5)	0.8 (0.7–0.9)	3
Aedes sp.[c,d]	10.0 (0.8–19.2)	53.4 (28.0–78.8)	0	NC	NC	NC
Crustacea						
Caecidotea sp.[c]	NC	NC	0	NC	<136.8	0
Ceriodaphnia dubia[d]	NC	>41 500	0	NC	NC	NC
Hyalella azteca	17.3 (10.9–23.6)	55.0 (43.0–67.0)	0	13.2 (9.6–16.9)	26.9 (22.3–31.5)	0
Oligochaeta						
Lumbriculus variegatus	29.9 (9.2–50.5)	33.8 (23.0–44.6)	0	NC	11.5 (8.1–16.2)	0

(*Continued*)

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TABLE 4: (*Continued*)

Species	LC10 ($\mu g\ L^{-1}$)	LC50 ($\mu g\ L^{-1}$)	Control mortality (%)[b]	EC10 ($\mu g\ L^{-1}$)	EC50 ($\mu g\ L^{-1}$)	Control immobilization (%)[b]
Thiamethoxam						
Insecta						
Ephemeroptera						
Caenis sp.[c]	61.6 (6.2–117.1)	381.9 (185.0–578.8)	0	NC	<23.3	0
Cloeon sp.[c]	815.5 (169.3–1461.7)	4633.6 (1835.8–7431.3)	0	NC	44.1 (31.2–62.4)	0
Ephemerella sp.[c]	45.1 (–13.4–103.8)	334.9 (135.9–533.9)	0	NC	<59.0	0
Hexagenia spp.	NC	>30 800.0	7	1.0 (-0.2–2.2)	35.8 (14.1–57.4)	10
Isonychia bicolor[c]	NC	>7120.0	0	NC	<445.0	0
McCaffertium sp.[c]	NC	>920.0	0	NC	81.7 (58.0–115.0)	0
Neocloeon triangulifer	NC	5.5 (3.9–7.8)	0	NC	5.5 (3.9–7.8)	0
Odonata						
Coenagrion sp.	1290.4 (–2327.2–4908.0)	15 061.8 (619.0–29 504.6)	20	NC	<4187.5	20
Plecoptera						
Agnetina, Paragnetina sp.[c]	NC	>7120.0	0	NC	<445.0	0
Hemiptera						
Trichocorixa sp.[c,d]	126.2 (–18.5–270.9)	1473.1 (176.3–2769.9)	0	NC	56.3 (34.3–68.6)	0
Trichoptera						
Cheumatopsyche sp.[c]	53.1 (–8.7–114.8)	170.1 (78.6–261.6)	14	NC	118.5 (108.8–218.0)	14
Micrasema sp.[c]	23.2 (16.7–29.8)	32.8 (26.4–39.2)	10	NC	18.5 (13.1–26.2)	10
Coleoptera						
Gyrinus sp.[c]	NC	31.0 (21.9–43.8)	0	4.3 (0.6–8.0)	14.0 (7.6–20.4)	0
Stenelmis sp.[c]	86.0 (50.7–121.3)	148.0 (109.6–186.4)	10	86.0 (50.7–121.3)	148 (109.6–186.4)	10
Diptera						
Chironomus dilutus	16.0 (9.5–22.5)	61.9 (45.4–78.4)	0	13.7 (9.3–18.2)	36.8 (29.4–44.3)	0
Aedes sp.[c,d]	23.2 (8.6–37.8)	67.4 (42.2–92.5)	0	NC	NC	NC
Crustacea						
Caecidotea sp.[c]	NC	>35 600	0	1512.0 (566.6–2457.4)	4775.4 (2976.3–6574.6)	10
Ceriodaphnia dubia[d]	NC	>80 000	0	NC	NC	NC
Daphnia magna[d]	NC	>80 000	0	NC	NC	NC
Hyalella azteca	183.8 (105.0–262.7)	801.0 (518.7–1083.3)	7	151.1 (102.4–199.7)	391.0 (312.1–469.9)	7
Oligochaeta						
Lumbriculus variegatus	1762.5 (1406.4–2118.6)	3438.2 (3025.5–3850.9)	0	1908.0 (607.1–3208.8)	2035.1 (1699.7–2370.6)	0

[a]The *x*% lethal concentration (LC*x*) and effect concentration (EC*x*; immobilization) values are given, with 95% confidence intervals in parentheses. All estimates are based on concentrations directly measured or calculated using a correction factor based on the percentage difference between nominal and measured concentrations.
[b]Control mortality/immobilization = percentage of negative control individuals exhibiting mortality/immobilization.
[c]Number of experimental replicates = 1. For all other tested species, replicates ≥ 3.
[d]Test duration 48 h. All other test durations 96 h.
NC = not calculable.

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laboratory-cultured species to be overestimated. Differential binding affinity at the *n*AChR is another potential source of variation. Neonicotinoids are thought to be highly selective in their binding to insect versus mammalian *n*AChRs due to structural differences in the receptor site that lead to changes in binding affinity (Tomizawa and Casida 2003). Within insects, variation in the type and number of *n*AChR subunits leads to differences in the way agonists, such as neonicotinoids, interact with the receptor (Jones et al. 2007). Conversely, different neonicotinoids have been shown to act differently at the same *n*AChR (Brown et al. 2006). For example, imidacloprid has been shown to act as a partial agonist, and clothianidin as a superagonist, on native *Drosophila* *n*AChRs (Brown et al. 2006).

Among the 7 mayfly species tested, response to neonicotinoids was highly variable, with LC50s spanning 4 orders of magnitude. *Neocloeon triangulifer* was the most sensitive mayfly, whereas the least sensitive in terms of lethality was *Hexagenia* spp., and the field-collected *Cloeon* sp. and *McCaffertium* sp., with LC50s $>890\,\mu g\,L^{-1}$; however, these species were immobilized at much lower concentrations, with most EC50s $<100\,\mu g\,L^{-1}$. In general, Ephemeroptera and Diptera exhibited immobility at concentrations much lower than those that caused lethality, as illustrated by the difference between median LC50 and EC50 estimates (Figure 1). An extreme example of this disparity occurred in the mayfly *Hexagenia* spp., which had EC50s of $<40\,\mu g\,L^{-1}$ for all neonicotinoids, but a concentration–response relationship for lethality could not be established even for concentrations 2 to 3 orders of magnitude greater. Although *Hexagenia* spp. was the only sediment-dwelling mayfly studied, all exposures were water-only, which therefore negated the influence of sediment on toxicity. Rubach et al. (2010, 2011) found some freshwater invertebrates that also showed a large sensitivity difference between immobility and lethality for the pesticide chlorpyrifos; through studying uptake and elimination kinetics, they showed that these species had high uptake rates and bioconcentration factors, and suggested differences in biotransformation or compensatory gene-regulation abilities as likely sources of variability (Rubach et al. 2010, 2011). Extrapolating to the present study, *Hexagenia* spp. may have bioconcentrated neonicotinoids to a lesser degree than the other mayflies, or may have been able to biotransform neonicotinoids once accumulated so to keep internal residues low, leading to constant immobility but not lethality.

Macroinvertebrates that become immobile as a result of neonicotinoid exposure may be subject to drift (in a lotic system). Berghahn et al. (2012) showed that repeated 12-h pulses of $12\,\mu g\,L^{-1}$ imidacloprid caused drift of insect larvae and young *Gammarus*. Immobile individuals may also be subject to increased predation due to reduced escape behaviors. Pestana et al. (2009) showed that exposure to a formulation of imidacloprid altered behavior in response to predation in the insects *C. riparius* (Diptera) and *Sericostoma vittatum* (Trichoptera). Both effects could reduce or remove these organisms from the community and make the endpoint of immobilization functionally equivalent to mortality. Endpoints that consider immobility, such as the EC50s in the present study, may therefore be a more relevant measure of real-world toxicity.

Recent studies have shown that neonicotinoid toxicity is influenced by temperature and seasonality. Camp and Buchwalter (2016) found a positive relationship among temperature, imidacloprid uptake, and toxicity for aquatic insects, most notably for mayflies. Van den Brink et al. (2016) also found a slight increase in toxicity at higher temperatures, as well as differences in sensitivity to imidacloprid between summer and overwintering generations of the mayfly *C. dipterum*. When

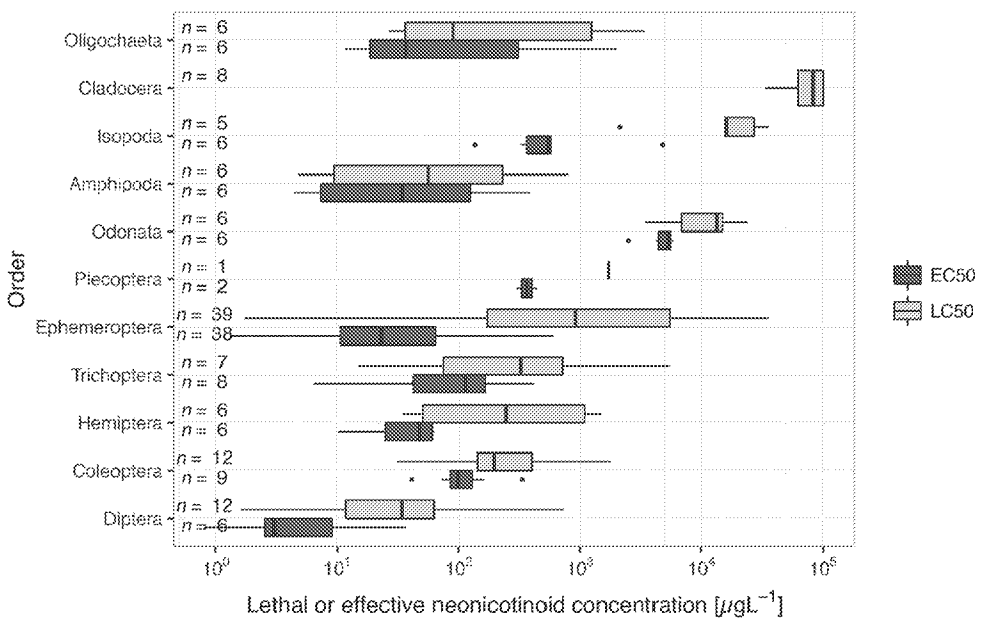

FIGURE 1: Distribution of median lethal (LC50) and effect (EC50) concentrations. Lower and upper threshold data (e.g., $>9600\,\mu g\,L^{-1}$) were treated as-is (e.g., $9600\,\mu g\,L^{-1}$). Data for 6 neonicotinoids are pooled.

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tested at the same temperature, summer generations were more sensitive in terms of immobilization, but not lethality, to imidacloprid than overwintering generations (Van den Brink et al. 2016). The present study used early summer (May) populations for lentic mayflies, and overwintering (November–December) populations for lotic mayflies. Assuming that the seasonal results of Van den Brink et al. (2016) are indicative of other mayfly species, the results for lotic mayflies in the present study could be underprotective. All field-collected organisms were tested at temperatures of 12 to 15 (±2) °C with the goal of mimicking field collection conditions in the laboratory. When compared with laboratory-cultured species, which were cultured and tested at 23 to 25 (±2) °C, field-collected species' toxicity could be underestimated due to lower testing temperatures, and associated lower uptake and toxicity. Additional studies characterizing neonicotinoid toxicity to the most sensitive taxa (e.g., the mayfly *N. triangulifer*) are required to understand how neonicotinoid toxicity is affected by temperature. This is especially needed for spring and early summer temperatures when neonicotinoid exposure is potentially at peak in streams, because transport to, and occurrence in, aquatic systems has been shown to be driven by crop planting and precipitation events (Hladik et al. 2014), both of which occur in spring and early summer.

With few exceptions, the data given in the present study are typically ≤1 order of magnitude compared with neonicotinoid toxicity data in the literature. In general, our median effect and lethal concentrations were greater than those from other studies, which may partially reflect our stricter criteria for the confirmation of lethality and use of technical rather than formulated product. There were some exceptions in which our data indicated toxicity that was >1 order of magnitude compared with published data. These include a reported 96-h LC50 for *C. dipterum* of 26.3 $\mu g\,L^{-1}$ imidacloprid (Roessink et al. 2013), a 44-fold difference compared with our value of 1152 $\mu g\,L^{-1}$ and a reported 48-h LC50 for *C. dubia* of 2.11 $\mu g\,L^{-1}$ (Chen et al. 2010), a 34 000-fold difference compared with our value of 72 124 $\mu g\,L^{-1}$. Both of these studies used formulated products, like many neonicotinoid toxicity studies in the literature (Alexander et al. 2007; Beketov and Liess 2008; Stoughton et al. 2008; Pestana et al. 2009; Roessink et al. 2013); perhaps these authors reasoned that because the formulated product is applied in crop protection it therefore provides a more realistic assessment of possible exposure in the environment. We used technical grade neonicotinoids in our study to facilitate comparison of the relative potency between neonicotinoids without the possible confounding toxicity of formulants. It is unclear whether there is a difference between technical and formulated neonicotinoid products because few studies have compared them. Stoughton et al. (2008) found a 3.75-fold difference in 96-h LC50s between technical imidacloprid and the formulation Admire® for *H. azteca*; however, the LC25s and LOECs were similar. The authors indicated that the difference in toxicity at higher concentrations may have been due to toxicity at higher concentrations of formulants, or combined toxicity between the formulants and the active ingredient (Stoughton et al. 2008). The same experiment with *Chironomus tentans* found no difference in toxicity between the technical and formulated product. In a separate study, a comparison of toxicity to *D. magna* found the commercial liquid formulation Confidor SL 200 (imidacloprid) to be 4× more toxic (by LOEC) than the active ingredient (Jemec et al. 2007). Further studies are needed to better understand the differences between formulated and technical neonicotinoid insecticides.

Hazard assessment

A hazard assessment was conducted to compare acute toxicity data with the maximum or 99th percentile of neonicotinoid surface water detections in Ontario. This assessment conservatively approximates short-term exposures such as pulse exposures. The HC5s determined from both lethality and immobilization-derived SSDs are presented in Table 3. The SSDs (Figure 2) yielded the following order of neonicotinoid toxicity based on the more robust lethality-derived SSDs: acetamiprid ≈ thiacloprid < imidacloprid ≈ clothianidin ≈ dinotefuran ≈ thiamethoxam. The HQs, calculated as the ratio of the HC5 to environmental concentrations for lethality-derived datasets for each neonicotinoid insecticide, indicated no acute hazard (HQ < 0.1) for dinotefuran, thiacloprid, and thiamethoxam, or low acute hazard (0.1 < HQ < 1.0) for acetamiprid, clothianidin, and imidacloprid. Immobilization-derived datasets (see SSDs in the Supplemental Data, Figure S13) indicated low acute hazard for acetamiprid, dinotefuran, thiacloprid, and thiamethoxam, and moderate acute hazard (HQ > 1.1) for imidacloprid. Hazard could not be determined for clothianidin, because none of the immobilization-derived SSDs constructed passed a goodness-of-fit test or visual inspection. Guidelines for interpreting HQs were based on Lemly (1996). When the more robust, but less sensitive endpoint of lethality is considered, this hazard assessment indicates a low or no acute hazard situation for 95% of the species tested exposed to the 99th percentile or the maximum of the Ontario surface water concentrations detected. Alternatively, when the less robust, but more sensitive and perhaps more environmentally relevant endpoint of immobilization is considered, this hazard assessment indicates a moderate hazard for the neonicotinoid imidacloprid. For the other neonicotinoids acetamiprid, dinotefuran, thiacloprid, and thiamethoxam, a conclusion of low or no acute hazard remained; for clothianidin, hazard could not be determined due to ill-fitting species sensitivity distribution models.

The results of this hazard assessment are comparable to those of other recent neonicotinoid hazard or risk assessments for aquatic invertebrates that are based on single-neonicotinoid SSDs. A recent probabilistic ecological risk assessment for imidacloprid by Whitfield-Aslund et al. (2017), which considered both acute lethal and immobility data, derived an acute HC5 of 1.73 μg active ingredient L^{-1}; when compared with exposure distributions for multiple use patterns, they categorized the risk to aquatic invertebrates as de minimis. Similarly, Finnegan et al. (2017) constructed an acute SSD for thiamethoxam and derived HC5s of 5.1 and 22.9 $\mu g\,L^{-1}$ using EC50s and LC50s, respectively. When compared with neonicotinoids in North American surface water samples, the risk was found to be low,

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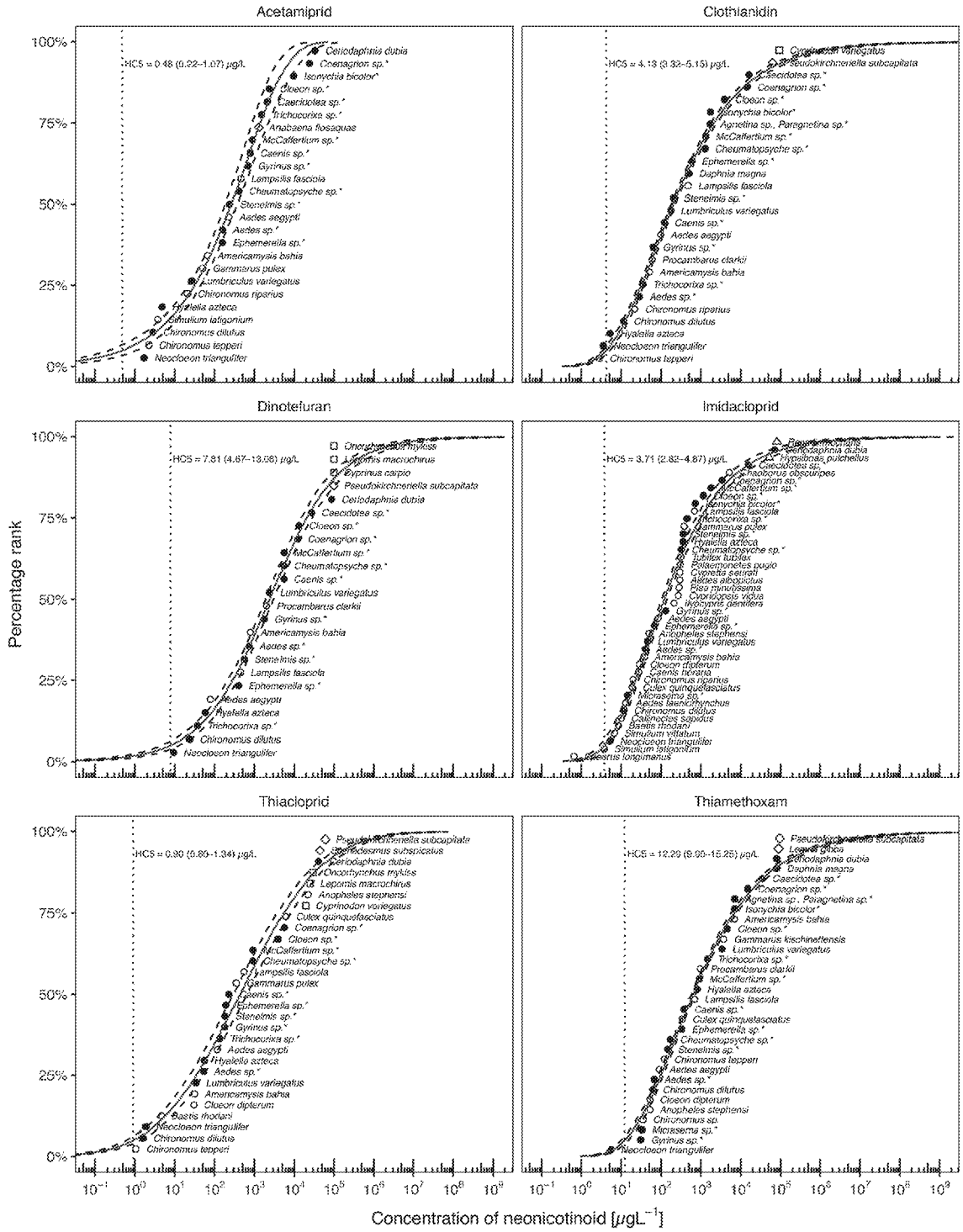

FIGURE 2: Species sensitivity distributions (SSDs) of acute (≤5 d) toxicity data for 6 neonicotinoids constructed using median lethal concentration (LC50) values for aquatic invertebrates generated from the present study and external literature data mined from the ECOTOX database. Closed (black) shapes represent data from the present study (internal), and open (white) shapes represent external data. Circles represent aquatic invertebrates, squares represent fish, triangles represent plants, and diamonds and triangles represent amphibians. For data from the present study, all field-collected species (indicated with an asterisk [*]) were derived from tests with a single replicate; the remaining internal data were laboratory-cultured species tested with a minimum of 3 replicates. Data were fit to regression models (red line) with a 95% confidence band for the regression (dashed line). Data for clothianidin, imidacloprid, and thiamethoxam were fit to Gumbel models; data for acetamiprid were fit to the extreme value model; data for dinotefuran were fit to the logistic model; and data for thiacloprid were fit to the normal model. Data for imidacloprid did not pass the Anderson–Darling goodness-of-fit test for any of the 4 possible models, but did pass visual inspection. The vertical dotted line illustrates the 5th percentile hazardous concentration (HC5), and the 95% confidence intervals are given in parentheses. The SSDs were modeled in CCME V3 Master software and plotted in R with ggplot2. Model parameters are given in the Supplemental Data, Table S10.

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with a likelihood of ~0.5% of exceeding the HC5. In contrast to these studies, an earlier assessment by Morrissey et al. (2015) derived an HC5 of 0.63 $\mu g\ L^{-1}$, which is lower than the results shown in the present study and by others (Finnegan et al. 2017; Whitfield-Aslund et al. 2017).

CONCLUSIONS

The present study contributes novel information to support the growing knowledge of neonicotinoid toxicity to aquatic invertebrates, especially for lesser studied aquatic invertebrates and poorly documented neonicotinoids such as acetamiprid, thiacloprid, and dinotefuran. The acute toxicity data presented in our study are relevant to short-term pulses of neonicotinoids in aquatic environments, which typically occur during heavy precipitation events and during spring freshet. Importantly, our observation of immobilization but not lethality for many species over the exposure periods used in the present study suggests that recovery may be possible following pulse exposure scenarios in the field. Future work in our laboratory will test this hypothesis. Furthermore, we found that sensitivity for many other species was often less than indicated in the literature. The derived HC5s, therefore, are greater than those estimated in previous work and may represent a more realistic assessment of acute neonicotinoid toxicity.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.4088.

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Data Availability—Data pertaining to this manuscript are deposited in figshare at DOI: 10.1002/etc.4088.

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Supplemental Data for:

"Acute toxicity of six neonicotinoid insecticides to freshwater invertebrates"

Melanie Raby, Monica Nowierski, Dmitri Perlov, Xiaoming Zhao, Chunyan Hao, David G. Poirier, Paul K. Sibley

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Contents:

Table S1. Summary of culture methods

Table S2. Physicochemical properties of dechlorinated water

S3. Neonicotinoid analysis methodology

S4. Neonicotinoid sample storage time correlation

Table S5. Correlation statistics for neonicotinoid sample storage time

Table S6. Test organism taxonomy

S7. Recirculating apparatus design

- **Figure S7-A.** Recirculating apparatus
- **Figure S7-B.** Motor, glass mixing chamber and ramp

Table S8. Water quality parameters for toxicity tests at test takedown

Table S9. Additional toxicity data mined from ECOTOX database for use in invertebrate lethality-derived species sensitivity distributions

Table S10. Summary of lethality (LC50)-derived species sensitivity distribution model parameters

Table S11. Additional toxicity data mined from ECOTOX database for use in invertebrate immobilization-derived species sensitivity distributions

Table S12. Summary of immobilization (EC50)-derived species sensitivity distribution model parameters

ED_002896_00040345-00017

Figure S13. Species sensitivity distributions (SSDs) of acute 48 or 96-h toxicity data for 6 neonicotinoids constructed using invertebrate immobilization (EC50) values

S14. Details on 3 Ontario neonicotinoid monitoring studies used in hazard assessment

Figure S15. Empirical cumulative distribution functions (ECDFs) for neonicotinoid monitoring dataset

Figure S16. *Hexagenia* spp. response after 96-h clothianidin exposure

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ED_002896_00040345-00018

Table S1. Summary of culture methods

	Neocloeon triangulifer	*Ceriodaphnia dubia*	*Daphnia magna*	*Hyalella azteca*	*Chironomus dilutus*	*Hexagenia spp.*	*Lumbriculus variegatus*
Reference	(Weaver et al. 2015)	(Environment Canada 2007)	(Ontario Ministry of the Environment and Climate Change Aquatic Toxicology Unit 2014)	(Ontario Ministry of the Environment and Climate Change Aquatic Toxicology Unit 2016a)	(Ontario Ministry of the Environment and Climate Change Aquatic Toxicology Unit 2016b)	(Ontario Ministry of the Environment and Climate Change Aquatic Toxicology Unit 2016c)	(Ontario Ministry of the Environment and Climate Change Aquatic Toxicology Unit 2016d)
Temperature (°C)	25 ± 2	25 ± 2	20 ± 2	23 ± 2	23 ± 2	23 ± 2	23 ± 2
Diurnal cycle	16:8 light: dark cycle						
Light intensity (lux)	100 – 500 lux	100 – 500 lux	400 – 800 lux	500 – 2500 lux	5000 – 1000 lux	100 – 1000 lux	100 – 1500 lux
Culture vessel and media	0 - ~10 d: 1 L flat-bottom glass dish with ~700 mL dechlorinated water ~10 d – emergence: 10 L tank with 3 L dechlorinated water	30 mL flat-bottom glass tubes with 15 mL selenium-spiked dechlorinated water	~2 L polyethylene containers, ~1.5 L culture water	~2 L polyethylene containers, filled with culture water	Breeding tanks: 20 L glass aquaria, half full with sand and dechlorinated water Rearing trays: white phenolic trays with several cm of dechlorinated water	10 L aquaria, with 1 L sediment and ~8 L dechlorinated water	>20 L glass aquaria, set-up with a flow-through system; flow rate approximately 200 mL/min
Substrate	None	None	None	2x5 cm pieces of cotton gauze	Breeding tanks: 1.6 L silica sand Rearing trays: none	Long Point sediment	Soaked, shredded, unbleached paper towel
Aeration	None	None	None	None	Breeding tanks: Constant gentle aeration	Constant gentle aeration	Constant gentle aeration

ED_002896_00040345-00019

					Rearing trays: none		
Feeding	Diatom (*Navicula* sp.) biofilms grown on frosted slides, added as needed	0.5 mL concentrated *Pseudokirchneriella subcapitata*, 0.1 mL filtered YCT daily	5 – 15% mixed algae (*Pseudokirchneriella subcapitata* and *Chlorella fusca*) in dechlorinated water	~20 mg ground Nutrafin daily	Slurry made of 3:2 cereal grass:ground Nutrafin in dechlorinated water; feeding as needed	Slurry as per *C. dilutus* feeding; 5-10 mL 1-2x weekly, based on a ramped feeding regime	6 g trout chow blended with dechlorinated water into a slurry, fed 3-7x/week
Water change frequency	Half refreshed 1-2x weekly	All refreshed 3x weekly	Half refreshed daily	Half refreshed 1-2x monthly	Partially refreshed as needed	Tanks topped up daily	Continuous flow
Additional details	None	Dechlorinated water spiked with 3 µg L^{-1} selenium	Selenium and vitamin B12 (1 µg L^{-1} each) added weekly	None	Egg cases collected from breeding tanks daily; hatched egg cases used to start rearing trays; breeding tank started with ~10-12 d old larvae	Eggs obtained from J. Ciborowski (University of Windsor) and stored at 7 ± 2°C for up to 12 months	Snails (*Planorbella pilbryi*) added to tanks as needed to reduce fungal contamination

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Table S2. Physicochemical properties of dechlorinated water used in all acute tests (years 2015-2017, n=5 or 6 for all properties)

Property	Unit	Mean	Std Dev	Range
Chloride	mg/L	29.03	3.62	24.90 - 36.80
Sulphate	mg/L	28.33	1.17	26.70 - 29.90
Conductivity	µS/cm	333	18.3	313 - 361
pH		8.01	0.15	7.72 - 8.21
Alkalinity	mg/L CaCO3	77.70	3.88	69.90 - 81.80
NH4+/NH3	mg/L	0.05	0.07	0.006 - 0.273
NO3-/NO2-	mg/L	0.58	0.15	0.268 - 0.718
Phosphorus/phosphate	mg/L	0.74	0.13	0.579 - 0.931
Dissolved organic carbon	mg/L	1.19	0.49	0.10 - 1.70
Dissolved inorganic carbon	mg/L	21.36	0.98	19.6 - 23.3
Silicon/reactive silicate	mg/L	0.56	0.07	0.46 - 0.66
Bromide	µg/L	4.65	3.08	1.21 - 10.30
Magnesium	mg/L	8.43	0.38	7.82 - 8.96
Potassium	mg/L	1.53	0.08	1.42 - 1.64
Sodium	mg/L	15.10	1.64	13.60 - 18.10
Copper	µg/L	0.90	0.45	0.26 - 1.80
Hardness	mg/L	122	4.94	112 - 128

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S3. Neonicotinoid analysis methodology

Analysis was performed by liquid chromatography/tandem mass spectrometry (LC-MS/MS) on a Shimadzu Prominence HPLC system and Sciex 5500QTrap mass spectrometer following MOECC routine method E3544 "The determination of neonicotinoids in water by liquid chromatography-tandem mass spectrometry analysis" (available upon request to laboratoryservicesbranch@ontario.ca). LC-MS/MS analysis was conducted using an electrospray ionization source (ESI) in positive ionization and Multiple Reaction Monitoring (MRM) scan mode. Identification and quantification of the target analytes are based on retention times (RTs) and the existence of the MRM transitions by running a sample against the prepared standards under the same instrumental conditions and comparing obtained responses of samples to those of the standards. Target compounds in aqueous environmental matrices were measured without sample concentration by direct aqueous injection (injection volume of 90 μL), and where results exceeded the calibration range of an analyte (500-2000 ng/L for different analytes, Table 2), samples were diluted as needed with HPLC Grade water.

S4. Neonicotinoid sample storage time correlation

Samples were stored for 1.6 – 16.6 weeks before analysis, with a mean storage time of 3.4 weeks (SD = 2.5 weeks, N = 149). A Spearman correlation analysis using cor.test in R (v. 3.3.2) was conducted for each neonicotinoid between storage time and the percent difference between nominal and measured concentrations. Storage time and percent difference were moderately correlated, but with no statistical significance ($\alpha = 0.05$) for clothianidin, dinotefuran, thiacloprid, and thiamethoxam; weakly correlated with statistical significance for imidacloprid; and moderately correlated with statistical significance (*rho* = 0.43, p = 0.06) for acetamiprid. No

ED_002896_00040345-00022

strong correlations were observed, indicating neonicotinoids did not degrade in storage between sampling and analysis. Spearman *rho* estimates and p-values are in Table S5. Neonicotinoid stability in solution under typical test temperature (23 ± 2°C) and light (500 – 1000 lux) conditions was evaluated for 3 neonicotinoids (imidacloprid, thiamethoxam, and clothianidin), each at 2 concentrations. The mean difference in analyte concentrations after 96 ± 4 h was -1.1% (SD = 4.7%, range -6.6% – 4.8%). Prosser et al. (2016) observed similar stability of imidacloprid, clothianidin, and thiamethoxam, with a mean percent difference of 3.7% over a 7-d exposure period.

Table S5. Correlation statistics for neonicotinoid sample storage time

Neonicotinoid	Spearman's *rho*	p-value	n	Interpretation
Acetamiprid	0.43	0.061	20	Moderate positive correlation, statistically significant
Clothianidin	-0.38	0.033	32	Moderate negative correlation, not statistically significant
Dinotefuran	0.57	0.02	16	Moderate positive correlation, not statistically significant
Imidacloprid	0.19	0.24	32	Very weak positive correlation, statistically significant
Thiacloprid	-0.5	0.023	20	Moderate negative correlation, not statistically significant
Thiamethoxam	0.41	0.024	29	Moderate positive correlation, not statistically significant

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Table S6. Test organism taxonomy

Common name	**Collection habitat**	**Ecological function** (Cummins and Merritt 1984)	**Phylum**	**Subphylum**	**Class**	**Order**	**Family**	**Genera**	**Species**
Water flea	Laboratory-cultured	Collectors: filter-feeders	Arthropoda	Crustacea	Branchiopoda	Cladocera	Daphniidae	*Ceriodaphnia*	*dubia*
Water flea			Arthropoda	Crustacea	Branchiopoda	Cladocera	Daphniidae	*Daphnia*	*magna*
Midge larvae		Shredders, collectors, scrapers	Arthropoda	Hexapoda	Insecta	Diptera	Chironomidae	*Chironomus*	*dilutus*
Mayfly nymph			Arthropoda	Hexapoda	Insecta	Ephemeroptera	Ephemeridae	*Hexagenia*	spp.
Scud			Arthropoda	Crustacea	Malacostraca	Amphipoda	Hyalellidae	*Hyalella*	*azteca*
Aquatic worm			Annelida	-	Clitellata	Lumbriculida	Lumbriculidae	*Lumbriculus*	*variegatus*
Mayfly nymph			Arthropoda	Hexapoda	Insecta	Ephemeroptera	Baetidae	*Neocloeon*	*triangulifer*
Aquatic pillbug	Lentic/pond		Arthropoda	Crustacea	Malacostraca	Isopoda	Asellidae	*Caecidotea*	sp.
Whirligig beetle			Arthropoda	Hexapoda	Insecta	Coleoptera	Gyrinidae	*Gyrinus*	sp.
Mayfly nymph			Arthropoda	Hexapoda	Insecta	Ephemeroptera	Caenidae	*Caenis*	sp.
Mayfly nymph			Arthropoda	Hexapoda	Insecta	Ephemeroptera	Baetidae	*Cloeon*	sp.
Water boatmen			Arthropoda	Hexapoda	Insecta	Hemiptera	Corixidae	*Trichocorixa*	sp.
Mosquito larvae			Arthropoda	Hexapoda	Insecta	Diptera	Culicidae	*Aedes*	sp.
Damselfly nymph		Predators	Arthropoda	Hexapoda	Insecta	Odonata	Coenagrionidae	*Coenagrion*	sp.
Mayfly nymph	Lotic/stream	Shredders, collectors, scrapers	Arthropoda	Hexapoda	Insecta	Ephemeroptera	Heptageniidae	*McCaffertium*	sp.
Mayfly nymph			Arthropoda	Hexapoda	Insecta	Ephemeroptera	Ephemerellidae	*Ephemerella*	sp.
Mayfly nymph			Arthropoda	Hexapoda	Insecta	Ephemeroptera	Isonychiidae	*Isonychia*	*bicolor*
Caddisfly larvae (net-spinning)			Arthropoda	Hexapoda	Insecta	Trichoptera	Hydropsychidae	*Cheumatopsyche*	sp.
Caddisfly larvae (case-building)			Arthropoda	Hexapoda	Insecta	Trichoptera	Brachycentridae	*Micrasema*	sp.
Beetle			Arthropoda	Hexapoda	Insecta	Coleoptera	Elmidae	*Stenelmis*	sp.
Stonefly nymph			Arthropoda	Hexapoda	Insecta	Plecoptera	Perlidae	*Agnetina, Paragnetina*	sp.

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S7. Recirculating apparatus design

The following recirculating apparatus (Figure S7-A) was modeled after Rodrigues & Kaushik (1984).

The apparatus was designed to mimic a stream for testing stream-dwelling (lotic) invertebrates such as mayflies, stoneflies, and beetles to toxicants under water-only acute (96 h) exposures.

The apparatus consisted of:

A. A 10 L (12" x 6" x 8") glass reservoir aquarium with 4-7 L of test solution. Aquaria were purchased from Miracles Aquariums (Toronto, Ontario, Canada).

B. A motor adapted from an aquarium filter (Aqueon™ AquaClear 20). The motor and its housing was removed from the rest of the filtration system, and a custom made acrylic fitting (Figure S6-B) and ½" PVC male barbed insert adapter was used to attach an approximately 80 cm piece of flexible tubing (Tygon® 3350, ½" internal diameter x 11/16" outer diameter).

C. A glass mixing chamber.

D. A downward angled (~5°) glass ramp.

The glass mixing chamber and ramp (Figure S7-B) were constructed using 2 mm glass and 100% silicone (GE Silicone, Clear Silicone Caulk, Lowe's Canada) as an adhesive.

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Figure S7-A. Recirculating apparatus in water bath. Motors connected to tubing (see Figure S7-B) are submerged in the reservoir aquaria.

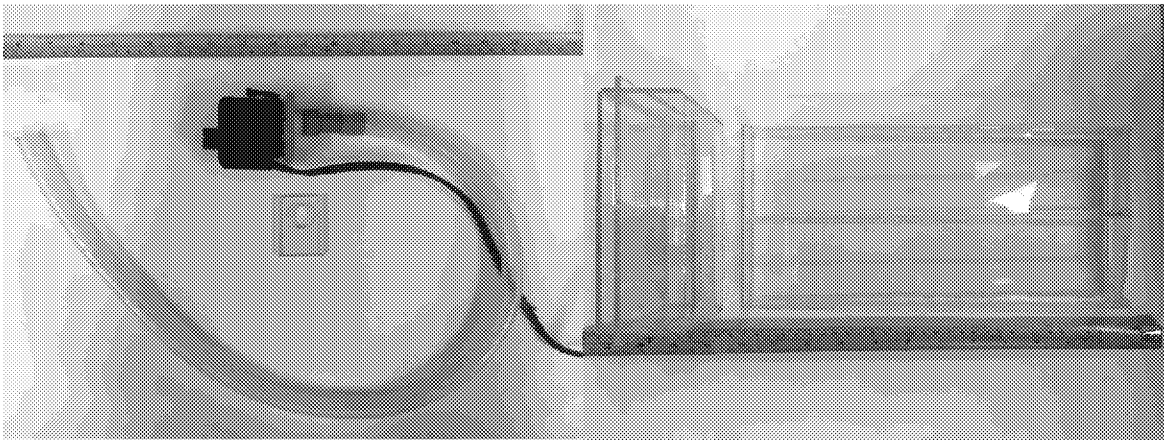

Figure S7-B. On left: Motor adapted from aquarium filter, with additional custom made acrylic fitting (6 mm thick x 3.8 cm wide x 5 cm long, with threaded hole sized to match male barbed insert adapter) shown. On right: glass mixing chamber and ramp.

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Table S8. Water quality parameters for toxicity tests at test takedown (t = 48 or 96 h) for 2-6 neonicotinoids

Species	n	pH				Conductivity (µS/cm)				Dissolved oxygen (mg/L)				Final temperature (°C)			
		Min	Max	Mean	Stdev	Min	Max	Mean	Stdev	Min	Max	Mean	Stdev	Min	Max	Mean	Stdev
Aedes sp.	6	7.8	8.1	8.0	0.11	313	445	346	35.8	7.7	11.2	9.6	1.1	12.0	15.5	13.8	1.46
Agnetina/Paragnetina sp.	2	8.1	8.2	8.2	0.06	311	341	327	15.2	10.1	10.9	10.5	0.5	14.3	14.5	14.4	0.12
Caecidotea sp.	6	*Final parameters not taken*												13.0	14.0	13.6	0.40
Caenis sp.	6	8.1	8.3	8.2	0.07	265	312	287	19.3	9.4	10.1	9.9	0.3	14.5	14.8	14.7	0.13
Ceriodaphnia dubia	6	8.2	8.8	8.4	0.20	319	363	336	13.8	8.0	9.4	8.7	0.6	22.2	24.0	23.0	0.65
Cheumatopsyche sp.	5[a]	8.1	8.2	8.2	0.04	312	348	327	13.8	10	11	10.4	0.4	13.9	17.0	14.5	0.97
Chironomus dilutus	6	7.7	8.1	8.0	0.14	310	414	363	34.8	6.0	7.5	6.8	0.6	21.0	23.4	22.2	0.95
Cloeon sp.	6	8.2	8.2	8.2	0.00	270	270	270	0.0	10.1	10.1	10.1	0.0	14.8	14.8	14.8	0.00
Coenagrion sp.	6	7.8	8.1	8.0	0.09	270	383	295	37.9	8.0	9.7	9.3	0.7	10.5	15.8	14.3	1.82
Daphnia magna	2	8.5	8.7	8.7	0.10	310	315	313	2.2	9.7	10.3	10.1	0.3	20.8	20.9	20.9	0.06
Ephemerella sp.	6	8.1	8.2	8.2	0.05	312	348	326	14.9	10.1	11	10.6	0.3	13.9	14.5	14.1	0.21
Gyrinus sp.	6	8.0	8.2	8.1	0.08	318	346	334	11.7	8.7	10.6	9.9	0.9	14.5	15.6	14.9	0.42
Hexagenia spp.	6	7.7	8.3	8.1	0.20	302	385	352	27.7	6.0	8.1	7.4	0.7	20.0	23.0	21.9	1.07
Hyalella azteca	6	7.5	7.9	7.8	0.11	310	423	360	28.5	4.5	7.4	6.3	0.9	21.2	23.8	22.4	0.91
Isonychia bicolor	4	8.1	8.2	8.2	0.04	310	341	329	11.0	10	10.9	10.3	0.4	14.1	17.0	15.0	1.15
Lumbriculus variegatus	6	8.1	8.3	8.2	0.07	326	375	348	15.7	7.6	8.5	8.1	0.3	21.0	22.5	21.7	0.61
McCaffertium sp.	6	8.1	8.2	8.2	0.04	312	348	330	12.9	10	11	10.4	0.4	13.9	17.0	14.6	1.10
Micrasema sp.	2	*Final parameters not taken*													15.0	15.0	0.00
Neocloeon triangulifer	6	8.5	8.8	8.7	0.10	325	385	363	19.9	8.0	8.6	8.4	0.2	21.5	27.0	24.1	1.92
Stenelmis sp.	2[b]	8.2	8.2	8.2	0.00	364	380	374	7.8	7.8	7.8	7.8	0.0	14.5	14.6	14.6	0.05
Trichocorixa sp.	6	8.0	8.2	8.1	0.07	310	334	322	8.1	8.3	11	10.1	0.8	13.1	19.9	15.2	2.42

[a] Final parameters not taken for *Cheumatopsyche* sp. test for thiamethoxam

[b] Final parameters not taken for *Stenelmis* sp. test for acetamiprid, clothianidin, dinotefuran, or thiacloprid

Note: For tests where lethality and immobilization tests were conducted separately (e.g. *Hexagenia* spp.), parameters are presented together

Table S9. Additional toxicity data mined from ECOTOX database for use in invertebrate lethality-derived species sensitivity distributions

Neonicotinoid	Taxa grouping	*Species*	Endpoint Type	Value (µg L^{-1})†	Duration (d)	Tolerant?*	Reference
Acetamiprid	Fish	*Cyprinodon variegatus*	LC50	100000	4	Y	(Environmental Fate and Effects Division, U.S. EPA, Washington 1992)
	Fish	*Oncorhynchus mykiss*	LC50	100000	4	Y	
	Fish	*Lepomis macrochirus*	LC50	119300	4	Y	
	Plant	*Anabaena flosaquae*	EC50	1300	5	N	
	Invertebrates	*Americamysis bahia*	LC50	66	4	N	
	Invertebrates	*Chironomus riparius*	LC50	20.9	2	N	(Beketov and Liess 2008)
	Invertebrates	*Gammarus pulex*	LC50	50	4	N	
	Invertebrates	*Simulium latigonium*	LC50	3.73	4	N	
	Invertebrates	*Aedes aegypti*	LC50	230	3	N	(Ahmed and Matsumura 2012)
	Invertebrates	*Chironomus tepperi*	LC50	2.22	1	N	(Stevens et al. 2005)
	Invertebrates	*Lampsilis fasciola*	LC50	456	2	N	(Prosser et al. 2016)
Clothianidin	Plant	*Pseudokirchneriella subcapitata*	EC50	64000	5	N	(Environmental Fate and Effects Division, U.S. EPA, Washington 1992)
	Fish	*Oncorhynchus mykiss*	LC50	105800	4	Y	
	Fish	*Lepomis macrochirus*	LC50	117000	4	Y	
	Fish	*Cyprinodon variegatus*	LC50	93600	4	N	
	Invertebrates	*Americamysis bahia*	LC50	51	4	N	
	Invertebrates	*Chironomus riparius*	LC50	22	2	N	(Barbee and Stout 2009)
	Invertebrates	*Procambarus clarkii*	LC50	59	4	N	
	Invertebrates	*Daphnia magna*	LC50	500	2	N	(de Perre et al. 2015)
	Invertebrates	*Chironomus tepperi*	LC50	2.83	1	N	(Stevens et al. 2005)
	Invertebrates	*Aedes aegypti*	LC50	98	3	N	(Ahmed and Matsumura 2012)
	Invertebrates	*Lampsilis fasciola*	LC50	478	2	N	(Prosser et al. 2016)
Dinotefuran	Fish	*Cyprinodon variegatus*	LC50	109000	4	Y	(Environmental Fate and Effects Division, U.S. EPA, Washington 1992)
	Plant	*Pseudokirchneriella subcapitata*	EC50	97600	4	N	
	Fish	*Cyprinus carpio*	LC50	99100	4	N	
	Fish	*Lepomis macrochirus*	LC50	99300	4	N	
	Fish	*Oncorhynchus mykiss*	LC50	99500	4	N	
	Invertebrates	*Americamysis bahia*	LC50	790	4	N	(Barbee and Stout 2009)
	Invertebrates	*Procambarus clarkii*	LC50	2032	4	N	
	Invertebrates	*Aedes aegypti*	LC50	78	3	N	(Ahmed and Matsumura 2012)
	Invertebrates	*Lampsilis fasciola*	LC50	447	2	N	(Prosser et al. 2016)
Imidacloprid	Fish	*Cyprinodon variegatus*	LC50	163000	4	Y	(Environmental Fate and Effects Division, U.S. EPA, Washington 1992)
	Fish	*Oncorhynchus mykiss*	LC50	229100	4	Y	
	Fish	*Lepomis macrochirus*	LC50	105001	4	Y	
	Invertebrates	*Americamysis bahia*	LC50	38	4	N	

	Amphibian/Reptile	*Rana limnocharis*	LC50	82000	4	N	(Feng et al. 2004)
	Amphibian/Reptile	*Pelophylax nigromaculatus*	LC50	129000	4	Y	(Feng et al. 2004)
	Amphibian/Reptile	*Hypsiboas pulchellus*	LC50	52622	4	N	(Pérez-Iglesias et al. 2014)
	Fish	*Danio rerio*	LC50	241000	4	Y	(Tišler et al. 2009)
	Plant	*Desmodesmus subspicatus*	IC50	389000	3	Y	(Tišler et al. 2009)
	Invertebrates	*Callinectes sapidus*	LC50	10.04	1	N	(Osterberg et al. 2012)
	Invertebrates	*Chydorus sphaericus*	LC50	132673	2	N	(Sánchez-Bayo and Goka 2006)
	Invertebrates	*Cypretta seurati*	LC50	301	2	N	(Sánchez-Bayo and Goka 2006)
	Invertebrates	*Cypridopsis vidua*	LC50	273	2	N	(Sánchez-Bayo and Goka 2006)
	Invertebrates	*Gammarus pulex*	LC50	386	4	N	(Van den Brink et al. 2016)
	Invertebrates	*Ilyocypris dentifera*	LC50	214	2	N	(Sánchez-Bayo and Goka 2006)
	Invertebrates	*Palaemonetes pugio*	LC50	308.8	4	N	(Key et al. 2007)
	Invertebrates	*Aedes aegypti*	LC50	84	3	N	(Paul et al. 2006)
	Invertebrates	*Aedes albopictus*	LC50	300	1	N	(Liu et al. 2004)
	Invertebrates	*Aedes taeniorhynchus*	LC50	13	2	N	(Song et al. 1997)
	Invertebrates	*Anopheles stephensi*	LC50	49	3	N	(Uragayala et al. 2015)
	Invertebrates	*Baetis rhodani*	LC50	8.49	2	N	(Beketov and Liess 2008)
	Invertebrates	*Caenis horaria*	LC50	28	4	N	(Van den Brink et al. 2016)
	Invertebrates	*Chaoborus obscuripes*	LC50	5226	4	N	(Van den Brink et al. 2016)
	Invertebrates	*Chironomus riparius*	LC50	19.9	2	N	(Azevedo-Pereira et al. 2011)
	Invertebrates	*Cloeon dipterum*	LC50	29	4	N	(Van den Brink et al. 2016)
	Invertebrates	*Culex quinquefasciatus*	LC50	19	3	N	(Bhan et al. 2014)
	Invertebrates	*Epeorus longimanus*	LC50	0.65	4	N	(Alexander et al. 2007)
	Invertebrates	*Plea minutissima*	LC50	287	4	N	(Van den Brink et al. 2016)
	Invertebrates	*Simulium latigonium*	LC50	3.73	4	N	(Beketov and Liess 2008)
	Invertebrates	*Simulium vittatum*	LC50	6.75	2	N	(Overmyer et al. 2005)
	Invertebrates	*Lampsilis fasciola*	LC50	688	2	N	(Prosser et al. 2016)
	Invertebrates	*Tubifex tubifex*	LC50	320	1	N	(Gerhardt 2009)
Thiacloprid	Fish	*Cyprinodon variegatus*	LC50	19700	4	N	
	Fish	*Lepomis macrochirus*	LC50	25200	4	N	
	Fish	*Oncorhynchus mykiss*	LC50	30200	4	N	(Environmental Fate and Effects Division, U.S. EPA, Washington 1992)
	Plant	*Scenedesmus subspicatus*	EC50	45000	3	N	
	Plant	*Pseudokirchneriella subcapitata*	EC50	60600	5	N	
	Fish	*Pimephales promelas*	LC50	104000	4	Y	
	Invertebrates	*Anopheles stephensi*	LC50	22511	3	N	(Uragayala et al. 2015)

	Invertebrates	*Baetis rhodani*	LC50	4.6	4	N	(Beketov and Liess 2008)
	Invertebrates	*Cloeon dipterum*	LC50	30	4	N	(Van den Brink et al. 2016)
	Invertebrates	*Culex quinquefasciatus*	LC50	6139	3	N	(Uragayala et al. 2015)
	Invertebrates	*Gammarus pulex*	LC50	350	4	N	(Beketov and Liess 2008)
	Invertebrates	*Lampsilis fasciola*	LC50	543	2	N	(Prosser et al. 2016)
Thiamethoxam	Fish	*Oncorhynchus mykiss*	LC50	100000	4	Y	
	Fish	*Cyprinodon variegatus*	LC50	111000	4	Y	(Environmental Fate and Effects Division, U.S. EPA, Washington 1992)
	Fish	*Lepomis macrochirus*	LC50	114000	4	Y	
	Plant	*Lemna gibba*	EC50	90000	7	N	
	Plant	*Pseudokirchneriella subcapitata*	EC50	97000	4	N	
	Invertebrates	*Anopheles stephensi*	LC50	52	3	N	(Uragayala et al. 2015)
	Invertebrates	*Culex quinquefasciatus*	LC50	343	3	N	(Uragayala et al. 2015)
	Invertebrates	*Cloeon dipterum*	LC50	52	4	N	(Van den Brink et al. 2016)
	Invertebrates	*Lampsilis fasciola*	LC50	691	2	N	(Prosser et al. 2016)

† Value used to construct species sensitivity distribution; actual L(EC) may be “>”

* Tolerant species (acute toxicity endpoint ≥ 100000 μg L^{-1}) were excluded from species sensitivity distributions.

Table S10. Summary of lethality (LC50)-derived species sensitivity distribution model parameters calculated using CCME SSD Master V3 software (Rodney et al. 2013)

Neonicotinoid	Parameter	Normal	Logistic	Extreme Value	Weibull*	Gumbel
	$\sum ei^2$	0.0326	0.0301	**0.0156**		0.0648
	MSE	0.0016	0.0015	**0.0008**		0.0032
	MSE Lower Tail	0.0206	0.0187	**0.0125**		0.0359
	Data from specified distribution? Anderson-Darling (n>5)	Yes	Yes	**Yes**		Yes
Acetamiprid	A^2	1.172	1.157	**0.969**		1.506
	HC50 (µg/L)	224.000	230.006	**262.742**		193.153
	HC5 (µg/L)	1.53	1.09	**0.48**		2.64
	Lower confidence limit on the mean (expected HC5)	1.02	0.62	**0.21**		1.45
	Upper confidence limit on the mean (expected HC5)	2.31	1.93	**1.07**		4.79
		Normal	**Logistic**	**Extreme Value**	**Weibull***	**Gumbel**
	$\sum ei^2$	0.0170	0.0185	0.0430		**0.0120**
	MSE	0.0008	0.0009	0.0020		**0.0006**
	MSE Lower Tail	0.0078	0.0098	0.0238		**0.0044**
	Data from specified distribution? Anderson-Darling (n>5)	Yes	Yes	Yes		**Yes**
Clothianidin	A^2	0.751	0.780	0.826		**0.848**
	HC50 (µg/L)	237.516	236.828	279.382		**203.976**
	HC5 (µg/L)	1.75	1.15	0.36		**4.13**
	Lower confidence limit on the mean (expected HC5)	1.19	0.65	0.11		**3.32**
	Upper confidence limit on the mean (expected HC5)	2.58	2.04	1.24		**5.15**
		Normal	**Logistic**	**Extreme Value**	**Weibull***	**Gumbel**
	$\sum ei^2$	0.0208	**0.0204**	0.0344		0.0387
	MSE	0.0010	**0.0010**	0.0016		0.0018
	MSE Lower Tail	0.0093	**0.0089**	0.0157		0.0213
	Data from specified distribution? Anderson-Darling (n>5)	Yes	**Yes**	Yes		Yes
Dinotefuran	A^2	0.363	**0.377**	0.418		0.609
	HC50 (µg/L)	2418.032	**2423.173**	2917.268		2034.050
	HC5 (µg/L)	11.51	**7.81**	1.75		29.92
	Lower confidence limit on the mean (expected HC5)	7.54	**4.67**	0.60		16.51
	Upper confidence limit on the mean (expected HC5)	17.56	**13.06**	5.10		54.24
		Normal	**Logistic**	**Extreme Value**	**Weibull***	**Gumbel**
	$\sum ei^2$	0.0581	0.0626	0.0651		**0.0609**
	MSE	0.0018	0.0020	0.0020		**0.0019**
	MSE Lower Tail	0.0122	0.0167	0.0289		**0.0035**
	Data from specified distribution? Anderson-Darling (n>5)	No	No	No		**No**
Imidacloprid	A^2	2.842	2.892	2.900		**2.925**
	HC50 (µg/L)	134.887	136.503	152.009		**120.826**
	HC5 (µg/L)	2.11	1.45	0.78		**3.71**
	Lower confidence limit on the mean (expected HC5)	1.71	0.99	0.42		**2.82**
	Upper confidence limit on the mean (expected	2.61	2.12	1.47		**4.87**

ED_002896_00040345-00031

	HC5)					
		Normal	**Logistic**	**Extreme Value**	**Weibull***	**Gumbel**
Thiacloprid	$\sum ei^2$	**0.0456**	0.0465	0.0803		0.0431
	MSE	**0.0018**	0.0018	0.0031		0.0017
	MSE Lower Tail	**0.0205**	0.0203	0.0424		0.0215
	Data from specified distribution? Anderson-Darling (n>5)	**Yes**	Yes	Yes		Yes
	A^2	**0.471**	0.494	0.674		0.652
	HC50 (µg/L)	**437.673**	427.587	562.638		355.018
	HC5 (µg/L)	**0.90**	0.55	0.07		3.56
	Lower confidence limit on the mean (expected HC5)	**0.60**	0.28	0.02		2.00
	Upper confidence limit on the mean (expected HC5)	**1.34**	1.10	0.33		6.33
		Normal	**Logistic**	**Extreme Value**	**Weibull***	**Gumbel**
Thiamethoxam	$\sum ei^2$	0.0399	0.0426	0.0828		**0.0208**
	MSE	0.0015	0.0016	0.0031		**0.0008**
	MSE Lower Tail	0.0274	0.0311	0.0531		**0.0139**
	Data from specified distribution? Anderson-Darling (n>5)	Yes	Yes	Yes		**Yes**
	A^2	0.814	0.864	0.984		**0.806**
	HC50 (µg/L)	768.604	761.890	926.824		**637.695**
	HC5 (µg/L)	4.87	3.11	0.86		**12.29**
	Lower confidence limit on the mean (expected HC5)	3.58	1.57	0.21		**9.90**
	Upper confidence limit on the mean (expected HC5)	6.63	6.16	3.48		**15.25**

*Weibull distribution can only be considered for arithmetic space (non-*log* transformed data) and is therefore not usable for this dataset.

Table S11. Additional toxicity data mined from ECOTOX database for use in invertebrate immobilization-derived species sensitivity distributions

Neonicotinoid	Taxa grouping	*Species*	Endpoint Type	Value (µg L^{-1})	Duration (d)	Tolerant?*	Reference
Acetamiprid	Fish	*Cyprinodon variegatus*	LC50	100000	4	Y	(Environmental Fate and Effects Division, U.S. EPA, Washington 1992)
	Fish	*Oncorhynchus mykiss*	LC50	100000†	4	Y	
	Fish	*Lepomis macrochirus*	LC50	119300†	4	Y	
	Plant	*Anabaena flosaquae*	EC50	1300†	5	N	
	Invertebrates	*Daphnia magna*	EC50	50000	2	N	
	Invertebrates	*Gammarus fasciatus*	EC50	80	4	N	
	Invertebrates	*Crassostrea virginica*	EC50	41000	4	N	
	Invertebrates	*Cheumatopsyche brevilineata*	EC50	3.35	2	N	(Yokoyama et al. 2009)
Clothianidin	Plant	*Pseudokirchneriella subcapitata*	EC50	64000	5	N	(Environmental Fate and Effects Division, U.S. EPA, Washington 1992)
	Fish	*Oncorhynchus mykiss*	LC50	105800†	4	Y	
	Fish	*Lepomis macrochirus*	LC50	117000†	4	Y	
	Fish	*Cyprinodon variegatus*	LC50	93600†	4	N	
	Invertebrates	*Daphnia magna*	EC50	119000	2	Y	
	Invertebrates	*Crassostrea virginica*	EC50	129140	2	Y	
	Invertebrates	*Cheumatopsyche brevilineata*	EC50	4.44	2	N	(Yokoyama et al. 2009)
Dinotefuran	Fish	*Cyprinodon variegatus*	LC50	109000†	4	Y	(Environmental Fate and Effects Division, U.S. EPA, Washington 1992)
	Plant	*Pseudokirchneriella subcapitata*	EC50	97600†	4	N	
	Fish	*Cyprinus carpio*	LC50	99100†	4	N	
	Fish	*Lepomis macrochirus*	LC50	99300†	4	N	
	Fish	*Oncorhynchus mykiss*	LC50	99500†	4	N	
	Invertebrates	*Daphnia magna*	EC50	968300	2	Y	
	Invertebrates	*Cheumatopsyche brevilineata*	EC50	10.4	2	N	(Yokoyama et al. 2009)
Imidacloprid	Fish	*Cyprinodon variegatus*	LC50	163000	4	Y	(Environmental Fate and Effects Division, U.S. EPA, Washington 1992)
	Fish	*Oncorhynchus mykiss*	LC50	229100	4	Y	
	Fish	*Lepomis macrochirus*	LC50	105000†	4	Y	
	Invertebrates	*Daphnia magna*	EC50	85200	2	N	
	Invertebrates	*Crassostrea virginica*	EC50	145000†	2	Y	
	Amphibian/Reptile	*Rana limnocharis*	LC50	82000	4	N	(Feng et al. 2004)
	Amphibian/Reptile	*Pelophylax nigromaculatus*	LC50	129000	4	Y	(Feng et al. 2004)
	Amphibian/Reptile	*Hypsiboas pulchellus*	LC50	52622	4	N	(Pérez-Iglesias et al. 2014)
	Fish	*Danio rerio*	LC50	241000	4	Y	(Tišler et al. 2009)
	Plant	*Desmodesmus subspicatus*	IC50	389000	3	Y	(Tišler et al. 2009)
	Invertebrates	*Asellus aquaticus*	EC50	78	4	N	(Van den Brink et al. 2016)
	Invertebrates	*Caenis horaria*	EC50	6	4	N	
	Invertebrates	*Chaoborus obscuripes*	EC50	3258	4	N	
	Invertebrates	*Cloeon dipterum*	EC50	18	4	N	
	Invertebrates	*Gammarus pulex*	EC50	49	4	N	
	Invertebrates	*Plea minutissima*	EC50	189	4	N	
	Invertebrates	*Cheumatopsyche brevilineata*	EC50	4.85	2	N	(Yokoyama et al. 2009)

ED_002896_00040345-00033

	Invertebrates	*Chydorus sphaericus*	EC50	832	2	N	
	Invertebrates	*Cypretta seurati*	EC50	1	2	N	(Sánchez-Bayo and Goka 2006)
	Invertebrates	*Cypridopsis vidua*	EC50	3	2	N	
	Invertebrates	*Ilyocypris dentifera*	EC50	3	2	N	
Thiacloprid	Fish	*Cyprinodon variegatus*	LC50	19700	4	N	(Environmental Fate and Effects Division, U.S. EPA, Washington 1992)
	Fish	*Lepomis macrochirus*	LC50	25200	4	N	
	Fish	*Oncorhynchus mykiss*	LC50	30200	4	N	
	Plant	*Scenedesmus subspicatus*	EC50	45000	3	N	
	Plant	*Pseudokirchneriella subcapitata*	EC50	60600	5	N	
	Fish	*Pimephales promelas*	LC50	104000†	4	Y	
	Invertebrates	*Daphnia magna*	EC50	22520	2	N	
	Invertebrates	*Crassostrea virginica*	EC50	4000	4	N	
	Invertebrates	*Cloeon dipterum*	EC50	10	4	N	(Van den Brink et al. 2016)
	Invertebrates	*Cheumatopsyche brevilineata*	EC50	5.27	2	N	(Yokoyama et al. 2009)
Thiamethoxam	Fish	*Oncorhynchus mykiss*	LC50	100000†	4	Y	(Environmental Fate and Effects Division, U.S. EPA, Washington 1992)
	Fish	*Cyprinodon variegatus*	LC50	111000†	4	Y	
	Fish	*Lepomis macrochirus*	LC50	114000†	4	Y	
	Plant	*Lemna gibba*	EC50	90000†	7	N	
	Plant	*Pseudokirchneriella subcapitata*	EC50	97000†	4	N	
	Invertebrates	*Daphnia magna*	EC50	106000†	2	Y	
	Invertebrates	*Crassostrea virginica*	EC50	119000†	4	Y	
	Invertebrates	*Cloeon dipterum*	EC50	20	4	N	(Van den Brink et al. 2016)

† Value used to construct species sensitivity distribution; actual L(EC) is “>”

Table S12. Summary of immobilization (EC50)-derived species sensitivity distribution model parameters calculated using CCME SSD Master V3 software (Rodney et al. 2013)

Neonicotinoid	Parameter	Normal	Logistic	Extreme Value	Weibull*	Gumbel
	$\sum ei^2$	0.0409	0.0426	0.0446		**0.0375**
	MSE	0.0051	0.0053	0.0056		**0.0047**
	MSE Lower Tail	0.0255	0.0272	0.0314		**0.0202**
	Data from specified distribution? Anderson-Darling (n>5)	Yes	Yes	Yes		**Yes**
Acetamiprid	A^2	1.399	1.415	1.440		**1.359**
	HC50 (µg/L)	32.232	32.704	36.519		**28.429**
	HC5 (µg/L)	0.24	0.15	0.08		**0.47**
	Lower confidence limit on the mean (expected HC5)	0.08	0.02	0.00		**0.16**
	Upper confidence limit on the mean (expected HC5)	0.70	1.14	1.26		**1.41**
		Normal	**Logistic**	**Extreme Value**	**Weibull***	**Gumbel**
	$\sum ei^2$	0.1259	0.1261	0.1478		**0.1018**
	MSE	0.0157	0.0158	0.0185		**0.0127**
	MSE Lower Tail	0.0662	0.0666	0.0811		**0.0507**
	Data from specified distribution? Anderson-Darling (n>5)	No	No	No		**No**
Clothianidin	A^2	2.289	2.235	2.546		**2.011**
	HC50 (µg/L)	248.929	220.124	471.269		**135.607**
	HC5 (µg/L)	0.01	0.00	0.00		**0.14**
	Lower confidence limit on the mean (expected HC5)	0.00	0.00	0.00		**0.01**
	Upper confidence limit on the mean (expected HC5)	0.11	6.31	420.81		**2.93**
		Normal	**Logistic**	**Extreme Value**	**Weibull***	**Gumbel**
	$\sum ei^2$	0.0890	0.0862	0.1263		**0.0606**
	MSE	0.0089	0.0086	0.0126		**0.0061**
	MSE Lower Tail	0.0430	0.0432	0.0619		**0.0331**
	Data from specified distribution? Anderson-Darling (n>5)	Yes	Yes	Yes		**Yes**
Dinotefuran	A^2	0.996	0.952	1.247		**0.869**
	HC50 (µg/L)	360.453	331.074	529.598		**270.272**
	HC5 (µg/L)	0.15	0.09	0.00		**0.86**
	Lower confidence limit on the mean (expected HC5)	0.04	0.00	0.00		**0.17**
	Upper confidence limit on the mean (expected HC5)	0.60	2.24	3.11		**4.43**
		Normal	**Logistic**	**Extreme Value**	**Weibull***	**Gumbel**
	$\sum ei^2$	0.0158	0.0182	0.0229		**0.0128**
	MSE	0.0011	0.0012	0.0015		**0.0009**
	MSE Lower Tail	0.0133	0.0157	0.0205		**0.0091**
	Data from specified distribution? Anderson-Darling (n>5)	No	No	No		**No**
Imidacloprid	A^2	3.045	3.069	3.118		**3.016**
	HC50 (µg/L)	39.352	39.570	41.775		**37.637**
	HC5 (µg/L)	0.68	0.48	0.30		**1.08**
	Lower confidence limit on the mean (expected HC5)	0.44	0.22	0.09		**0.82**
	Upper confidence limit on the mean (expected	1.07	1.04	0.97		**1.43**

	HC5)					
		Normal	**Logistic**	**Extreme Value**	**Weibull***	**Gumbel**
Thiacloprid	$\sum ei^2$	0.0737	0.0772	0.0966		**0.0549**
	MSE	0.0053	0.0055	0.0069		**0.0039**
	MSE Lower Tail	0.0340	0.0364	0.0557		**0.0166**
	Data from specified distribution? Anderson-Darling (n>5)	Yes	Yes	Yes		**Yes**
	A^2	0.766	0.790	0.919		**0.632**
	HC50 (µg/L)	296.410	286.134	451.639		**197.461**
	HC5 (µg/L)	0.05	0.02	0.00		**0.30**
	Lower confidence limit on the mean (expected HC5)	0.02	0.00	0.00		**0.07**
	Upper confidence limit on the mean (expected HC5)	0.15	0.25	0.14		**1.25**
		Normal	**Logistic**	**Extreme Value**	**Weibull***	**Gumbel**
Thiamethoxam	$\sum ei^2$	**0.0057**	0.0062	0.0084		0.0054
	MSE	**0.0010**	0.0010	0.0014		0.0009
	MSE Lower Tail	**0.0050**	0.0053	0.0065		0.0053
	Data from specified distribution? Anderson-Darling (n>5)	**Yes**	Yes	No		No
	A^2	**1.926**	1.921	1.945		1.975
	HC50 (µg/L)	**81.854**	81.518	83.093		81.652
	HC5 (µg/L)	**6.09**	4.89	3.58		8.33
	Lower confidence limit on the mean (expected HC5)	**3.07**	3.33	1.88		5.89
	Upper confidence limit on the mean (expected HC5)	**12.08**	7.18	6.81		11.80

*Weibull distribution can only be considered for arithmetic space (non-*log* transformed data) and is therefore not usable for this dataset.

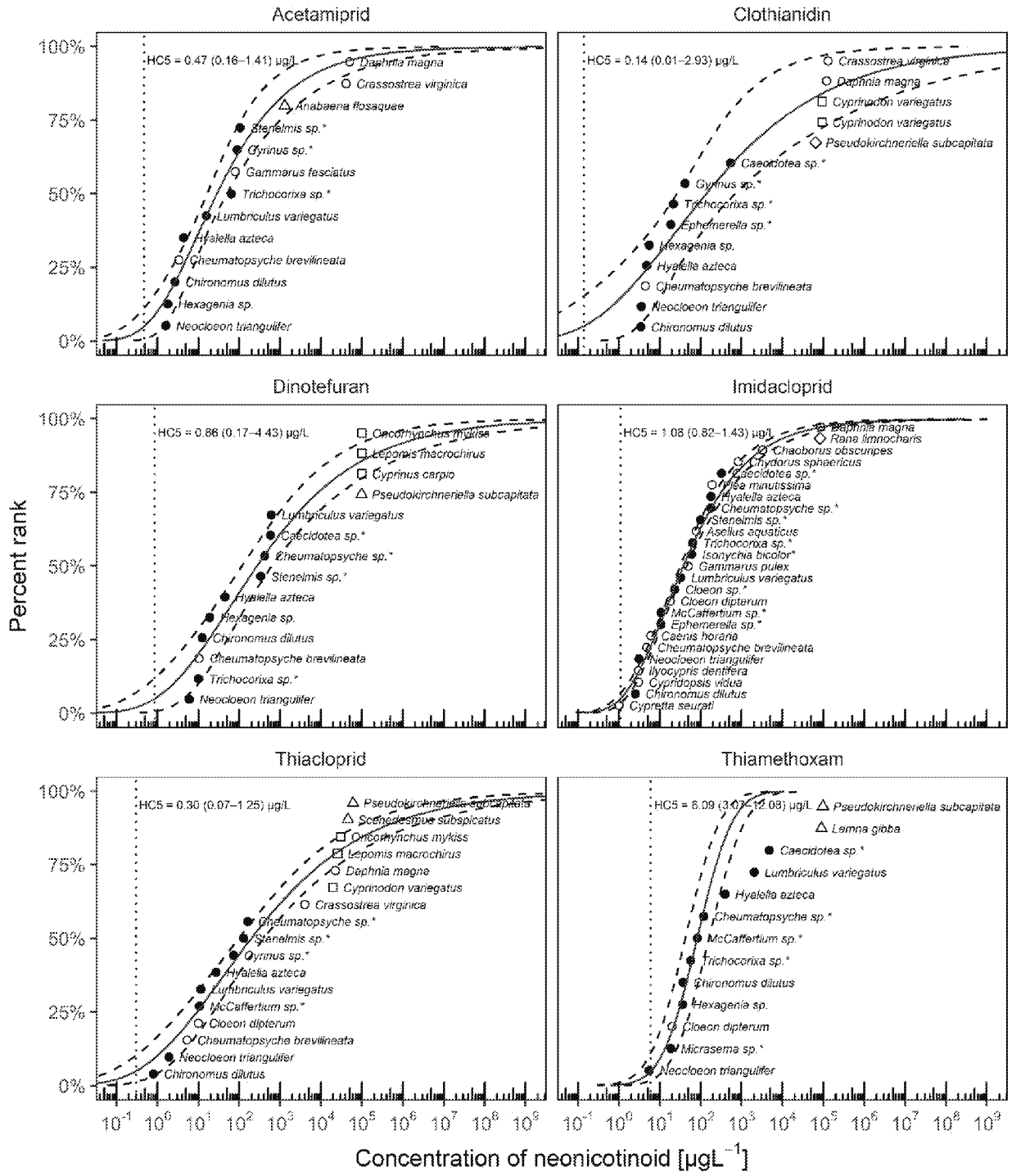

Figure S13. Species sensitivity distributions (SSDs) of acute (≤5 d) toxicity data for 6 neonicotinoids constructed using immobilization (EC50) values for aquatic invertebrates generated from the present study and external literature data mined from ECOTOX database. See

Table S11 for external data included in these SSDs. Closed (black) shapes represent data from the present study (internal) and open (white) shapes represent external data. Circles represent aquatic invertebrates, squares represent fish, triangles represent plants, and diamonds triangles represent amphibians. For data from the present study, all field-collected species (indicated with an asterisk [*]) was derived from tests with a single replicate; remaining internal data were lab-cultured species tested with a minimum of 3 replicates. Data were fit to Gumbel regression models (red line) for all neonicotinoids except thiamethoxam, which was fit to a normal model, with 95% confidence band for the regression (dashed line). However, the SSD for clothianidin did not pass Anderson-Darling goodness-of-fit test or visual inspection for any of the four possible models. The vertical dotted line illustrates the 5th percentile hazardous concentration (HC5), with 95% CI. SSDs were modeled in CCME V3 Master software and plotted in R with *ggplot2*. Model parameters are in Supplemental Data, Table S12.

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S14. Details on 3 Ontario neonicotinoid monitoring studies used in hazard assessment

1. *Ontario stream neonicotinoid monitoring study for 2015 (Full raw data and metadata available at: ontario.ca/data/stream-neonicotinoid-monitoring-study).* Characterized neonicotinoids in 5 Ontario watersheds from April to November 2015 using grab and wet weather triggered sampling methods. Each of the 5 sites were sampled approximately 30 times for a total of 149 samples collected. Samples were analyzed for 8 neonicotinoids: acetamiprid (MDL 5 ng/L), clothianidin (MDL 5 ng/L), dinotefuran (MDL 5 ng/L), flonicamid (MDL 10 ng/L), imidacloprid (MDL 5 ng/L), nitenpyram (MDL 5 ng/L), thiacloprid (MDL 2 ng/L) and thiamethoxam (5 ng/L) by the Ontario Ministry of the Environment and Climate Change, Laboratory Services Branch, Organic Contaminants Section, per methods outlined in this Supplemental Data, S3.
2. *Ontario Ministry of Agriculture, Food and Rural Affairs/MOECC Joint Pesticide Network, 2012-2015.* Characterized pesticides, including neonicotinoids, in 17-19 sites in (depending on the year) in Ontario using grab sampling methods. Sites were sampled throughout the year, with the majority of sampling occurring from spring to fall. Each sample was analyzed for 6 neonicotinoids: acetamiprid, clothianidin, dinotefuran, imidacloprid, thiacloprid, and thiamethoxam. Detection limits, limits of quantification, and sampling locations available upon request to corresponding author.
3. *Survey of southern Ontario watersheds, D. Poirier, June 16 2016.* Characterized neonicotinoids in 10 watersheds at outflow to a great lake (Lake Huron, Lake Erie, or Lake Ontario) using a single grab sample. Samples were analyzed as for Ontario stream neonicotinoid monitoring study. Sampling locations available upon request to corresponding author.

ED_002896_00040345-00039

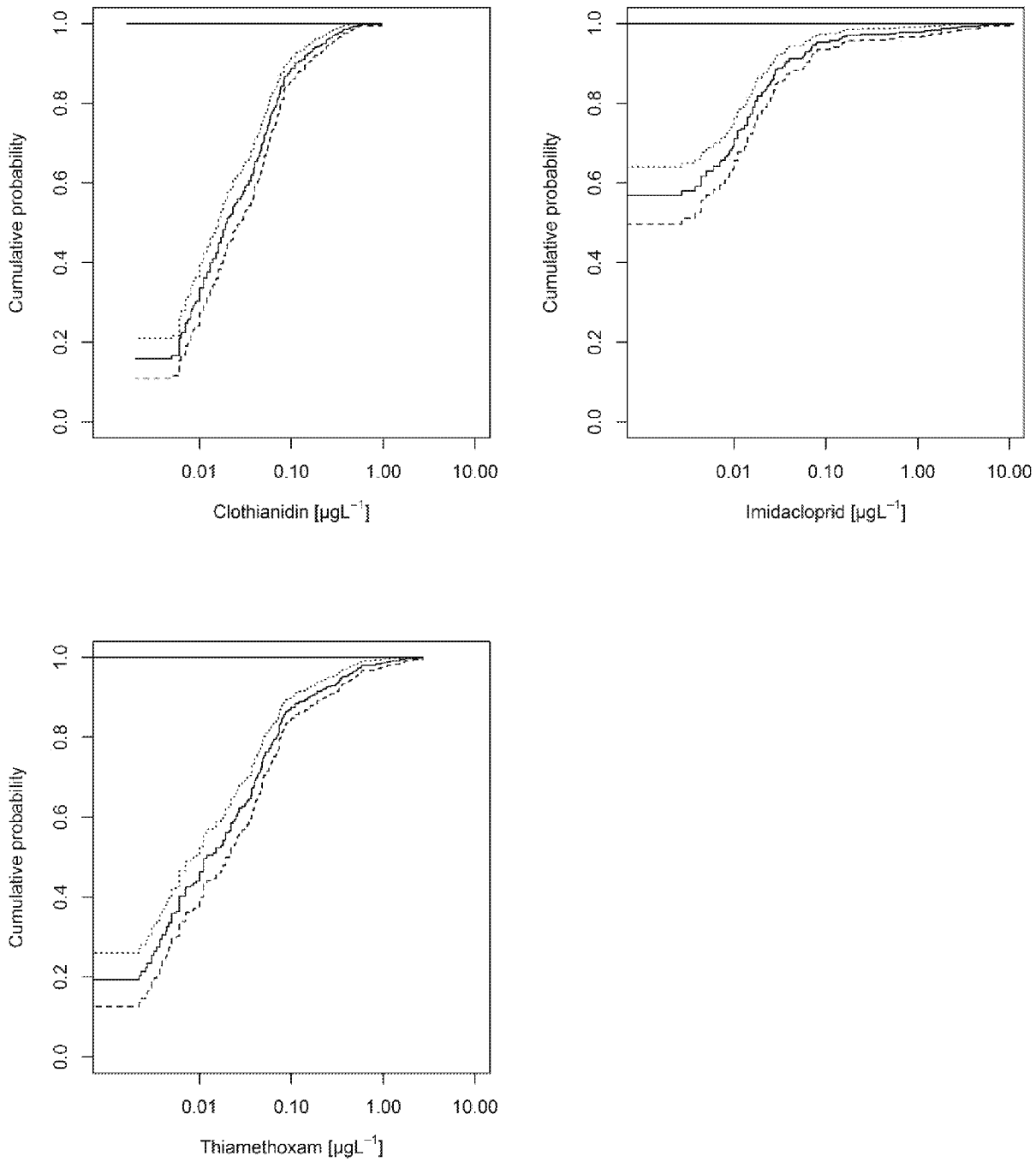

Figure S15. Empirical cumulative distribution functions for neonicotinoid surface water monitoring data from three studies in Ontario. Distributions were estimated using Kaplan-Meier method in 'NADA' package (v. 1.6-1) in R (v. 3.3.2)

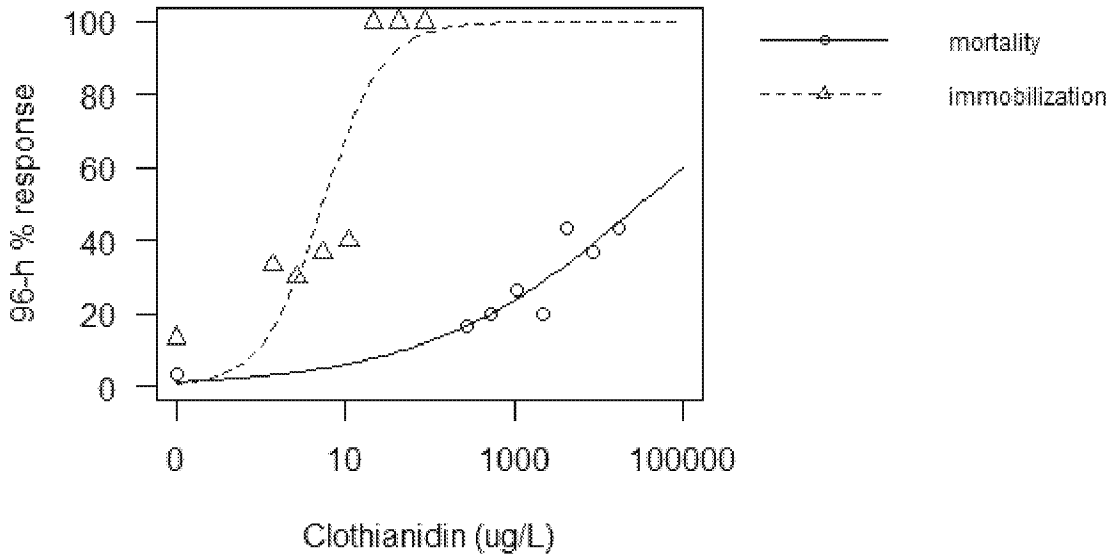

Figure S16. *Hexagenia* spp. response after 96-h clothianidin exposure; mortality (blue line, open circles) and immobilization (red line, open triangles) tests conducted separately. Points represent mean response at each concentration in 3 replicates. 96-h EC50 = 5.5 µg L^{-1}. 96-h LC50 > 17400 µg L^{-1}, as discussed below.

This plot is a visualization of the commonly observed response of *Hexagenia* spp. to neonicotinoids in the present study. *Hexagenia* spp. were immobilized at concentrations orders of magnitude lower than what caused mortality. The dose-response curve for *Hexagenia* spp. mortality was shallow, and >50% mortality was not observed even at the highest concentrations tested for all neonicotinoids except imidacloprid; therefore, an LC50 could not be calculated for all neonicotinoids except imidacloprid.

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Ontario Ministry of the Environment and Climate Change Aquatic Toxicology Unit. 2016c. Standard Operating Procedure: *Hexagenia* spp. Culturing, SOP HX1.v7.

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Message

From: Rossmeisl, Colleen [Rossmeisl.Colleen@epa.gov]
Sent: 3/15/2019 3:52:26 PM
To: Garber, Kristina [Garber.Kristina@epa.gov]
CC: Peck, Charles [Peck.Charles@epa.gov]
Subject: ESA revised probabilistic analysis
Attachments: ESA Probabilistic Analysis_updated_3-14-19.docx

Flag: Follow up

Hi Kris –

The revised probabilistic section is attached. I am hoping I captured the revisions you recommended and we discussed yesterday. I think it flows better now and gives some base/background. Chuck has reviewed changes made to the aquatic part. It is a clean version but if you want to be reminded of comments, etc. those are still all in the revised methods version on SharePoint.

I will check email again late today. I will be working on the revised document on Monday and can drop it back into the main body then with any other edits or comments you have. I will also be working on reading through the whole document Monday, making any other edits needed from RTC and adding language on drift adjustments to the usage section. When it does go to Brian review Monday/Tuesday, the team will also have the chance to review again then as well.

Thanks!
Colleen

Message

From: Blankinship, Amy [Blankinship.Amy@epa.gov]
Sent: 5/24/2018 10:58:42 AM
To: Garber, Kristina [Garber.Kristina@epa.gov]
Subject: RE: Diazinon (057801) National and State Summary Use and Usage Summary

Thanks for the quick reply

From: Garber, Kristina
Sent: Thursday, May 24, 2018 5:39 AM
To: Blankinship, Amy <Blankinship.Amy@epa.gov>
Subject: Re: Diazinon (057801) National and State Summary Use and Usage Summary

HiAmy,

The SUUM has not been revised for diazinon.

Kris

On May 23, 2018, at 5:15 PM, Blankinship, Amy <Blankinship.Amy@epa.gov> wrote:

> Hi Kris,
>
> Josh is working on some drinking water refinements for diazinon and was asking about usage data. I mentioned BEAD had done some work for us for ESA. I have the data/memo from the email below, but I thought I would check with you to make sure I wasn't missing a more recent update.
>
> Thanks,
> Amy
>
> ---
>
> **From:** Garber, Kristina
> **Sent:** Tuesday, September 05, 2017 2:41 PM
> **To:** Connolly, Jennifer <Connolly.Jennifer@epa.gov>; Lennartz, Steven <Lennartz.Steven@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Blankinship, Amy <Blankinship.Amy@epa.gov>; Donovan, Elizabeth <Donovan.Elizabeth@epa.gov>; Kanarek, Andrew <Kanarek.Andrew@epa.gov>; Eckel, William <Eckel.William@epa.gov>
> **Subject:** FW: Diazinon (057801) National and State Summary Use and Usage Summary
>
> Attached is the refined usage data for diazinon
>
> ---
>
> **From:** Garber, Kristina
> **Sent:** Tuesday, September 05, 2017 2:36 PM
> **To:** Echeverria, Marietta <Echeverria.Marietta@epa.gov>; 'Anderson, Brian' <anderson.brian@epa.gov>; Villanueva, Philip <Villanueva.Philip@epa.gov>; Pease, Anita <Pease.Anita@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>
> **Subject:** FW: Diazinon (057801) National and State Summary Use and Usage Summary

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

From: Garber, Kristina
Sent: Wednesday, October 26, 2016 10:09 AM
To: Pease, Anita <Pease.Anita@epa.gov>
Subject: Fwd: Diazinon (057801) National and State Summary Use and Usage Summary

Here's the final memo from BEAD on the refined usage analysis for diazinon.

Begin forwarded message:

> **From:** "Russell, CarolynY" <Russell.Carolyny@epa.gov>
> **To:** "Garber, Kristina" <Garber.Kristina@epa.gov>, "Nguyen, Khue" <Nguyen.Khue@epa.gov>
> **Cc:** "Paisley-Jones, Claire" <Paisley-Jones.Claire@epa.gov>, "Miller, Wynne" <Miller.Wynne@epa.gov>, "Jarboe, Stephen" <Jarboe.Steve@epa.gov>, "Doucoure, Cynthia" <Doucoure.Cynthia@epa.gov>, "Prieto, Rafael" <Prieto.Rafael@epa.gov>, "Shah, Aruna" <Shah.Aruna@epa.gov>, "Atwood, Donald" <Atwood.Donald@epa.gov>
> **Subject: Diazinon (057801) National and State Summary Use and Usage Summary**
>
> Your message is ready to be sent with the following file or link attachments:
>
> Diazinon.pdf
>
> Note: To protect against computer viruses, e-mail programs may prevent sending or receiving certain types of file attachments. Check your e-mail security settings to determine how attachments are handled.

Message

From: Blankinship, Amy [Blankinship.Amy@epa.gov]
Sent: 5/23/2018 9:15:07 PM
To: Garber, Kristina [Garber.Kristina@epa.gov]
Subject: RE: Diazinon (057801) National and State Summary Use and Usage Summary

Hi Kris,

Josh is working on some drinking water refinements for diazinon and was asking about usage data. I mentioned BEAD had done some work for us for ESA. I have the data/memo from the email below, but I thought I would check with you to make sure I wasn't missing a more recent update.

Thanks,
Amy

From: Garber, Kristina
Sent: Tuesday, September 05, 2017 2:41 PM
To: Connolly, Jennifer <Connolly.Jennifer@epa.gov>; Lennartz, Steven <Lennartz.Steven@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Blankinship, Amy <Blankinship.Amy@epa.gov>; Donovan, Elizabeth <Donovan.Elizabeth@epa.gov>; Kanarek, Andrew <Kanarek.Andrew@epa.gov>; Eckel, William <Eckel.William@epa.gov>
Subject: FW: Diazinon (057801) National and State Summary Use and Usage Summary

Attached is the refined usage data for diazinon

From: Garber, Kristina
Sent: Tuesday, September 05, 2017 2:36 PM
To: Echeverria, Marietta <Echeverria.Marietta@epa.gov>; 'Anderson, Brian' <anderson.brian@epa.gov>; Villanueva, Philip <Villanueva.Philip@epa.gov>; Pease, Anita <Pease.Anita@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>
Subject: FW: Diazinon (057801) National and State Summary Use and Usage Summary

Deliberative Process / Ex. 5

From: Garber, Kristina
Sent: Wednesday, October 26, 2016 10:09 AM
To: Pease, Anita <Pease.Anita@epa.gov>
Subject: Fwd: Diazinon (057801) National and State Summary Use and Usage Summary

Here's the final memo from BEAD on the refined usage analysis for diazinon.

Begin forwarded message:

From: "Russell, CarolynY" <Russell.Carolyny@epa.gov>
To: "Garber, Kristina" <Garber.Kristina@epa.gov>, "Nguyen, Khue" <Nguyen.Khue@epa.gov>
Cc: "Paisley-Jones, Claire" <Paisley-Jones.Claire@epa.gov>, "Miller, Wynne" <Miller.Wynne@epa.gov>, "Jarboe, Stephen" <Jarboe.Steve@epa.gov>, "Doucoure, Cynthia" <Doucoure.Cynthia@epa.gov>, "Prieto, Rafael" <Prieto.Rafael@epa.gov>, "Shah, Aruna" <Shah.Aruna@epa.gov>, "Atwood, Donald" <Atwood.Donald@epa.gov>
Subject: Diazinon (057801) National and State Summary Use and Usage Summary

Your message is ready to be sent with the following file or link attachments:

Diazinon.pdf

Note: To protect against computer viruses, e-mail programs may prevent sending or receiving certain types of file attachments. Check your e-mail security settings to determine how attachments are handled.

Message

From: Kanarek, Andrew [Kanarek.Andrew@epa.gov]
Sent: 4/30/2018 7:52:56 PM
To: Kyle, Lee [Kyle.Lee@epa.gov]; Garber, Kristina [Garber.Kristina@epa.gov]
Subject: draft of slides for pop mod (con't) briefing with Marietta
Attachments: popmod_briefing_5.9.18.pptx

Attached.

Andrew R Kanarek, PhD
Ecological Modeler, Population Biologist
Environmental Fate and Effects Division
Office of Pesticide Programs, PYS-10242
US Environmental Protection Agency
1200 Pennsylvania Ave, NW (7507P)
Washington, DC 20460
Phone: (703) 347-8223
Fax: (703) 305-0204

Applications of Population Modeling in Ecological Risk Assessment of Pesticides (part II)

May 9, 2018

Objective of this briefing

- Provide EFED management update on population model development
- Discuss ORD collaborations related to population modeling
- Get EFED management input on next steps for population model development efforts

Deliberative Process / Ex. 5

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Deliberative Process / Ex. 5

Message

From: Kanarek, Andrew [Kanarek.Andrew@epa.gov]
Sent: 5/23/2018 2:36:07 PM
To: Garber, Kristina [Garber.Kristina@epa.gov]
Subject: FW: EPA endangered species work
Attachments: CA ESA Case Study 5-24-18_CDPR.PPTX

Do you think it's worth sending this around to Sujatha, Lee, Kimberley, or others so they can get a better sense of this project?

Hope you feel better ☺

From: Raimondo, Sandy
Sent: Wednesday, May 23, 2018 10:20 AM
To: Moreno, Polo@CDPR <Polo.Moreno@cdpr.ca.gov>; Singhasemanon, Nan@CDPR <Nan.Singhasemanon@cdpr.ca.gov>; Luo, Yuzhou@CDPR <Yuzhou.Luo@cdpr.ca.gov>; Jennifer Teerlink <jennifer.teerlink@cdpr.ca.gov>; Annette.Narzynski@cdpr.ca.gov; Denton, Debra <Denton.Debra@epa.gov>; Etterson, Matthew <Etterson.Matthew@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>; Justice, JamesR <Justice.JamesR@epa.gov>; Kanarek, Andrew <Kanarek.Andrew@epa.gov>; Martin, Roy <Martin.Roy@epa.gov>; Oliver, Leah <Oliver.Leah@epa.gov>; Purucker, Tom <Purucker.Tom@epa.gov>; Sharpe, Leah <sharpe.leah@epa.gov>; Thursby, Glen <Thursby.Glen@epa.gov>; Waits, Eric <Waits.Eric@epa.gov>; Yuan, Yongping <Yuan.Yongping@epa.gov>
Cc: TenBrook, Patti <TenBrook.Patti@epa.gov>
Subject: RE: EPA endangered species work

Hi folks,

Here is a link to the webinar for tomorrow's meeting. I have also attached the slides in the event that anyone has difficulties accessing the webinar system.

http://epawebconferencing.acms.com/r1iy3ul6di0/

Looking forward to our conversation tomorrow,
Sandy

-----Original Appointment-----
From: Raimondo, Sandy
Sent: Wednesday, May 09, 2018 8:51 AM
To: Raimondo, Sandy; Moreno, Polo@CDPR; Singhasemanon, Nan@CDPR; Luo, Yuzhou@CDPR; Jennifer Teerlink; Annette.Narzynski@cdpr.ca.gov; Denton, Debra; Etterson, Matthew; Garber, Kristina; Justice, JamesR; Kanarek, Andrew; Martin, Roy; Oliver, Leah; Purucker, Tom; Sharpe, Leah; Thursby, Glen; Waits, Eric; Yuan, Yongping
Cc: TenBrook, Patti
Subject: EPA endangered species work
When: Thursday, May 24, 2018 1:00 PM-2:00 PM (UTC-06:00) Central Time (US & Canada).
Where: (202) 991-0477 code 1664316

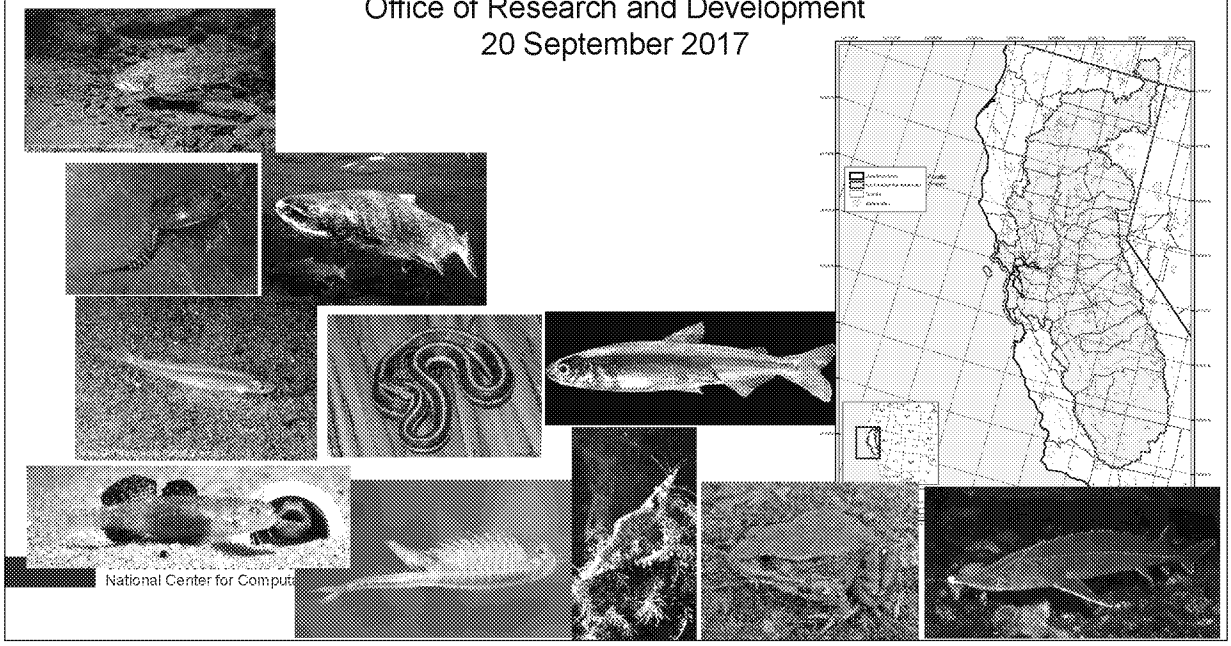
Thanks for finding the time to meet with me to discuss EPA's work on endangered species pesticide risk assessment. I will send a link to a webinar the week of the meeting and start the discussion with some project overview slides.

Looking forward to speaking with everyone,
Sandy

United States Environmental Protection Agency

Chemical Safety for Sustainability
California Endangered Species Case Study

Sandy Raimondo
Office of Research and Development
20 September 2017



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The EPA Team

ORD/NHEERL

- Gulf Ecology Division (GED)
  - Sandy Raimondo (task lead)
  - Leah Oliver
  - Leah Sharpe
- Mid-Continent Ecology Division (MED)
  - Matthew Etterson
  - Nathan Pollesch
- Atlantic Ecology Division (AED)
  - Glen Thursby

Region 9

- Debra Denton

ORD/NERL

- Environmental Sciences Division (ESD)
  - Yongping Yuan
- Ecosystems Research Division (ERD)
  - Tom Purucker
  - Sumathy Sinnathamby
- Ecological Exposure Research Division (EERD)
  - Roy Martin
  - Eric Waits

- OPP/EFED
  - Kris Garber
  - Andrew Kanarek

Collaborators

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Water Boards

USDA

California Fish & Wildlife

NOAA — National Oceanic and Atmospheric Administration, U.S. Department of Commerce

Department of Water Resources, State of California

CalEPA California Environmental Protection Agency

USGS

Pacific States Marine Fisheries Commission

SWAMP Surface Water Ambient Monitoring Program

DWR · USBR · USFWS · SWRCB · DFW · USEPA · USACE · NMFS · USGS

UCDAVIS

Cramer Fish Sciences

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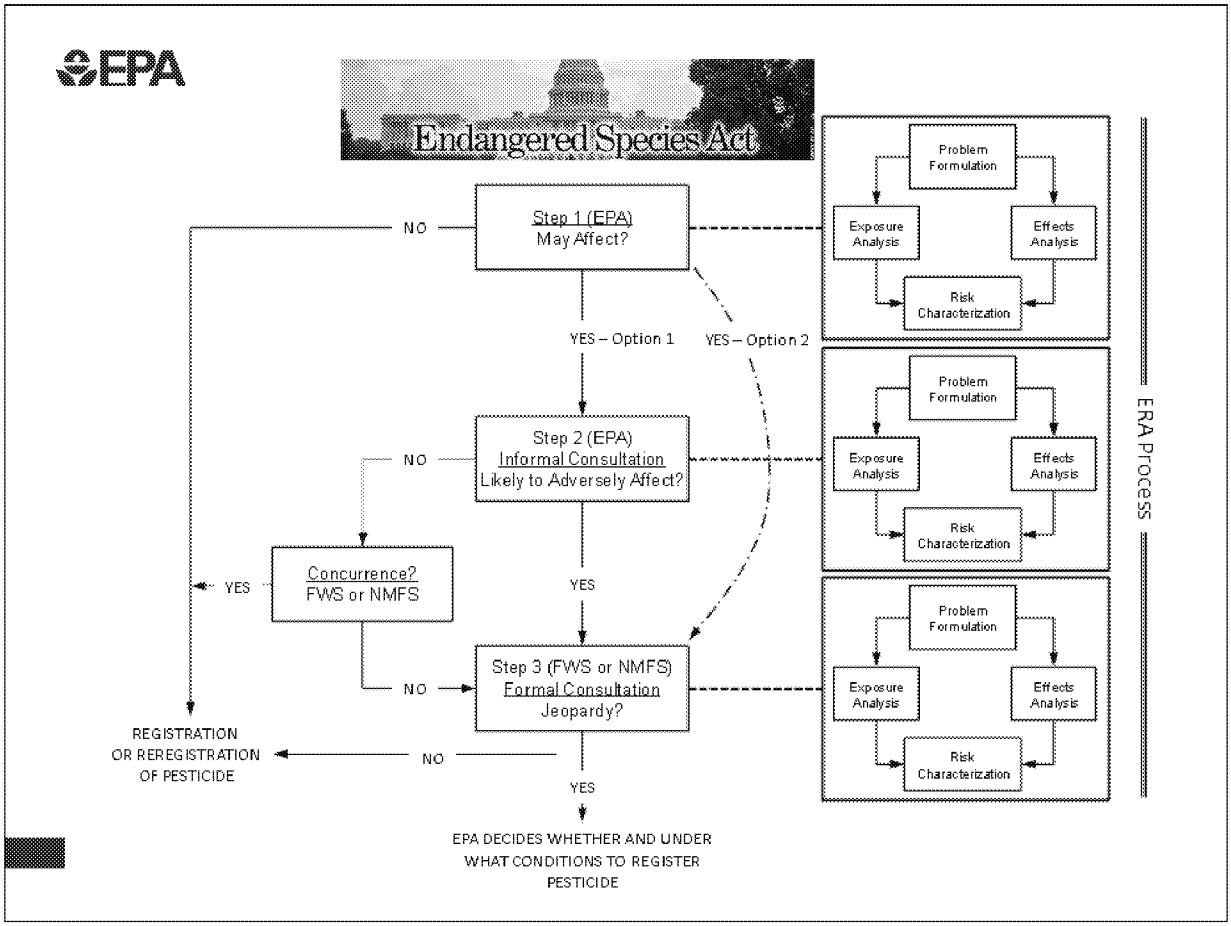
Task Drivers and Goals

- EPA research priorities highlighted in the National Academy of Science's report (2013) "Assessing Risks to Endangered and Threatened Species from Pesticides"
- Develop, evaluate, and demonstrate approaches that improve endangered species risk assessments and reduce knowledge-based uncertainties
- Approaches that transition from the current EPA process toward probabilistic risk assessment for threatened and endangered (T&E) species
- Develop, evaluate, and provide guidance on approaches that make use of minimal data, increasing the number and types of exposure scenarios, and implementing models that project population-level impacts to listed species.


EPA
Endangered Species Act
Step 1 (EPA)
May Affect?
NO
YES – Option 1
YES – Option 2
Step 2 (EPA)
Informal Consultation
Likely to Adversely Affect?
NO
YES
Concurrence?
FWS or NMFS
YES
NO
Step 3 (FWS or NMFS)
Formal Consultation
Jeopardy?
NO
YES
REGISTRATION
OR REREGISTRATION
OF PESTICIDE
EPA DECIDES WHETHER AND UNDER
WHAT CONDITIONS TO REGISTER
PESTICIDE
Problem Formulation
Exposure Analysis
Effects Analysis
Risk Characterization
Problem Formulation
Exposure Analysis
Effects Analysis
Risk Characterization
Problem Formulation
Exposure Analysis
Effects Analysis
Risk Characterization
ERA Process

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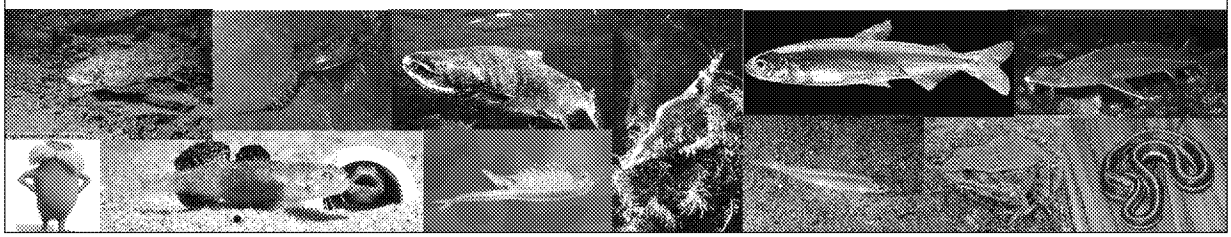
Anticipated Project Outcomes

- Reduced uncertainties associated with predicting pesticide impacts to endangered species
- Provide improved and defensible approaches available for Section 7 consultation
- Develop framework for integrating exposure and effects into probabilistic assessments
- Minimize impacts to listed species from pesticide exposure that provides greater protection of other species



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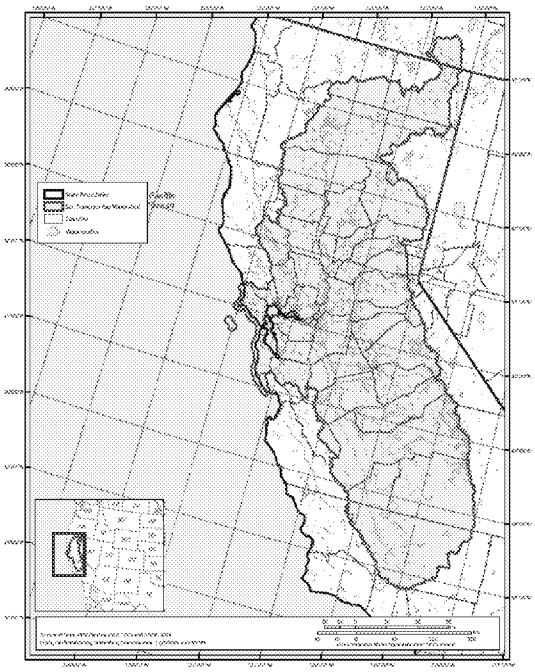
California Central Valley

- Diversity
  - Listed species
  - Pesticide use
  - Habitat types
- Relatively "abundant" data
  - Pesticide use
  - Environmental monitoring
  - Species data
- Region & State partnership
- Historical processes for ESA well documented



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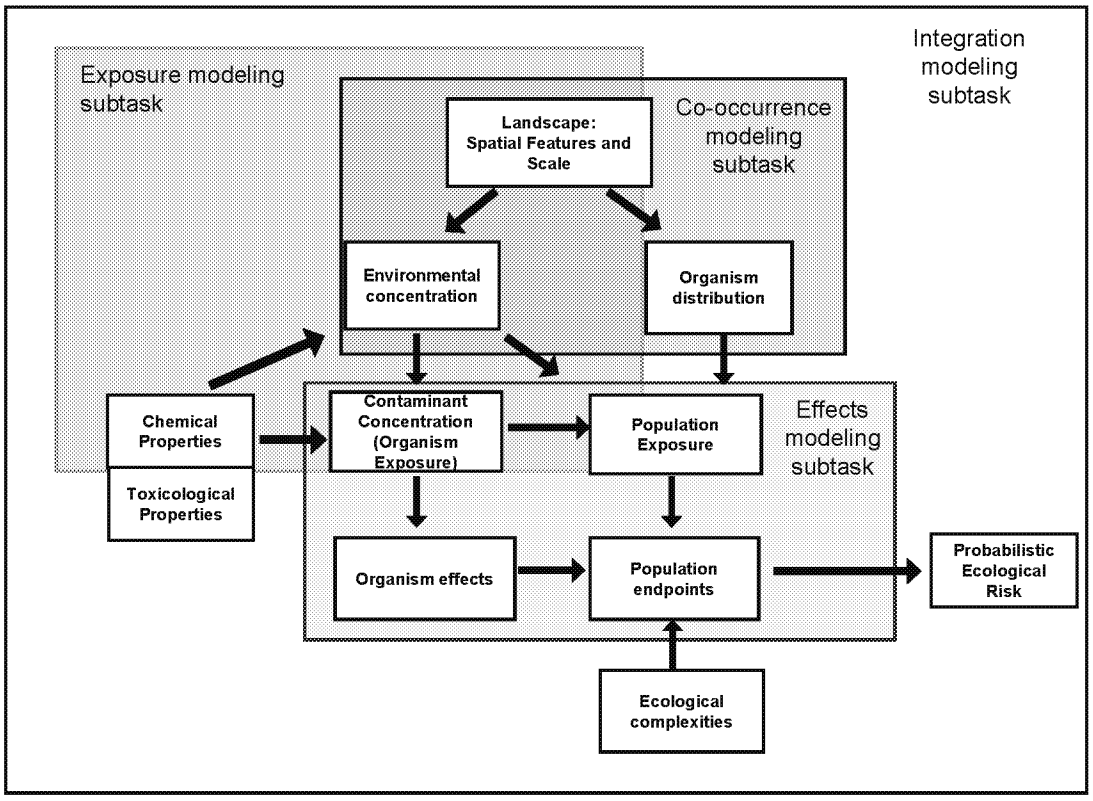
2015 Project Scoping Meeting

- Understand processes required of the Agency, Services, and States to comply with the Endangered Species Act (ESA)
- Overview species and pesticide usage within the study area
- Engagement with representatives of the Regional and State Offices
  - EPA Office of Pesticide Programs
  - EPA Office of Water
  - EPA Region 9
  - US Fish and Wildlife
  - CA State Water Resource Board (Rich Breuer)
  - CA Department of Pesticide Registration (Yuzhou Luo, Rich Marovich)
  - CA Department of Fish and Wildlife (Stephen Louie)
- Task framework established

Task Conceptual Model and Structure



California Central Valley Species

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Tier 1

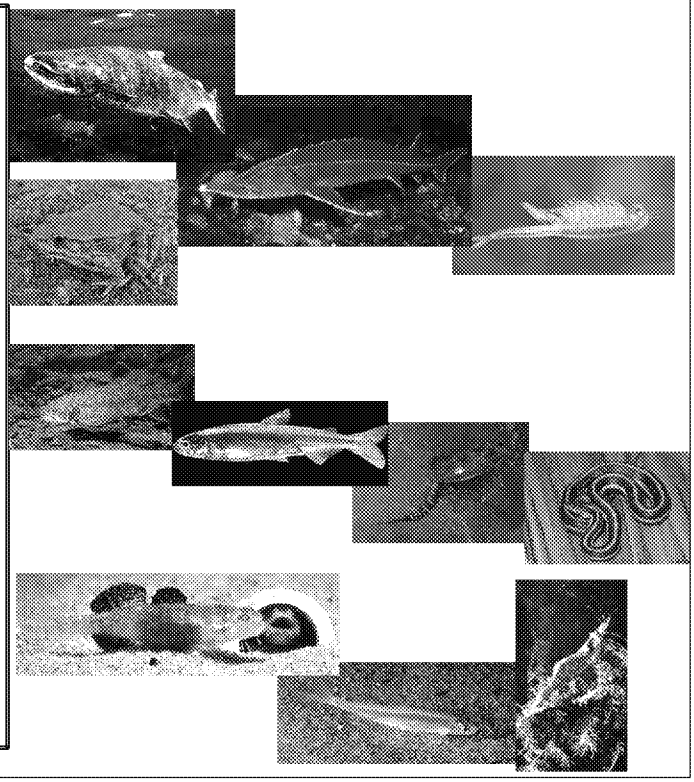
- Chinook Salmon (*Oncorhynchus tshawytscha*)
  - Sacramento River winter-run
  - Central Valley spring-run
- Southern North American Green Sturgeon (*Acipenser medirostris*)
- Fairy shrimp (*Branchinecta lynchi*)
- California Red-legged Frog (*Rana draytonii*)

Tier 2

- Central Valley steelhead (*Onchoryncus mykiss*)
- Delta Smelt (*Hypomesus transpacificus*)
- Tadpole shrimp (*Lepidurus packardi*)
- Garter snake (*Thamnophis gigas*)

Tier 3

- Tidewater goby (*Eucyclogobius newberryi*)
- San Francisco Longfin Smelt (*Spirinchus thaleichthys*)
- California Freshwater Shrimp (*Syncaris pacifica*)



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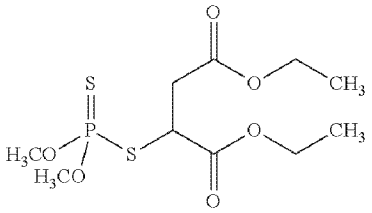
Priority Chemicals

- 40 chemicals, priority tiered
- OPP/Region 9 priorities
- Diverse chemical class
- Varying available data



Malathion

Chemical	CAS	Use pattern	Chemical Class	Tier
Bifenthrin	82657043	Insecticide	pyrethroid	1
Carbaryl	63252	Insecticide	carbamate	1
Chlorpyrifos	2921882	Insecticide	organophosphate	1
Cypermethrin	52315078	Insecticide	pyrethroid	1
Diazinon	333415	Insecticide	organophosphate	1
Fipronil	120068373	Insecticide	phenylpyrazole	1
Imidacloprid	138261413	Insecticide	neonicotinoid	1
Malathion	121755	Insecticide	organophosphate	1
Methomyl	16752775	Insecticide	carbamate	1
Permethrin	52645531	Insecticide	pyrethroid	1
Thiobencarb	28249776	Herbicide	carbamate	1
Deltamethrin	52918635	Insecticide	pyrethroid	2
Captan	133062	Fungicide	thiophthalimide	2
Chlorothalonil	1897456	Fungicide	chloronitrile	2

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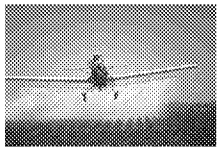
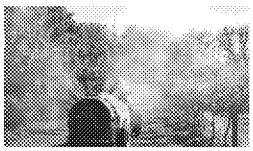
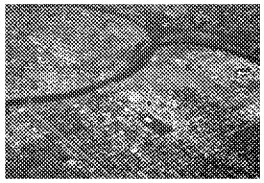
Exposure Modeling Subtask





Pesticide Use
- Row crops
- Orchards
- Urban
- Rice

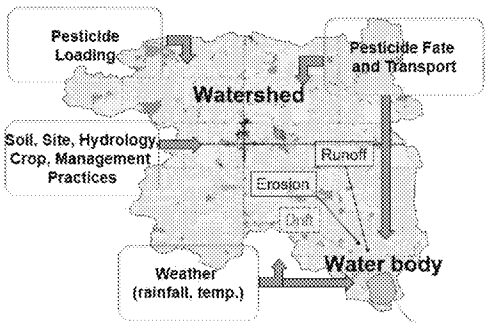


Habitat type
- Streams
- Delta
- Vernal pools

Exposure Modeling Subtask

- Spatial Aquatic Model (SAM; agriculture)
  - Watershed based model for surface water
  - Improve approach for higher-tiered assessments
  - Where and when exceedance will occur
  - Daily estimates

- Pesticide in Water Calculator (PWC)
  - Urban, vernal pool applications
  - Watershed delineation using Soil and Water Assessment Tool (SWAT)
  - Crop parameters
  - Weather data
  - Physicochemical properties



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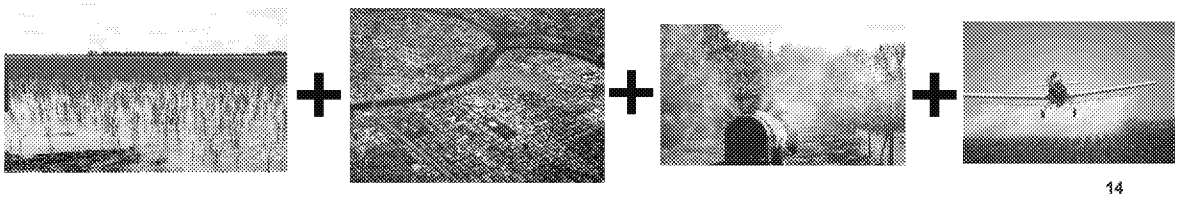
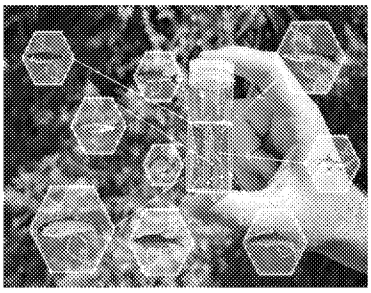
Exposure Modeling Subtask

- Rice
  - Collaborations: Martin Locke, Ron Bingner, USDA; Minghua Zhang, Ruoyu Wang, UC-Davis
  - RiceRQ; Pesticide in Flooded Application Model (PFAM) and other candidate models
- Comparison to monitoring data
- Cumulative exposure
  - additive exposure from multiple applications

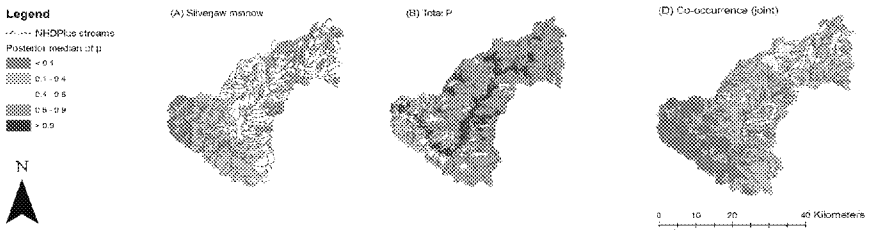


Co-Occurrence Subtask



- Environmental DNA (eDNA)
  - Species occurrence
  - Evaluate the use of eDNA to validate and improve risk models for specific endangered species
  - Comparison to predicted occupancy and existing monitoring data.

- Co-occurrence modeling
  - Data-driven, joint distribution modeling to predict and map coincidence of contaminants and sensitive species
  - Integrate with exposure models
  - High-resolution perspective (spatio-temporal dynamics / uncertainty)
    - Daily estimates of probability of co-occurrence across catchments



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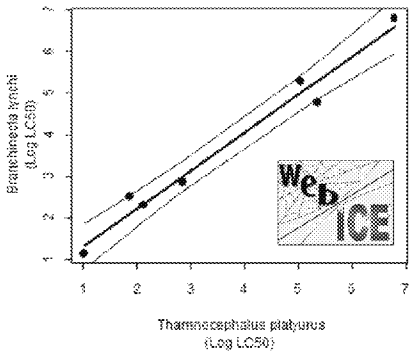
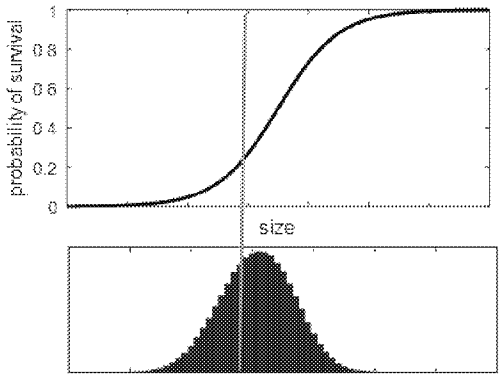
Effects Modeling Subtask

- Web-based Interspecies Correlation Estimation
  - Sensitivity of untested species
- Fish translator
  - model that integrates the results of FIFRA (Part 158) studies to estimate population-relevant endpoints for fish exposed to pesticides
  - Apply existing FIFRA – based studies (ELS & LC50) in population-level risk assessment
- Surrogacy and minimal data
  - Scenario specific



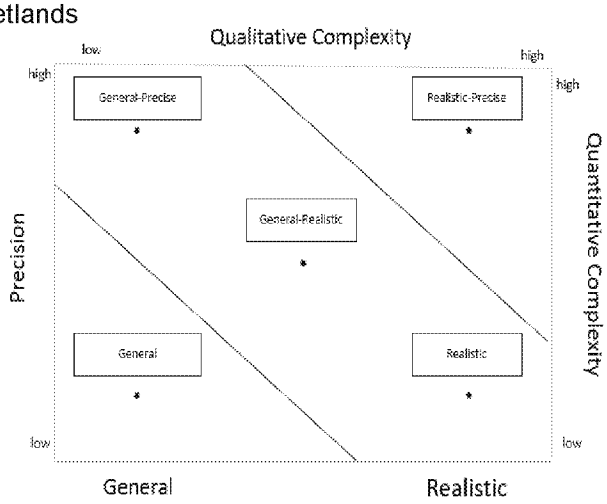


Integration Subtask

- Population models
  - Crustacean population model for time-varying exposures
  - Salmon population model in collaboration with NOAA
  - *A Framework for Linking Population Model Development with Ecological Risk Assessment Objectives.*

- Critical habitat – Geographically Isolated Wetlands
  - Vernal pools

- Weight of Evidence
  - Decision Science



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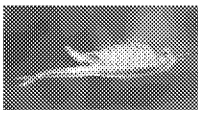
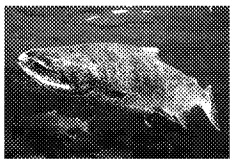
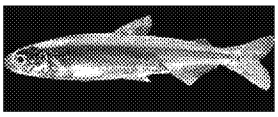
Pulling Pieces Together: Probabilistic Assessments

Probabilistic species risk assessment for one or more endangered California aquatic species

- Fairy shrimp (critical habitat focus)
  - PWC
  - eDNA
  - Lab, field, Web-ICE
  - Population model
- Salmon
  - Cumulative exposure from all modeling sources
  - Population model vs. “traditional” analysis
- Delta smelt
  - Cumulative exposure from all modeling sources
  - Co-occurrence model
  - eDNA
  - Fish translator







Tier 1 chemicals: Carbaryl, methomyl, bifenthrin, thiobencarb

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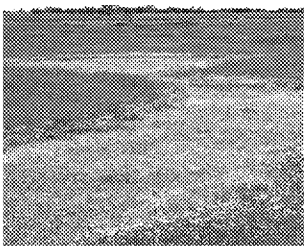
Fairy Shrimp & Vernal Pools

Exposure estimates
- PWC
- RiceRQ
- Field samples for chemical detection (water, soil)



Co-occurrence
- eDNA

Effects estimates
- Toxicity testing
- Web-ICE estimates

Integration
- VP fairy shrimp (meta)population model
- Framework for critical habitat assessment

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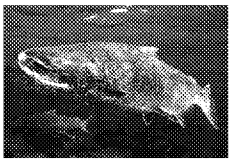
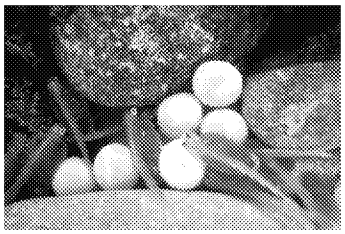
Salmon



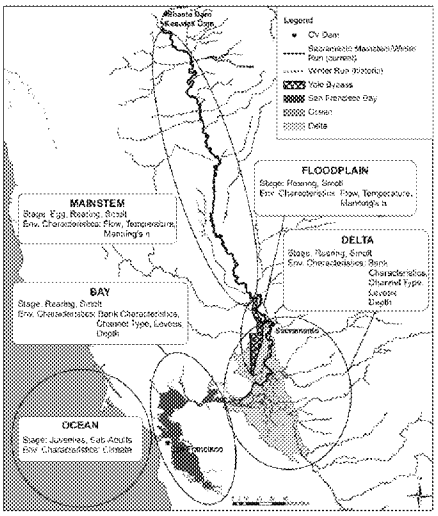


Exposure estimates
- SAM
- RiceRQ
- PWC for Urban applications
- Monitoring data

Co-occurrence
- NA

Effects estimates
- Literature

Integration
- Population model with NOAA collaboration
- Demonstration of models of varying complexity
- Comparison to traditional ERA



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National Center for Computational Toxicology

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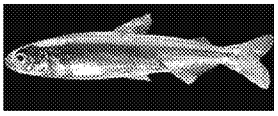
Delta Smelt

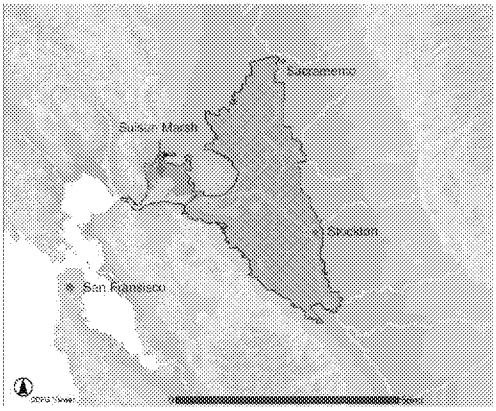
Exposure estimates
- Cumulative exposure from all sources
- Monitoring data

Co-occurrence
- Probabilistic modeling
- eDNA

Effects estimates
- Fish Translator

Integration
- Do we need population models?





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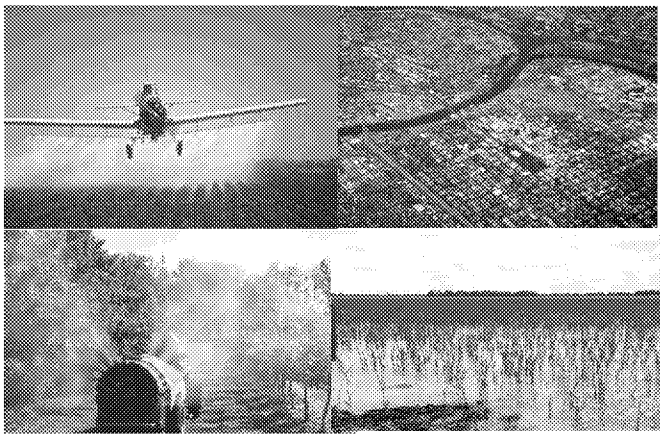
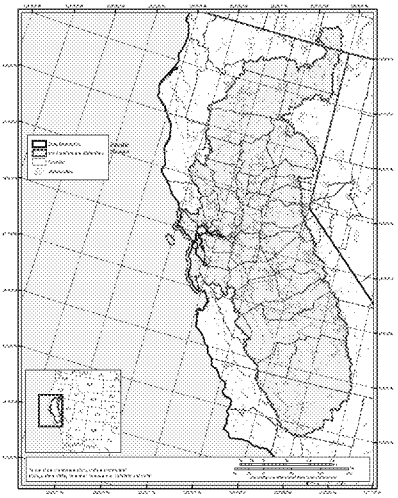
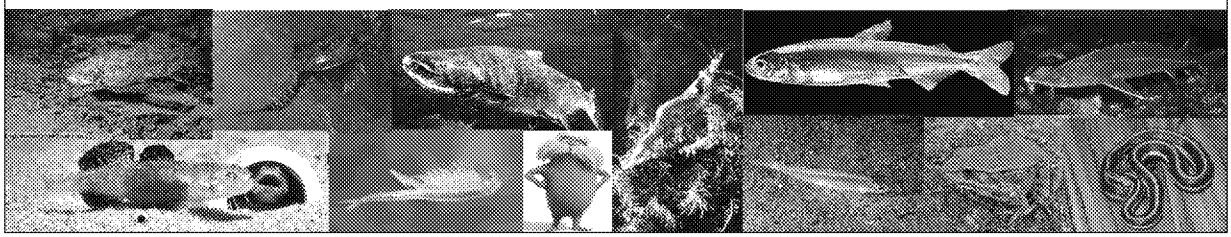
Questions?







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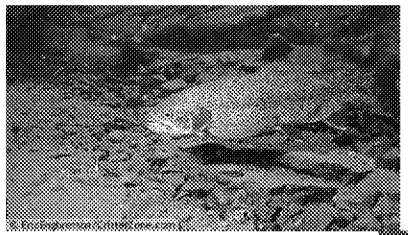
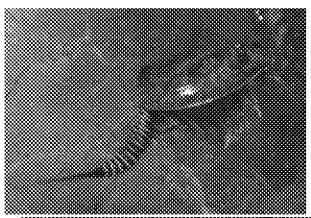
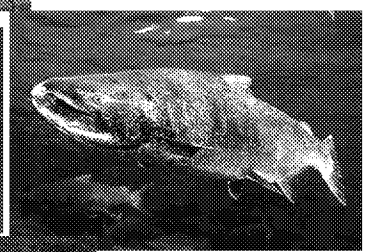
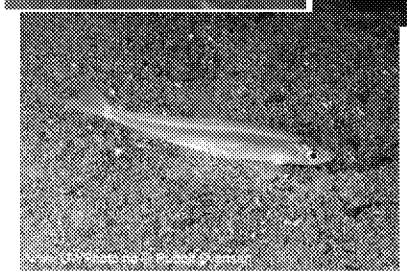
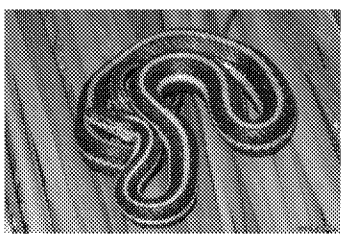
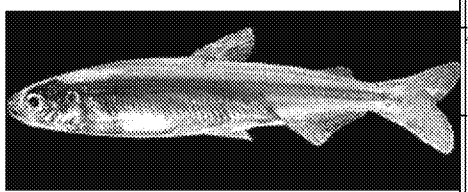
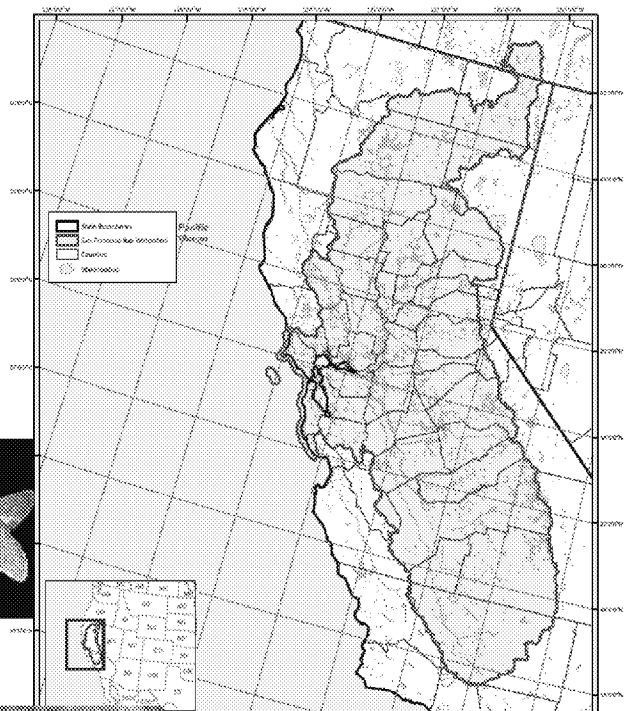
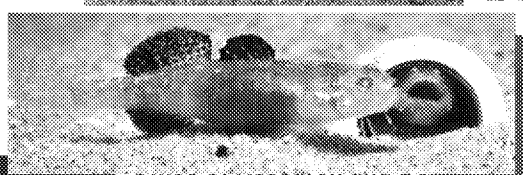
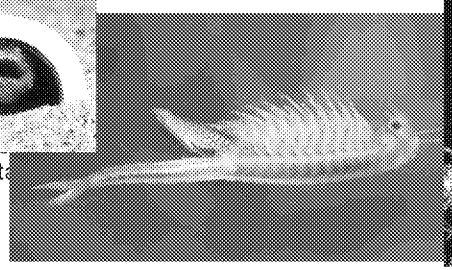
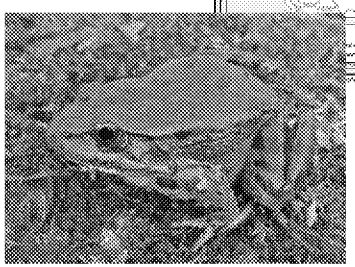
Chemical Safety for Sustainability
California Endangered Species Case Study

Sandy Raimondo
Office of Research and Development
20 September 2017



National Center for Comput

United States Environmental Protection Agency

The EPA Team

ORD/NHEERL

- Gulf Ecology Division (GED)
  - Sandy Raimondo (task lead)
  - Leah Oliver
  - Leah Sharpe
- Mid-Continent Ecology Division (MED)
  - Matthew Etterson
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- Atlantic Ecology Division (AED)
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Region 9

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- Ecosystems Research Division (ERD)
  - Tom Purucker
  - Sumathy Sinnathamby
- Ecological Exposure Research Division (EERD)
  - Roy Martin
  - Eric Waits

- OPP/EFED
  - Kris Garber
  - Andrew Kanarek

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EPA
United States
Environmental Protection
Agency

Stakeholders

Water Boards

USDA

CALIFORNIA DEPARTMENT OF FISH & WILDLIFE

NOAA
NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION
U.S. DEPARTMENT OF COMMERCE

DEPARTMENT OF WATER RESOURCES
STATE OF CALIFORNIA

CalEPA
California Environmental
Protection Agency

USGS

PACIFIC STATES MARINE FISHERIES COMMISSION

SWAMP
Surface Water
Ambient Monitoring
Program

DWR • USBR • USFWS • SWRCB • DFW • USEPA • USACE • NMFS • USGS

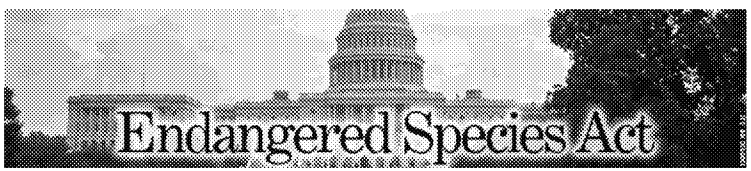
UCDAVIS

CRAMER
FISH SCIENCES
Oregon • California • Washington • Idaho • Alaska

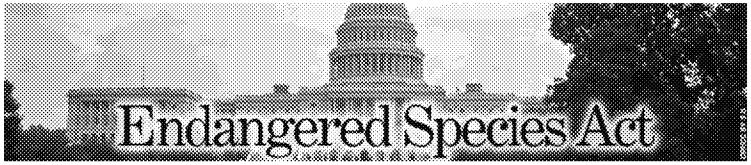
United States Environmental Protection Agency

Task Drivers and Goals

- EPA research priorities highlighted in the National Academy of Science's report (2013) "Assessing Risks to Endangered and Threatened Species from Pesticides"
- Develop, evaluate, and demonstrate approaches that improve endangered species risk assessments and reduce knowledge-based uncertainties
- Approaches that transition from the current EPA process toward probabilistic risk assessment for threatened and endangered (T&E) species
- Develop, evaluate, and provide guidance on approaches that make use of minimal data, increasing the number and types of exposure scenarios, and implementing models that project population-level impacts to listed species.



EPA



Step 1 (EPA)
May Affect?

NO

YES – Option 1

YES – Option 2

Step 2 (EPA)
Informal Consultation
Likely to Adversely Affect?

NO

YES

Concurrence?
FWS or NMFS

YES

NO

Step 3 (FWS or NMFS)
Formal Consultation
Jeopardy?

NO

REGISTRATION
OR REREGISTRATION
OF PESTICIDE

YES

EPA DECIDES WHETHER AND UNDER
WHAT CONDITIONS TO REGISTER
PESTICIDE

Problem Formulation
Exposure Analysis
Effects Analysis
Risk Characterization

Problem Formulation
Exposure Analysis
Effects Analysis
Risk Characterization

Problem Formulation
Exposure Analysis
Effects Analysis
Risk Characterization

ERA Process

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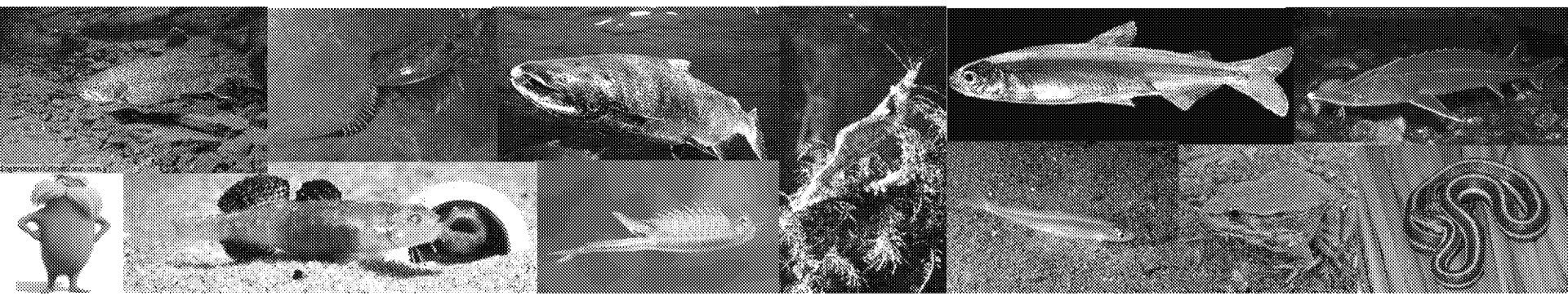
Anticipated Project Outcomes

- Reduced uncertainties associated with predicting pesticide impacts to endangered species
- Provide improved and defensible approaches available for Section 7 consultation
- Develop framework for integrating exposure and effects into probabilistic assessments
- Minimize impacts to listed species from pesticide exposure that provides greater protection of other species



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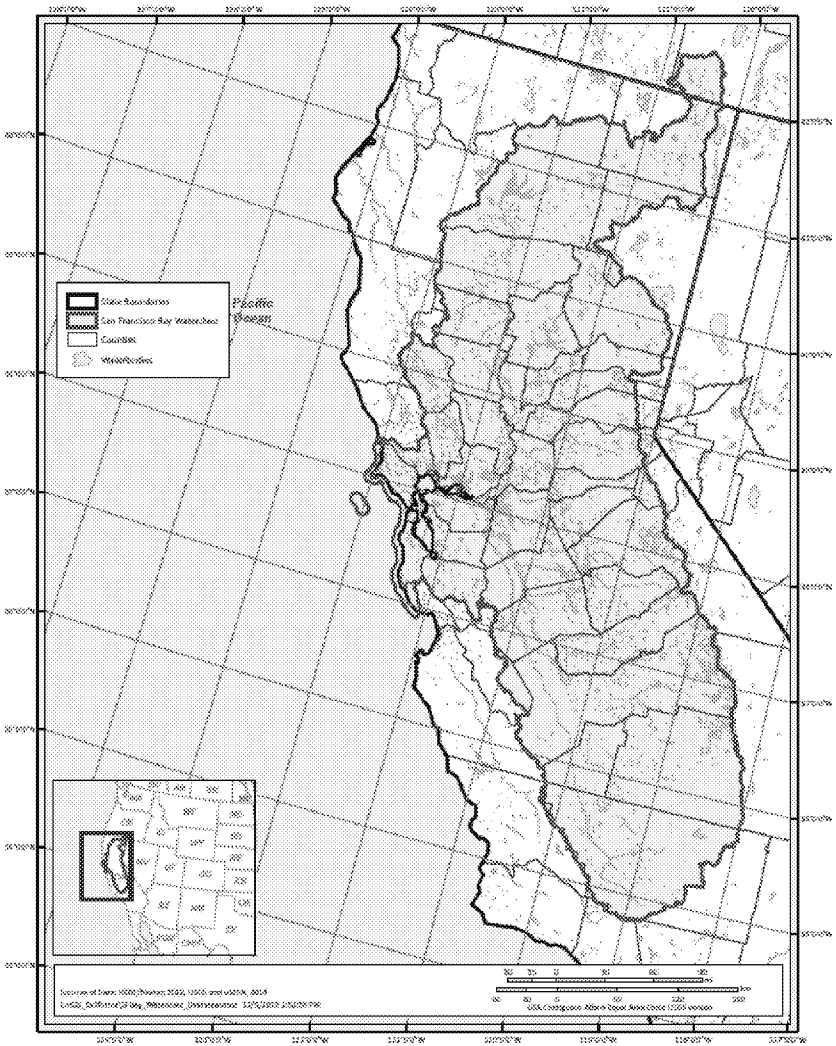
California Central Valley

- Diversity
  - Listed species
  - Pesticide use
  - Habitat types
- Relatively “abundant” data
  - Pesticide use
  - Environmental monitoring
  - Species data
- Region & State partnership
- Historical processes for ESA well documented



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2015 Project Scoping Meeting

- Understand processes required of the Agency, Services, and States to comply with the Endangered Species Act (ESA)
- Overview species and pesticide usage within the study area
- Engagement with representatives of the Regional and State Offices
  - EPA Office of Pesticide Programs
  - EPA Office of Water
  - EPA Region 9
  - US Fish and Wildlife
  - CA State Water Resource Board (Rich Breuer)
  - CA Department of Pesticide Registration (Yuzhou Luo, Rich Marovich)
  - CA Department of Fish and Wildlife (Stephen Louie)
- Task framework established

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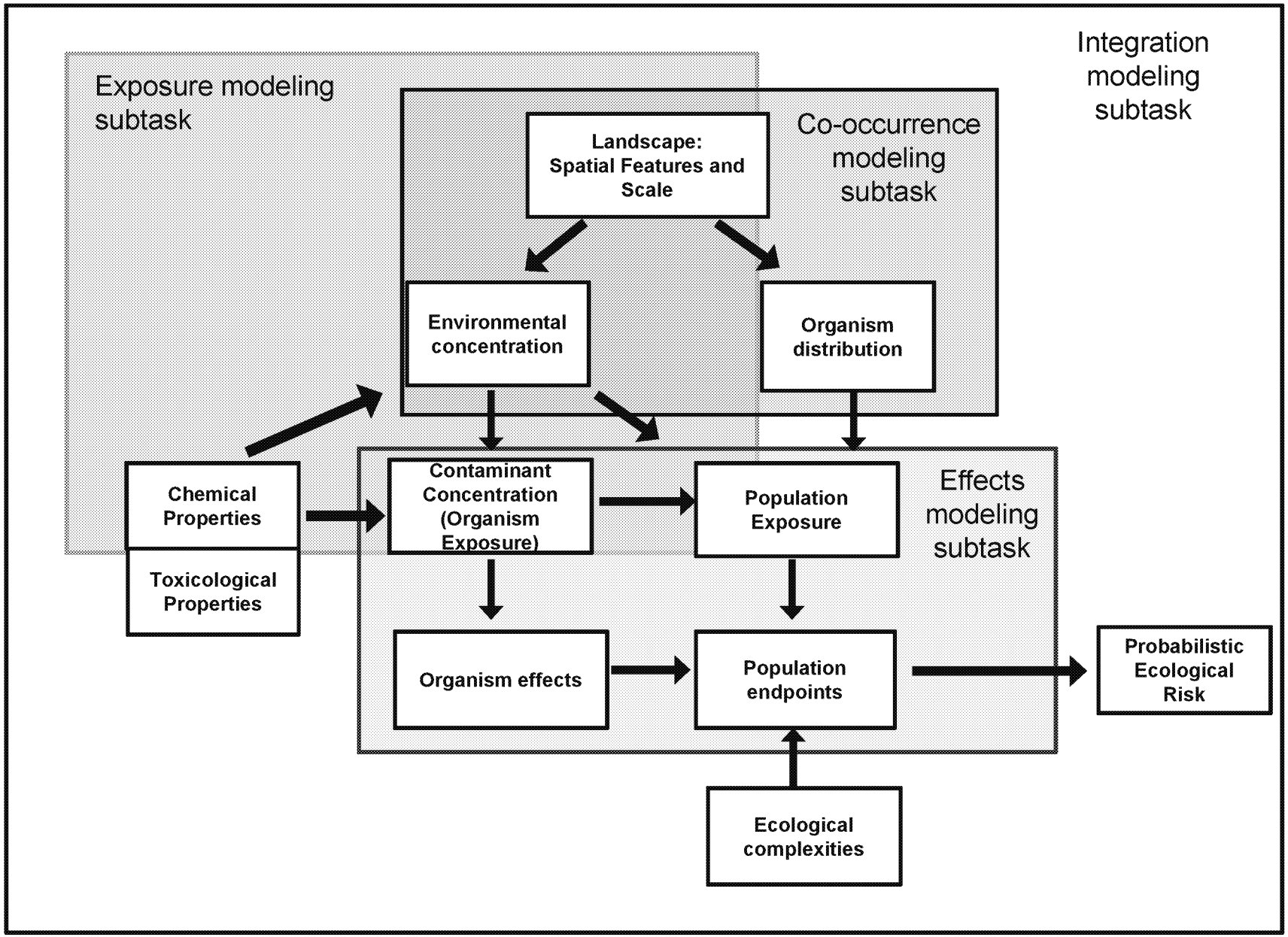
Task Conceptual Model and Structure



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California Central Valley Species

Tier 1

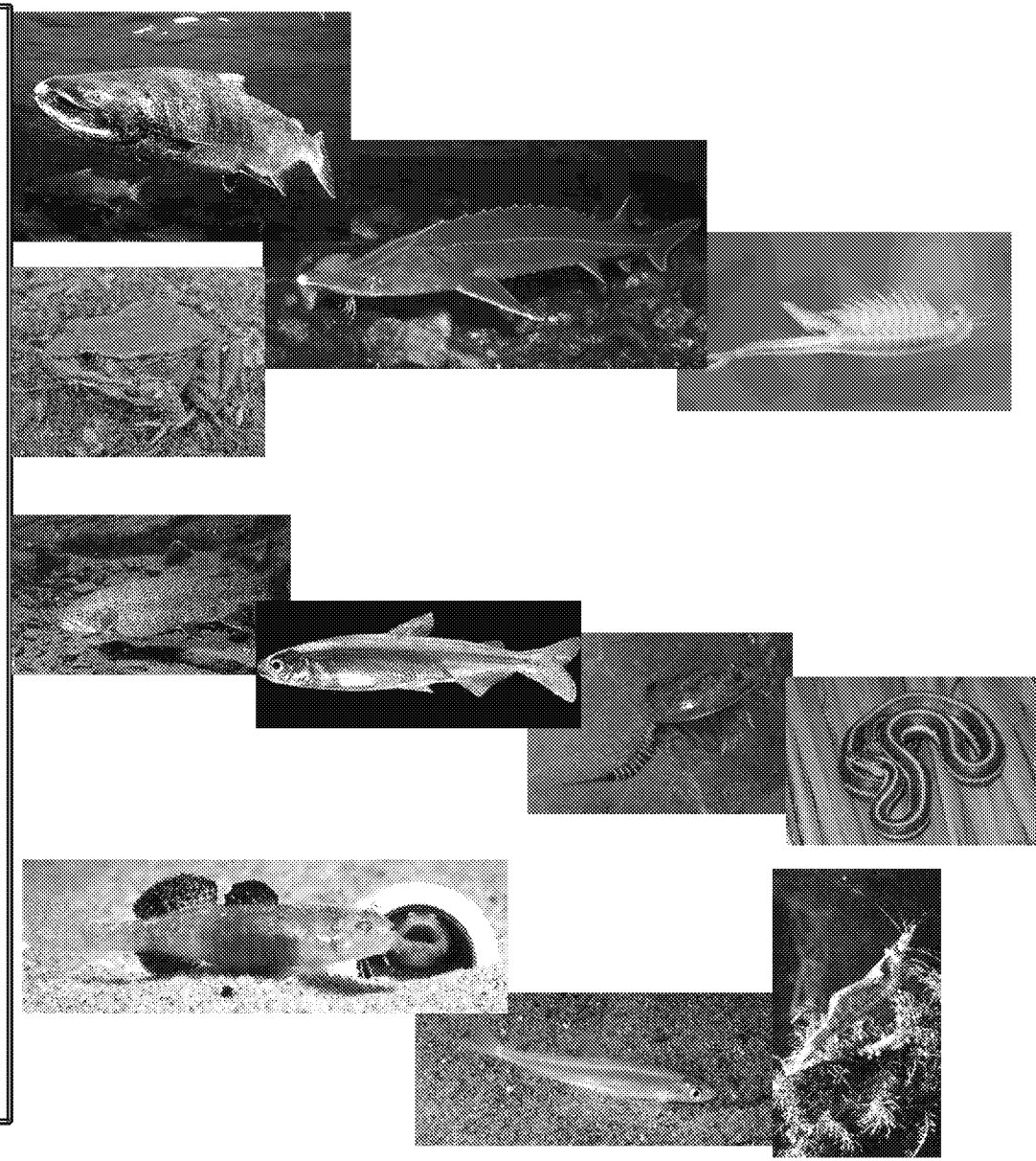
- Chinook Salmon (*Oncorhynchus tshawytscha*)
  - Sacramento River winter-run
  - Central Valley spring-run
- Southern North American Green Sturgeon (*Acipenser medirostris*)
- Fairy shrimp (*Branchinecta lynchi*)
- California Red-legged Frog (*Rana draytonii*)

Tier 2

- Central Valley steelhead (*Onchoryncus mykiss*)
- Delta Smelt (*Hypomesus transpacificus*)
- Tadpole shrimp (*Lepidurus packardi*)
- Garter snake (*Thamnophis gigas*)

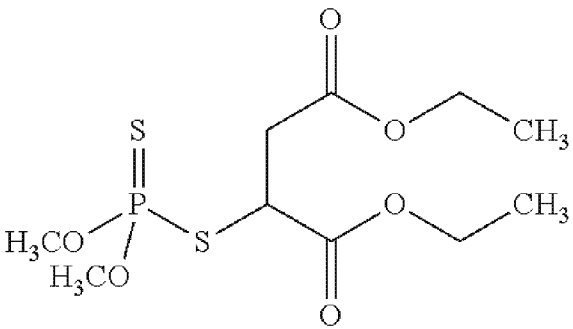
Tier 3

- Tidewater goby (*Eucyclogobius newberryi*)
- San Francisco Longfin Smelt (*Spirinchus thaleichthys*)
- California Freshwater Shrimp (*Syncaris pacifica*)



Priority Chemicals

- 40 chemicals, priority tiered
- OPP/Region 9 priorities
- Diverse chemical class
- Varying available data



Malathion

Chemical	CAS	Use pattern	Chemical Class	Tier
Bifenthrin	82657043	Insecticide	pyrethroid	1
Carbaryl	63252	Insecticide	carbamate	1
Chlorpyrifos	2921882	Insecticide	organophosphate	1
Cypermethrin	52315078	Insecticide	pyrethroid	1
Diazinon	333415	Insecticide	organophosphate	1
Fipronil	120068373	Insecticide	phenylpyrazole	1
Imidacloprid	138261413	Insecticide	neonicotinoid	1
Malathion	121755	Insecticide	organophosphate	1
Methomyl	16752775	Insecticide	carbamate	1
Permethrin	52645531	Insecticide	pyrethroid	1
Thiobencarb	28249776	Herbicide	carbamate	1
Deltamethrin	52918635	Insecticide	pyrethroid	2
Captan	133062	Fungicide	thiophthalimide	2
Chlorothalonil	1897456	Fungicide	chloronitrile	2

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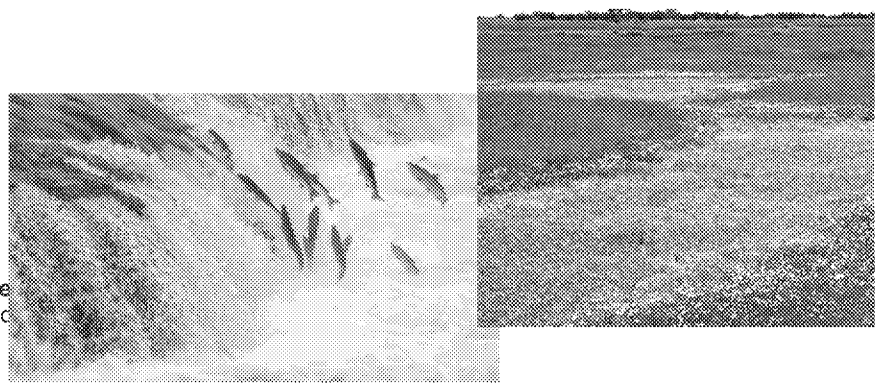
Exposure Modeling Subtask

Pesticide Use

- Row crops
- Orchards
- Urban
- Rice

Habitat type

- Streams
- Delta
- Vernal pools



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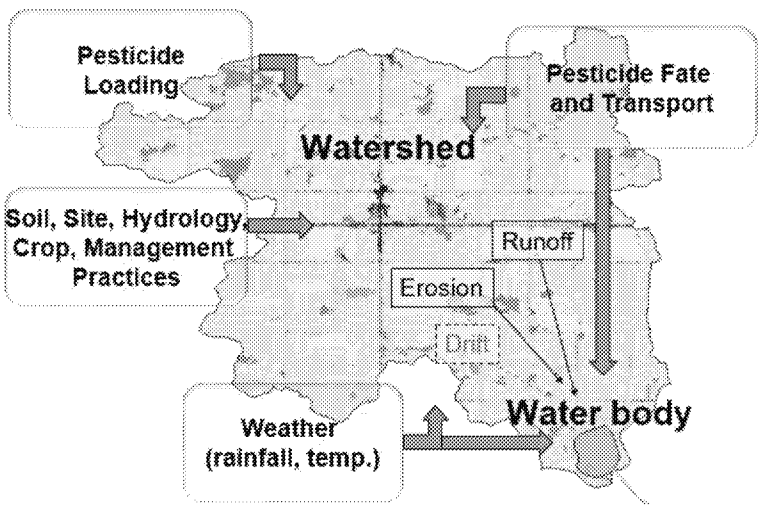
Exposure Modeling Subtask

- Spatial Aquatic Model (SAM; agriculture)
  - Watershed based model for surface water
  - Improve approach for higher-tiered assessments
  - Where and when exceedance will occur
  - Daily estimates

- Pesticide in Water Calculator (PWC)
  - Urban, vernal pool applications
  - Watershed delineation using Soil and Water Assessment Tool (SWAT)
  - Crop parameters
  - Weather data
  - Physicochemical properties



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Exposure Modeling Subtask

- Rice
  - Collaborations: Martin Locke, Ron Bingner, USDA; Minghua Zhang, Ruoyu Wang, UC-Davis
  - RiceRQ; Pesticide in Flooded Application Model (PFAM) and other candidate models
- Comparison to monitoring data
- Cumulative exposure
  - additive exposure from multiple applications

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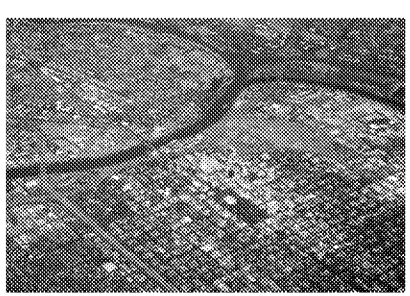
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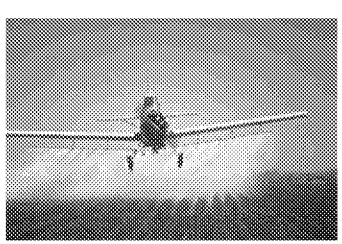

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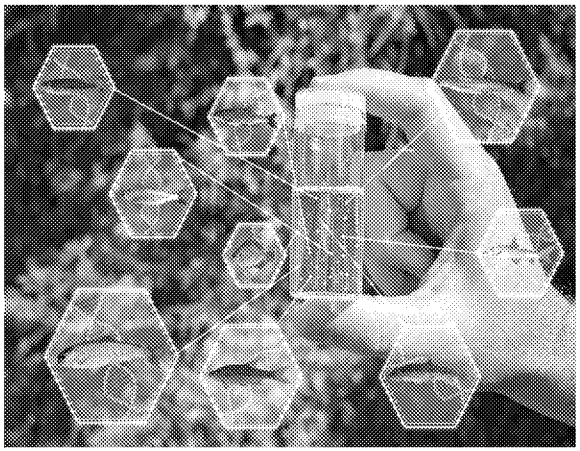
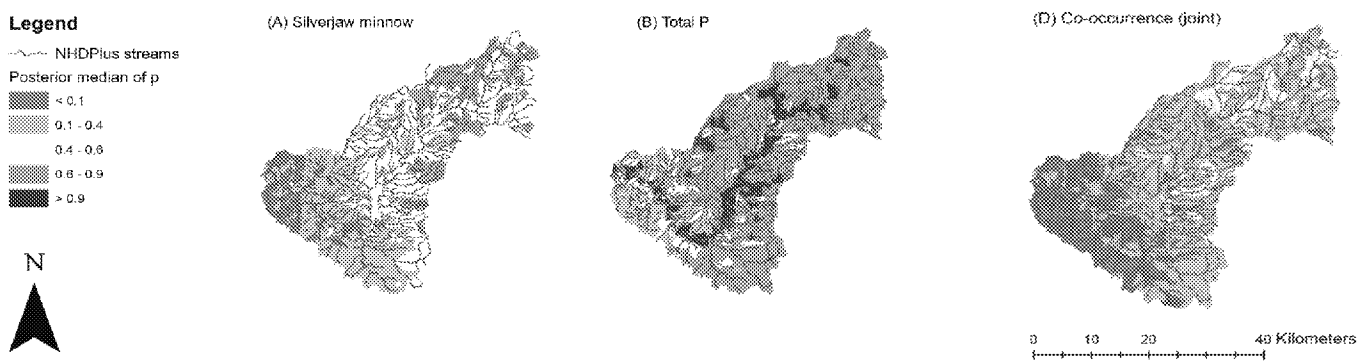
Co-Occurrence Subtask



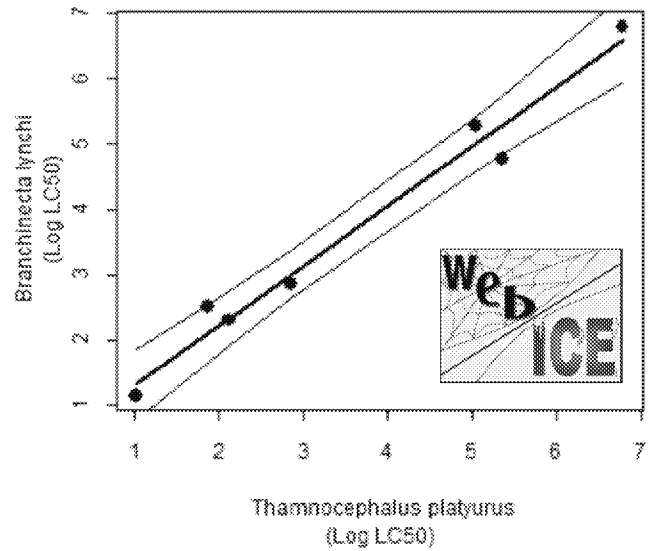
- Environmental DNA (eDNA)
  - Species occurrence
  - Evaluate the use of eDNA to validate and improve risk models for specific endangered species
  - Comparison to predicted occupancy and existing monitoring data.

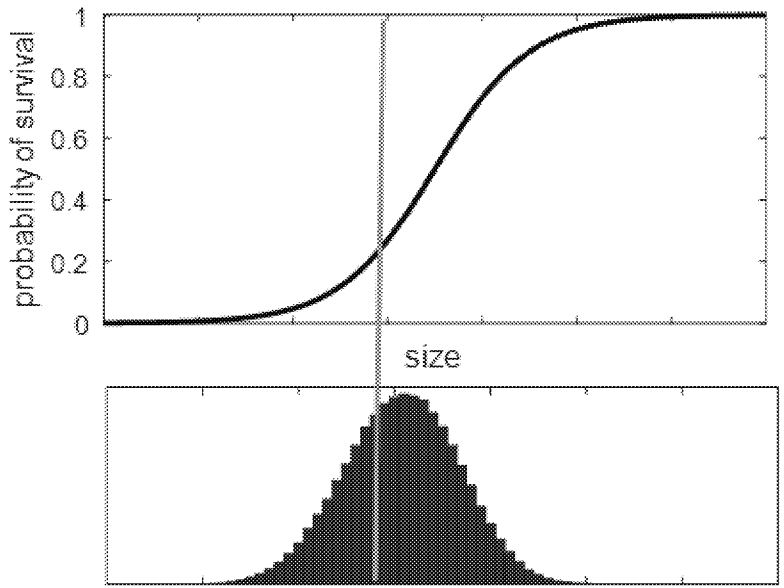
- Co-occurrence modeling
  - Data-driven, joint distribution modeling to predict and map coincidence of contaminants and sensitive species
  - Integrate with exposure models
  - High-resolution perspective (spatio-temporal dynamics / uncertainty)
    - Daily estimates of probability of co-occurrence across catchments



Effects Modeling Subtask

- Web-based Interspecies Correlation Estimation
  - Sensitivity of untested species
- Fish translator
  - Model that integrates the results of FIFRA (Part 158) studies to estimate population-relevant endpoints for fish exposed to pesticides
  - Apply existing FIFRA – based studies (ELS & LC50) in population-level risk assessment
- Surrogacy and minimal data
  - Scenario specific





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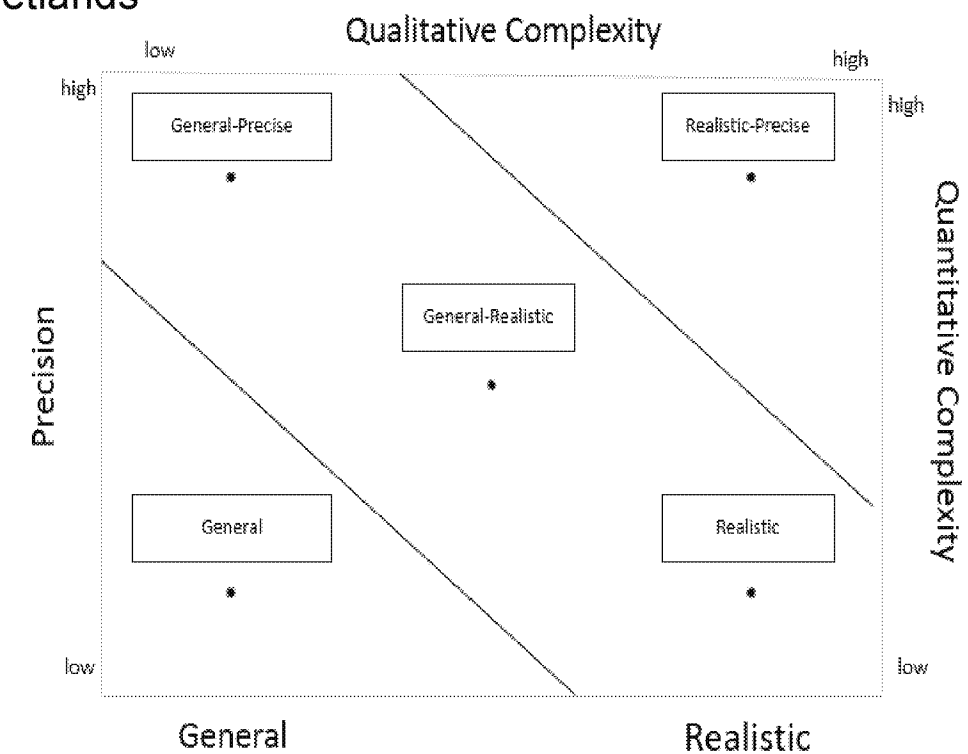
Integration Subtask

- Population models
  - Crustacean population model for time-varying exposures
  - Salmon population model in collaboration with NOAA
  - *A Framework for Linking Population Model Development with Ecological Risk Assessment Objectives.*

- Critical habitat – Geographically Isolated Wetlands
  - Vernal pools

- Weight of Evidence
  - Decision Science



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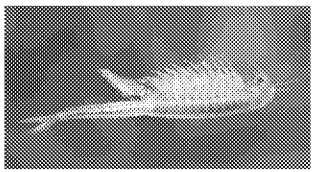
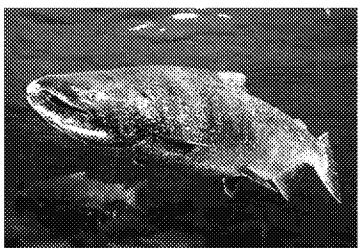
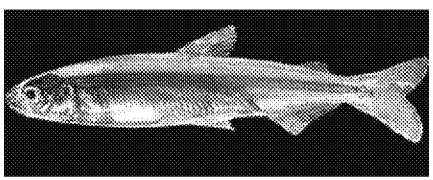
Pulling Pieces Together: Probabilistic Assessments

Probabilistic species risk assessment for one or more endangered California aquatic species

- Fairy shrimp (critical habitat focus)
  - PWC
  - eDNA
  - Lab, field, Web-ICE
  - Population model
- Salmon
  - Cumulative exposure from all modeling sources
  - Population model vs. “traditional” analysis
- Delta smelt
  - Cumulative exposure from all modeling sources
  - Co-occurrence model
  - eDNA
  - Fish translator







Tier 1 chemicals: Carbaryl, methomyl, bifenthrin, thiobencarb

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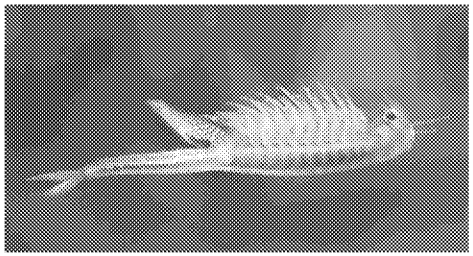
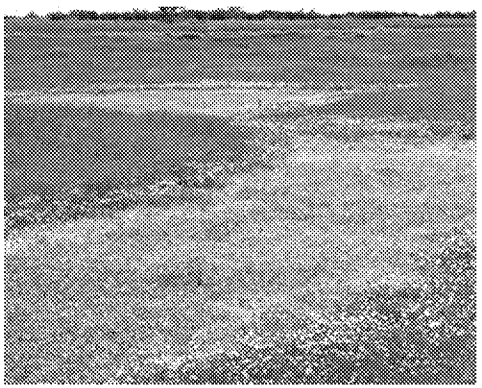
Fairy Shrimp & Vernal Pools



Exposure estimates
- PWC
- RiceRQ
- Field samples for chemical detection (water, soil)



Co-occurrence
- eDNA

Effects estimates
- Toxicity testing
- Web-ICE estimates

Integration
- VP fairy shrimp (meta)population model
- Framework for critical habitat assessment

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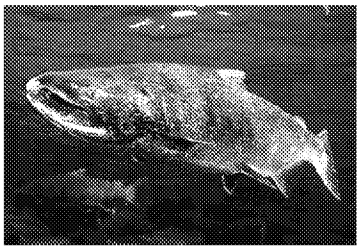
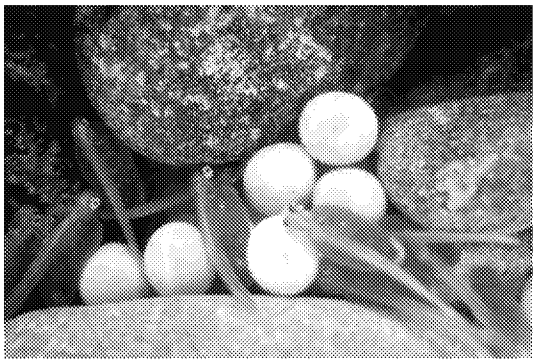
Salmon





Exposure estimates

- SAM
- RiceRQ
- PWC for Urban applications
- Monitoring data

Co-occurrence

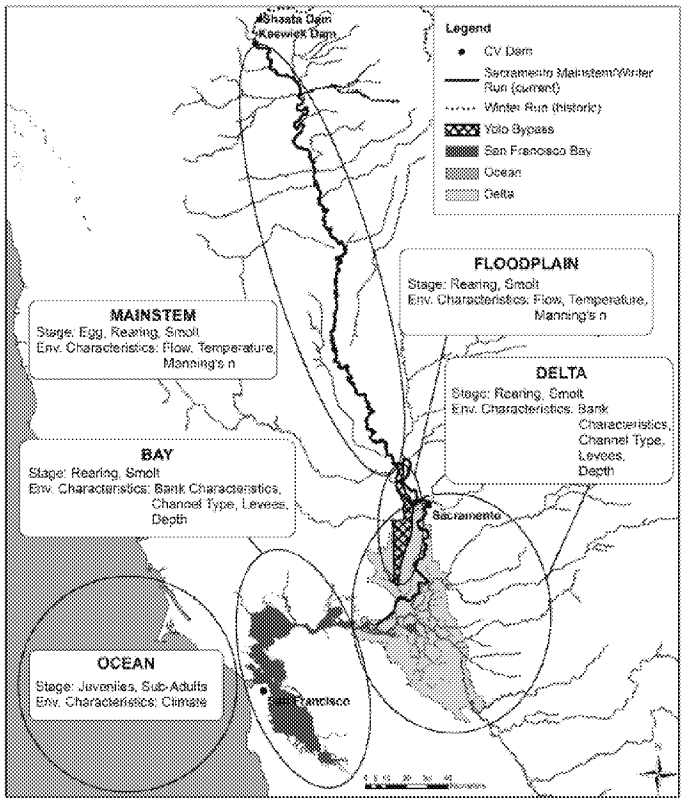
- NA

Effects estimates

- Literature

Integration

- Population model with NOAA collaboration
- Demonstration of models of varying complexity
- Comparison to traditional ERA



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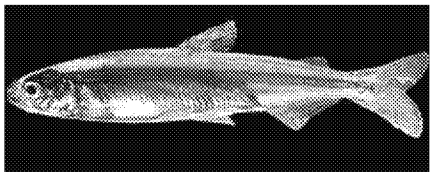
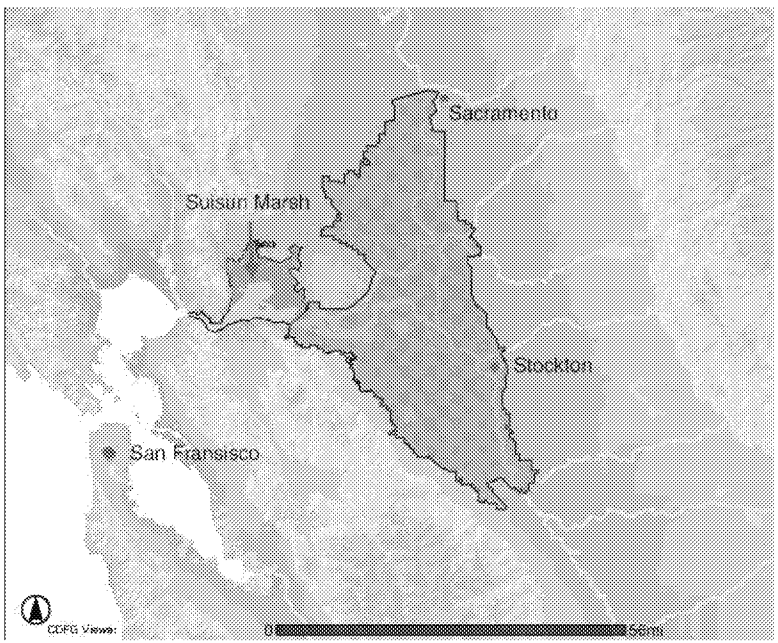
Delta Smelt

Exposure estimates
- Cumulative exposure from all sources
- Monitoring data



Co-occurrence
- Probabilistic modeling
- eDNA



Effects estimates
- Fish Translator

Integration
- Do we need population models?

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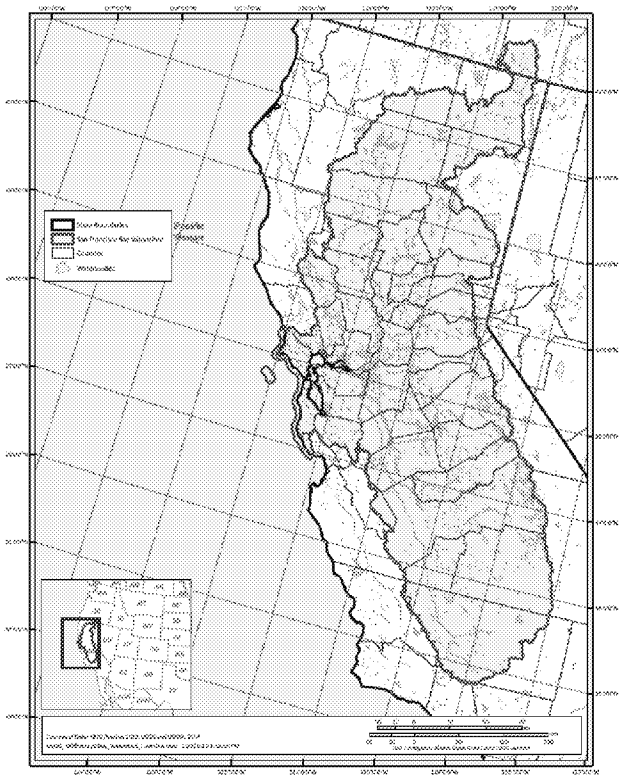
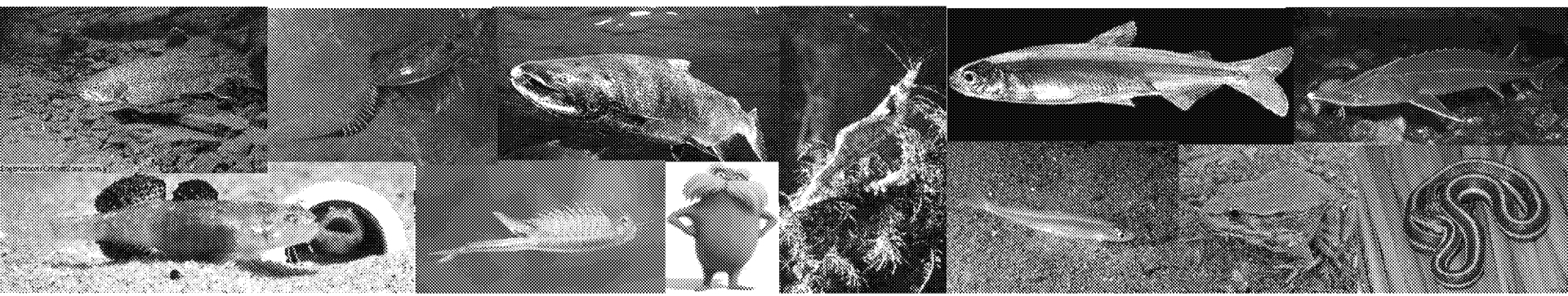
Questions?





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Environmental Toxicology

Acute Toxicity of 6 Neonicotinoid Insecticides to Freshwater Invertebrates

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[d]Laboratory Services Branch, Ontario Ministry of the Environment and Climate Change, Toronto, Ontario, Canada

Abstract: Neonicotinoids are a group of insecticides commonly used in agriculture. Due to their high water solubility, neonicotinoids can be transported to surface waters and have the potential to be toxic to aquatic life. The present study assessed and compared the acute (48- or 96-h) toxicity of 6 neonicotinoids (acetamiprid, clothianidin, dinotefuran, imidacloprid, thiacloprid, and thiamethoxam) to 21 laboratory-cultured and field-collected aquatic invertebrates spanning 10 aquatic arthropod orders. Test conditions mimicked species' habitat, with lentic taxa exposed under static conditions, and lotic taxa exposed under recirculating systems. Median lethal concentrations (LC50s) and median effect concentrations (EC50s; immobility) were calculated and used to construct separate lethal- and immobilization-derived species sensitivity distributions for each neonicotinoid, from which 5th percentile hazard concentrations (HC5s) were calculated. The results showed that the most sensitive invertebrates were insects from the orders Ephemeroptera (*Neocloeon triangulifer*) and Diptera (*Chironomus dilutus*), whereas cladocerans (*Daphnia magna*, *Ceriodaphnia dubia*) were the least sensitive. The HC5s were compared with neonicotinoid environmental concentrations from Ontario (Canada) monitoring studies. For all neonicotinoids except imidacloprid, the resulting hazard quotients indicated little to no hazard in terms of acute toxicity to aquatic communities in Ontario freshwater streams. For the neonicotinoid imidacloprid, a moderate hazard was found when only invertebrate immobilization, and not lethality, data were considered. *Environ Toxicol Chem* 2018;37:1430–1445. © 2018 SETAC

Keywords: Insecticides; Pesticides; Aquatic invertebrates; Species sensitivity distributions; Toxic effects

INTRODUCTION

Neonicotinoids are a class of pesticides widely used in agriculture as plant protectants. All are water soluble, can be applied as seed or spray treatments, and act systemically to protect the entire plant (Jeschke et al. 2011). They bind strongly to the nicotinic acetylcholine receptor (*n*AChR) found in the central nervous system of insects, causing constant nerve stimulation that eventually leads to death or other toxic effects (Morrissey et al. 2015).

The first neonicotinoid brought to market was imidacloprid in 1991, and 6 others followed in the next decade: acetamiprid (1995), nitenpyram (1995), thiamethoxam (1998), thiacloprid (2000), clothianidin (2002), and dinotefuran (2002; Jeschke and Nauen 2008). Their systemic actions, combined with low mammalian toxicity, effectiveness against pests, and low application volumes, have made neonicotinoids the most popular class of insecticides worldwide (Jeschke et al. 2011; Simon-Delso et al. 2015). Due to their widespread use, high water solubility, and persistence, neonicotinoid residues are therefore frequently detected in surface waters that drain agricultural lands (Morrissey et al. 2015).

Transport of neonicotinoids to aquatic environments is typically driven by precipitation events (Hladik and Kolpin 2016; Struger et al. 2017), snowmelt (Main et al. 2016), and contaminated dust (Limay-Rios et al. 2016). Median neonicotinoid surface water concentrations are on the order of ng L^{-1}, with peak concentrations rising to low $\mu g\ L^{-1}$. Struger et al. (2017) detected the 3 most common neonicotinoids—imidacloprid, thiamethoxam, and clothianidin—in >90% of samples from more than half of their 15 Ontario (Canada) surface water sites. Median concentrations in that study ranged from <0.00128 to 0.585 $\mu g\ L^{-1}$, and the maximum detection was 10.4 $\mu g\ L^{-1}$ imidacloprid. Detections were correlated with registered uses; thiamethoxam and clothianidin were correlated

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with row crops, imidacloprid and acetamiprid with vegetable crops, greenhouses, and other agriculture, and thiacloprid with fruit production (Struger et al. 2017). In addition, neonicotinoid detections showed seasonality, with maximum concentrations occurring in the late spring and fall (Struger et al. 2017). Monitoring by the Ontario Ministry of the Environment and Climate Change in 2015 also showed the most common detections to be imidacloprid, thiamethoxam, and clothianidin, with median concentrations from 0.018 to 0.050 $\mu g\,L^{-1}$ and maximum concentrations of 2.3 $\mu g\,L^{-1}$ imidacloprid and 2.7 $\mu g\,L^{-1}$ thiamethoxam (Ontario Ministry of the Environment and Climate Change 2016). Environmental concentrations are similar on a global scale; Morrissey et al. (2015) surveyed 29 surface water neonicotinoid monitoring studies from 9 countries and reported mean surface water concentrations of 0.13 $\mu g\,L^{-1}$, with a geometric mean for peak concentrations of 0.63 $\mu g\,L^{-1}$.

Neonicotinoids are acutely toxic to aquatic macroinvertebrates at concentrations that span 6 orders of magnitude, with insects generally showing greater sensitivity than crustaceans (Morrissey et al. 2015). However, within the Insecta, sensitivity varies widely. Roessink et al. (2013) reported 96-h median lethal concentrations (LC50s) for imidacloprid of between 6.68 $\mu g\,L^{-1}$ for *Caenis horaria* (Ephemeroptera) and >10 000 $\mu g\,L^{-1}$ for *Notonecta* spp. (Hemiptera) and *Sialis lutaria* (Megaloptera). Reported 96-h LC50s for mayflies vary from 0.65 $\mu g\,L^{-1}$ for *Epeorus longimanus* (Alexander et al. 2007) to 154 $\mu g\,L^{-1}$ for *Cloeon dipterum* (Van den Brink et al. 2016). Such variability in toxicity also exists in the Crustacea, with 48-h LC50s and EC50s (median effect concentration) >10 000 $\mu g\,L^{-1}$ reported for *Daphnia magna* (Song et al. 1997; Hayasaka et al. 2012), compared with 48-h LC50 of 2.07 $\mu g\,L^{-1}$ (Chen et al. 2010) and 48-h EC50 of 571.62 $\mu g\,L^{-1}$ (Hayasaka et al. 2012) for *Ceriodaphnia dubia*. This wide range in sensitivity within and between groups highlights the need for testing across different aquatic invertebrate orders, including those outside the typical model species, to better understand the range of neonicotinoid toxicity and hence potential risks in aquatic ecosystems.

A few government regulatory bodies around the world have derived water quality guidelines for neonicotinoids, as summarized by Morrissey et al. (2015). In North America, the US Environmental Protection Agency has published acute aquatic life benchmarks for 6 neonicotinoids, ranging from 10.5 $\mu g\,L^{-1}$ for acetamiprid to >484 150 $\mu g\,L^{-1}$ for dinotefuran (US Environmental Protection Agency 2017). The Canadian Council of Ministers of the Environment (CCME) has an interim water quality guideline for imidacloprid of 0.23 $\mu g\,L^{-1}$. This guideline is based on a 28-d lowest-observed-effect concentration (LOEC) for *Chironomus riparius* of 2.25 $\mu g\,L^{-1}$ imidacloprid, with a 0.1 safety factor applied (Canadian Council of Ministers of the Environment 2007a). Individual water quality guidelines for other neonicotinoids do not exist in Canada, partly because of the lack of toxicity data and minimum data requirements (minimum of 3 aquatic invertebrates, ideally with 1 being a mayfly, caddisfly, or stonefly) stipulated by the CCME for constructing species sensitivity distributions (SSDs; Canadian Council of Ministers of the Environment 2007b). In 2015, Morrissey et al. constructed an acute SSD using a combined dataset whereby toxicity data for all neonicotinoids were standardized and weighted by molecular mass to imidacloprid to establish a recommended short-term acute guideline of 0.2 $\mu g\,L^{-1}$. However, although this is a useful interim approach, it is based on the assumption that the most toxic neonicotinoid across all aquatic species is imidacloprid, which, as we show in the present study, is not always the case.

The objective of the present study was to evaluate the acute toxicity of 6 neonicotinoid insecticides (imidacloprid, thiamethoxam, clothianidin, acetamiprid, thiacloprid, and dinotefuran) to a suite of aquatic invertebrate species ($n = 21$), with the goal of better defining the range of sensitivity, creating a comprehensive dataset to characterize the potential risk of the 6 neonicotinoids, and developing rigorous short-term acute water quality guidelines. The invertebrates tested in the present study included both laboratory-cultured model aquatic invertebrates and field-collected species; they represent both lotic and lentic habitats, have broad representation within the Arthropoda, and include several functional feeding groups (Merritt and Cummings 1996). All species tested are relevant to freshwaters near agricultural areas where neonicotinoids could be applied.

MATERIALS AND METHODS

Toxicity tests: Test solution preparation

Toxicity tests were conducted with 6 technical-grade neonicotinoids purchased from Sigma-Aldrich: acetamiprid (99.9%, CAS 135410-20-7), clothianidin (99.9%, CAS 210880-92-5), dinotefuran (98.6–98.8%, CAS 165252-70-0), imidacloprid (99.9%, CAS 138261-41-3), thiacloprid (99.9%, CAS 111988-49-9), and thiamethoxam (99.6%, CAS 153719-23-4). Imidacloprid (99.8%) was also obtained from Bayer CropScience and thiamethoxam (technical grade, purity not stated) from Syngenta Crop Protection. Stock solutions were prepared by dissolving up to 100 mg of chemical/1 L of dechlorinated municipal tap water (physicochemical properties provided in the Supplemental Data, Table S2) in class A glassware. The solutions were covered with tin foil to limit photodegradation stirred overnight, and then stored in 1-L amber glass bottles at 4 ± 2 °C. Test solutions were prepared by either spiking the appropriate volume of stock into a known volume of dechlorinated water or by serial dilutions in which 5 to 7 test solutions were prepared for each test in a 50 or 70% geometric series. Each test included a negative control of dechlorinated water. Conductivity, pH, dissolved oxygen, and temperature were measured on at least the negative control and the highest test concentration before and after each toxicity test. Temperature was monitored throughout the test.

Test species

Laboratory-cultured species. Acute toxicity tests were performed with 7 laboratory-cultured species: *Ceriodaphnia dubia*, *Chironomus dilutus*, *Daphnia magna*, *Hexagenia* spp., *Hyalella azteca*, *Lumbriculus variegatus*, and *Neocloeon triangulifer*. Culturing methods are outlined in the Supplemental Data, Table S1. Test methods were based on standard Ontario

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Ministry of the Environment and Climate Change and literature-derived methods (Rodrigues and Kaushik 1984; Environment Canada 2007a, 2007b; Soucek and Dickinson 2015; Ontario Ministry of the Environment and Climate Change Aquatic Toxicology Unit 2016). Tests were conducted with 5 or 10 organisms/replicate, with 3 to 5 replicates/treatment using a regression design. Test parameters are described in Table 1. Endpoints of immobility and mortality were observed at 96 ± 4 h; obvious mortalities (i.e., individuals starting to decompose) during the tests were counted, and the dead organisms were removed daily. Mortality was classified as complete cessation of organism movement even after gentle prodding with a pipette and was confirmed under a dissecting microscope (×2–4 magnification). Immobilization responses included lethargy, limited swimming behaviors, and muscle spasms, as described by Camp and Buchwalter (2016). In preliminary tests, we had noted that immobile individuals that did not respond to prodding were alive when observed under a microscope, often with mouthparts, legs, and/or gills moving. We therefore confirmed all mortality under a microscope, and only scored individuals as dead when there was complete cessation of all organism movement. Other studies noting the effects of neonicotinoids on aquatic invertebrates have generally counted what we considered to be immobility as death, and this should be kept in mind when the results of the present study are compared with those of other studies. For example, it is possible that the LC50s reported in other literature in which immobility was taken to mean death are equivalent to the median EC50s reported in the present study. Organisms were considered immobile if the customary swimming response or movement was not observed over a period of ≥15 s after gentle agitation of the solution or gentle stimulation with a probe. Tests with laboratory-cultured species were considered invalid if control mortality exceeded 10%, except for *Hexagenia* spp., for which the acceptability criterion was 20% mortality due to inherent increased variability when these sediment-burrowing mayflies were tested in water-only exposures.

Field-collected species. Test organisms were collected from ponds and streams around Guelph (ON, Canada) and Ottawa (ON, Canada; Table 1). Grab samples taken from one of the ponds and the Eramosa River (Guelph) during one of the organism collecting events showed that all neonicotinoid levels were less than the method detection limit (ranging from 5–10 ng L^{-1}, as shown in Table 2).

Toxicity tests were conducted with 14 field-collected taxa from the orders Ephemeroptera (*Caenis* sp., *Cloeon* sp., *Ephemerella* sp., *Isonychia bicolor*, *McCaffertium* sp.), Trichoptera (*Cheumatopsyche* sp., *Micrasema* sp.), Coleoptera (*Stenelmis* sp., *Gyrinus* sp.), Isopoda (*Caecidotea* sp.), Hemiptera (*Trichocorixa* sp.), Odonata (*Coenagrion* sp.), Diptera (*Aedes* sp.), and Plecoptera (*Agnetina* sp. and *Paragnetina* sp.). *Agnetina* sp. and *Paragnetina* sp. were tested together and treated as a single test unit due to low collection numbers for the individual genera. All field-collected taxa were nymphs/juveniles, with the exception of *Caecidotea* sp., *Trichocorixa* sp., *Gyrinus* sp., and *Stenelmis* sp., which were tested as adults. An attempt was made to collect and test species within a similar visual size range. Animals were collected using a kick-sampling technique and a 500-μm D-net, or careful removal from the underside of stream rocks. After collection, organisms were transferred to aerating dechlorinated water and maintained at 12 to 20 °C (depending on the water temperature at the collection site) for 1 to 5 d prior to testing. A selection of aquatic vegetation and/or leaf litter was provided for food and substrate during the holding period. Species were chosen to represent a diverse set of lentic and lotic invertebrate taxa known to occur in agricultural landscapes in Ontario.

Tests with pond (lentic)-dwelling species were conducted under the static conditions just described in the *Laboratory-cultured species* section. Tests were conducted with 7 to 10 organisms/treatment, with 1 replicate/treatment, using a regression design (see *Concentration–response models* section). Tests with stream (lotic)-dwelling species were conducted in recirculating exposure systems that mimic stream flow (Rodrigues and Kaushik 1984). Details concerning the construction of the flow-through exposure system are provided in the Supplemental Data, S7. Tests were conducted with 7 to 20 organisms/treatment in each simulated stream (flow rate 25 ± 6 mL/s), with 1 replicate/treatment, using a regression design (see *Concentration–response models* section). Water depth in the artificial streams was maintained at approximately 2 cm using a glass partial wall and 280-μm Nitex screen on the lower end of the ramp. Test temperatures were 12 to 15 (±2) °C to mimic collection conditions and reduce thermal stress. In all tests, endpoints of immobility and mortality were observed and recorded as described in the *Laboratory-cultured species* section. Organisms were removed from the stream apparatus and examined under microscopy to confirm mortality at test completion, or when mortality was obvious (i.e., decomposition).

Test acceptability criteria was set at 20% mortality due to higher inherent variability in field-collected species. Any tests with >20% mortality in the controls were rejected except for clothianidin toxicity to *Aedes* sp., for which the control mortality was 2/9 = 22%.

Chemical analysis

Samples from the exposure treatments were collected and analyzed to confirm nominal concentrations using liquid chromatography–tandem mass spectrometry. Samples (40 mL) of 1 to 4 exposure treatments/test were collected at the time of initial organism exposure and stored in amber glass bottles at 4 ± 2 °C prior to analysis. Across all toxicity tests, a total of 149 samples were collected to measure actual concentrations. Complete details of the chemical analyses are provided in the Supplemental Data, S3.

Statistical analysis

Concentration–response models. Endpoints were tallied as percentage of total organisms in each treatment exhibiting

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TABLE 1: Summary of tested species and associated test conditions used to assess the acute toxicity of 6 neonicotinoid insecticides

Species	*Ceriodaphnia dubia*	*Daphnia magna*	*Chironomus dilutus* *Hexagenia* spp. *Hyalella azteca* *Lumbriculus variegatus*	*Neocloeon triangulifer*	*Caecidotea* sp. *Gyrinus* sp. *Caenis* sp. *Cloeon* sp.	*Trichocorixa* sp. *Aedes* sp.	*Coenagrion* sp.	*McCaffertium* sp. *Ephemerella* sp. *Isonychia bicolor* *Cheumatopsyche* sp. *Micrasema* sp. *Stenelmis* sp. *Agnetina/Paragnetina* sp.
Origin	MOECC laboratory culture	MOECC laboratory culture	MOECC laboratory culture	MOECC laboratory culture	Field-collected in ponds in Guelph, ON, Canada; *Gyrinus* sp. from Lac Gerard, QC, Canada	Field-collected in quiet eddies of Speed River and ponds in Guelph, ON	Field-collected in pond in Guelph, ON	Field-collected in Speed River, Eramosa River, Guelph, ON
Test protocol	Environment Canada (2007a)	Environment Canada (2007b)	Ontario Ministry of the Environment and Climate Change Aquatic Toxicology Unit (2016)	Soucek and Dickinson (2015)	Adapted from Ontario Ministry of the Environment and Climate Change Aquatic Toxicology Unit (2016)	Adapted from Ontario Ministry of the Environment and Climate Change Aquatic Toxicology Unit (2016)	Adapted from Ontario Ministry of the Environment and Climate Change Aquatic Toxicology Unit (2016)	Adapted from Rodrigues and Kaushik (1984)
Life stage	<24 h old	<24 h old	*Chironomus*: 3rd instar, as measured by mean head capsule width (0.33–0.45 mm) *Hexagenia:* mean wet wt ~4–6 mg *Hyalella:* 2–9 d old, within 2 d of each other *Lumbriculus:* age-synchronized to 7 d	<24 h old	*Caecidotea* sp.: adults *Gyrinus* sp.: adults *Caenis* sp.: nymphs *Cloeon* sp.: nymphs	*Trichocorixa* sp.: adults *Aedes* sp.: larvae	Nymphs	All nymphs, except *Stenelmis* sp.
Exposure duration (h)	48 ± 2	48 ± 2	96 ± 4	96 ± 4	96 ± 4	48 ± 2	96 ± 4	96 ± 4
Exposure temp. (°C)	25 ± 2	20 ± 2	23 ± 2	25 ± 2	15 ± 2	14 ± 2, except thiamethoxam to *Trichocorixa* sp., 18 ± 2	13 ± 3	15 ± 2
Light intensity (lux)	All tests conducted at 500–1000 lux, 16:8-h light:dark photoperiod							
Test vessel	15 mL solution in 30-mL flat-bottom glass tube	50 mL solution in 50-mL glass tube	200 mL solution in 250-mL glass beaker	200 mL solution in 250-mL glass beaker	200 mL solution in 250 mL glass beaker	200 mL solution in 250-mL glass beaker	20 mL solution in 30-mL flat-bottom glass tube	Recirculating apparatus
Minimum no. of treatments	8	8	8	8	8	8	6	6
No. of test replicates	10	10	3	4	1	1	10	1
No. of animals/vessel/replicate	1	1	10	5	10	10	1	10

(*Continued*)

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TABLE 1: (*Continued*)

Species	*Ceriodaphnia dubia*	*Daphnia magna*	*Chironomus dilutus* *Hexagenia* spp. *Hyalella azteca* *Lumbriculus variegatus*	*Neocloeon triangulifer*	*Caecidotea* sp. *Gyrinus* sp. *Caenis* sp. *Cloeon* sp.	*Trichocorixa* sp. *Aedes* sp.	*Coenagrion* sp.	*McCaffertium* sp. *Ephemerella* sp. *Isonychia bicolor* *Cheumatopsyche* sp. *Micrasema* sp. *Stenelmis* sp. *Agnetina/Paragnetina* sp.
Feeding during test	0.5 mL *Pseudokirchneriella subcapitata*, 0.1 mL YCT	0.5 mL *P. subcapitata*, 0.5 mL *Chlorella* sp.	*Chironomus*: 1.25 mL 3:2 ratio cereal grass: ground Nutrafin *Hexagenia*: None *Hyalella*: 2 mg ground Nutrafin *Lumbriculus*: None	0.3 mL *Navicula* sp.: free diatoms	None	None	None	None
Test substrate	None	None	*Chironomus*: 20 mL silica sand *Hexagenia*: glass tubes *Hyalella*: 5 × 5 cm cotton gauze *Lumbriculus*: None	None	None	None	3 × 1 cm 500 μm Nitex screen	280 μm Nitex screen

MOECC = Ontario Ministry of the Environment and Climate Change; YCT = yeast, cereal leaves, and Tetramin.

mortality or immobilization. The number of treatment replicates for each test varied depending on organism availability; tests with laboratory-cultured organisms used a minimum of 3 replicates, whereas tests with field-collected organisms used 1 replicate due to the logistical constraint of collecting large numbers of organisms in the field. All tests were conducted using a regression design, with 5 to 7 treatments/test in a dilution series. Nominal concentrations were corrected using the mean percentage difference between the nominal and measured concentrations taken from each test and the corrected concentrations used to model the concentration–response relationships.

Concentration–response relationships were modeled for datasets containing one or more partial values using a 4-parameter log-logistic regression model (Equation 1) in R Ver 3.3.3 with the *drc* package (Ritz and Streibig 2005). The lower limit was fixed at 0, and the upper limit at 100, representing the minimum and maximum possible effects, respectively. The variables *b*, *c*, *d*, and *e* represent the relative slope around the LC/EC value, the lower and upper limits, and the LC/EC value, respectively.

$$\text{Mortality or immobilization} = c + \frac{d - c}{1 + \exp\left\{b\left(\log(concentration) - \log(e)\right)\right\}} \quad (1)$$

Datasets containing no partial values were analyzed using the binomial method (Environment Canada 2007c). Lethal and effective concentrations for the 10th and 50th percentiles (LC10/LC50 and EC10/EC50, respectively) and associated 95% confidence intervals were calculated from regression models. The LC/EC10s were not calculated for binomial distributions.

SSDs and hazard assessment. The dataset of 112 neonicotinoid/species combinations was used to construct short-term SSDs for each neonicotinoid. These SSDs allow for comparison of toxicity among aquatic taxa and among the 6 neonicotinoids. The SSDs were constructed according to CCME type A guidelines (Canadian Council of Minister of the Environment 2007b). Data from the present study were used to fulfill the dataset requirement for aquatic invertebrate species, and data from the ECOTOX database were used to fulfill requirements for fish (3 species, at least 1 salmonid and 1 nonsalmonid), suggested plant and amphibian species requirements, and to strengthen the invertebrate dataset from the present study. To be included in the SSDs, external data from the ECOTOX database had to be derived from tests using the active ingredient and not a formulated product and had to have an acute exposure duration (≤4 d for invertebrates, and ≤5 d for plants). If multiple entries were present for the same species, the lowest value was included. If multiple exposure durations were present for the same species from the same study, the duration closest to 4 d was chosen to maintain consistency with the present study exposure duration. Fish, plant, and invertebrate data were available for all neonicotinoids, but amphibian data were only available for imidacloprid. The SSDs were generated

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TABLE 2: Summary of percentage difference between nominal and measured neonicotinoid concentrations

	Method performance[a]	Sample analysis		
Neonicotinoid	Method detection limit ($\mu g\,L^{-1}$)	Mean % difference	SD	No.
Acetamiprid	0.010	11.0	7.1	20
Clothianidin	0.010	9.6	6.5	32
Dinotefuran	0.010	1.5	15.8	16
Imidacloprid	0.010	8.5	5.8	32
Thiacloprid	0.005	12.9	8.4	20
Thiamethoxam	0.010	16.0	7.5	29

[a]Estimated uncertainty for neonicotinoid analysis method is 9 to 13%.
SD = standard deviation.

using CCME SSD Ver 3 (2010) software (Rodney et al. 2013). Two short-term SSDs were generated for each neonicotinoid, one with invertebrate LC50 values (lethality derived), and one with invertebrate EC50 values (immobilization derived). Both SSDs used external, ECOTOX database EC50/median inhibitory concentration values for plant data and LC50s for fish and amphibian data. The LC50 or EC50 values that fell below the smallest concentration in a test (e.g., $<600\,\mu g\,L^{-1}$) were not used in the dataset. The LC50 or EC50 values that were greater than the largest concentration in a test (e.g., $>9600\,\mu g\,L^{-1}$) were included as-is (e.g., $9600\,\mu g\,L^{-1}$), in a conservative approach. Any species with LC50 or EC50 values $\geq 100\,000\,\mu g\,L^{-1}$ were considered tolerant, and were used to calculate plotting positions but were not fit to the model. Although they are less environmentally relevant, LC50s and EC50s were chosen due to the inherent increased statistical robustness and the greater abundance of LC50s and EC50s compared with the application of LOEC, no-observed-effect concentration, or L(E)Cx (where x = value other than 50) values.

To construct the SSDs, the datasets were fit to 4 models: normal, logistic, extreme value, and Gumbel. Models were assessed for goodness-of-fit using mean squared error for the entire dataset and for the lower tail using the Anderson–Darling (A^2 statistic) test and visual inspection. The model with the lowest mean squared error for the lower tail (the A^2 statistic) and best visual inspection was chosen. If a model failed a visual inspection and the Anderson–Darling test for goodness-of-fit, it was not chosen; if all models failed both tests, that SSD was not included in the hazard assessment. The chosen model was plotted in R with the ggplot2 (Ver 2.2.1) package. The 5th percentile of the distribution, termed the HC5 (hazardous concentration, 5%), and its associated 95% confidence intervals were calculated from the model.

The HC5s for each neonicotinoid were compared with environmental concentrations of associated neonicotinoids in Ontario surface waters in a hazard assessment. To assess environmental concentrations, monitoring data from 3 Ontario studies spanning the years 2012 to 2016 (see Supplemental Data, S14, for details on each study) with a total of 2790 observations on the 6 neonicotinoids were compiled. Due to the highly left-censored nature of the data (i.e., values below method detection or quantitation limits, or nondetects) coupled with multiple detection limits, the nonparametric Kaplan–Meier method was used to model the data as empirical cumulative distribution functions (ECDFs) using the NADA package (Ver 1.6-1) in R (Ver 3.3.2; Helsel 2012). The ECDFs were estimated for environmental monitoring data for clothianidin, imidacloprid, and thiamethoxam, but were not estimated for acetamiprid, dinotefuran, and thiacloprid due to low numbers of detections (>98% of observations were censored). The ECDF summary statistics are shown in Table 3, and ECDF plots are presented in the Supplemental Data, Figure S15.

Hazard quotients (HQs) were calculated for each neonicotinoid where HQ equals exposure divided by effects. Exposure was either the maximum environmental concentration (acetamiprid, dinotefuran, and thiacloprid, where ECDFs could not be calculated because there were too few detections) or the 99th percentile of the ECDF (clothianidin, imidacloprid, and thiamethoxam). Effects was the associated HC5 calculated for each compound from SSDs.

RESULTS AND DISCUSSION

Solution chemistry

Water chemistry parameters in exposure solutions were not affected by addition of neonicotinoids. For all tests at test initiation, measured pH values ranged from 7.9 to 8.4, conductivity ranged from 240 to 360 μS/cm, and dissolved oxygen ranged from 8.1 to 10.5 mg/L, or within 90 to 110% saturation at test temperatures varying by $<2\,^{\circ}C$ ($<3\,^{\circ}C$ for *Trichocorixa* sp.) from the intended temperatures (range 11–25 °C, depending on the test). For all tests at test completion, pH ranged from 7.5 to 8.8, conductivity ranged from 260 to 450 μS/cm, and dissolved oxygen ranged from 4.5 to 11.2 mg/L, or within 60 to 110% saturation at the same temperatures as test initiation. Summarized water chemistry parameters for all tests at test termination are available in the Supplemental Data, Table S8.

Mean percentage differences between measured and nominal neonicotinoid concentrations for the 149 water samples are summarized in Table 2. Measured concentrations were generally within 20% of nominal concentrations.

Toxicity

Many studies have assessed the acute toxicity of neonicotinoid insecticides, but most have focused on only 1 or 2 neonicotinoids and generally involved single or a small number of species. The novel contributions of the present study are that

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TABLE 3: Estimated Kaplan–Meier ECDF summary statistics, and hazard assessment for neonicotinoid concentrations in Ontario (Canada) surface waters from select monitoring studies

Neonicotinoid	No.	% Censored	Maximum ($\mu g\,L^{-1}$)	ECDF median ($\mu g\,L^{-1}$)	ECDF mean (95% CI) ($\mu g\,L^{-1}$)	ECDF SD ($\mu g\,L^{-1}$)	99th Percentile	Endpoint: immobilization HC5 (95% CI) ($\mu g\,L^{-1}$)	Endpoint: immobilization HQ	Endpoint: immobilization Hazard	Endpoint: lethality HC5 (95% CI) ($\mu g\,L^{-1}$)	Endpoint: lethality HQ	Endpoint: lethality Hazard
Acetamiprid	348	98.3	0.100		Not calculated			0.47 (0.16–1.41)	0.21	Low	0.48 (0.21–1.07)	0.21	Low
Clothianidin	544	60.3	0.970	0.020	0.052 (0.044–0.060)	0.093	0.44	0.14 (0.01–2.93)	No model passed goodness-of-fit test or visual inspection		4.13 (3.32–5.15)	0.11	Low
Dinotefuran	348	99.4	0.019		Not calculated			0.86 (0.17–4.43)	0.020	Low	7.81 (4.67–13.06)	<0.005	None
Imidacloprid	544	79.8	11.000	N/A[a]	0.091 (0.037–0.145)	0.645	2.70	1.08 (0.82–1.43)	1.92	Moderate	3.71 (2.82–4.87)	0.73	Low
Thiacloprid	462	99.8	0.002		Not calculated			0.30 (0.07–1.25)	0.0070	Low	0.90 (0.60–1.34)	<0.005	None
Thiamethoxam	544	63.4	2.700	0.012	0.077 (0.058–0.097)	0.230	1.20	6.09 (3.07–12.08)	0.20	Low	12.29 (9.90–15.25)	0.010	None

[a]Empirical cumulative distribution function median is not applicable for imidacloprid due to probability of detection > 0.5.
ECDF = empirical cumulative distribution function; CI = confidence interval; SD = standard deviation; HC5 = 5th percentile hazard concentration; HQ = hazard quotient.

we assessed and compared the acute toxicity of 6 neonicotinoid insecticides to aquatic invertebrates; that we included species from 10 aquatic arthropod orders, many of which have not previously been tested; and that we constructed individual SSDs for each compound. A total of 112 neonicotinoid/species combinations were tested, and the derived LC and EC values and control mortality are presented in Table 4.

The cladocerans *D. magna* and *C. dubia* were the least sensitive taxa, with effects only observed at concentrations >40 000 $\mu g\,L^{-1}$. Insects were among the most sensitive taxa tested, with 63% of the calculated LC50s, and 91% of the calculated or threshold estimated EC50s ≤1000 $\mu g\,L^{-1}$. The most sensitive species for both lethality and immobilization endpoints were the midge *C. dilutus* and the mayfly *N. triangulifer*, with LC50s < 65 $\mu g\,L^{-1}$ and as low as 1.7 $\mu g\,L^{-1}$, and EC50s < 40 $\mu g\,L^{-1}$ and as low as 0.8 $\mu g\,L^{-1}$, respectively. The amphipod *Hyalella azteca* also showed high sensitivity to clothianidin and acetamiprid, with LC50s and EC50s of approximately 5 $\mu g\,L^{-1}$. These results are consistent with published studies in which Diptera, especially *Chironomus* spp., and Ephemeroptera have been reported to be the most sensitive insect taxa to neonicotinoids (Roessink et al. 2013; Morrissey et al. 2015; Smit et al. 2015; Camp and Buchwalter 2016; Cavallaro et al. 2017). The greater sensitivity of insect taxa relative to noninsect taxa was not unexpected because neonicotinoids are designed to target and bind strongly with the insect *n*AChR.

Although on average the insects were most sensitive, toxicity values spanned 4 orders of magnitude, indicating wide variation in relative sensitivity within this group. Variation in the sensitivity of aquatic organisms to pesticides is a function of the toxicokinetic and toxicodynamic relationship between the organism and the pesticide (Rico and Van den Brink 2015). Interspecies differences in uptake rate and transport to the site of action, structure of the *n*AChR, and metabolism or detoxification mechanisms all have the potential to introduce variation in the expression of toxic effects. For example, uptake of the pesticide chlorpyrifos has been shown to be a function of insect body size and respiratory strategy, with small insects that rely on aqueous gas exchange to respire (such as Ephemeroptera, Trichoptera, and some Diptera) typically showing greater uptake (Buchwalter et al. 2002). Furthermore, and specifically for neonicotinoids, highly sensitive mayfly species such as *N. triangulifer* have been shown to bioconcentrate more imidacloprid per mass than less sensitive stoneflies or gastropods; uptake rates also increased with temperature (Camp and Buchwalter 2016). In the present study, species that were small at test initiation and that were tested at higher temperatures (23–25 °C), such as *N. triangulifer* and *H. azteca*, showed greater sensitivity relative to similar taxa (e.g., *Cloeon* sp., and *Caecidotea* sp., respectively). Many of these small, sensitive species were laboratory cultured and showed greater sensitivity than field-collected species, which were likely collected at later developmental stages (instars were not determined), which could be more resilient. As a result of differences in developmental stage and testing temperature, there is the potential in the present study for toxicity to field-collected species to be underestimated, and toxicity to

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TABLE 4: Summary of acute toxicity values for 6 neonicotinoids[a]

Species	LC10 ($\mu g\,L^{-1}$)	LC50 ($\mu g\,L^{-1}$)	Control mortality (%)[b]	EC10 ($\mu g\,L^{-1}$)	EC50 ($\mu g\,L^{-1}$)	Control immobilization (%)[b]
Acetamiprid						
Insecta						
Ephemeroptera						
Caenis sp.[c]	270.2 (106.6–433.8)	782.8 (499.3–1066.2)	0	NC	<138.8	0
Cloeon sp.[c]	201.0 (–0.5–402.5)	2369.7 (805.0–3934.4)	0	<16.6	<16.6	0
Ephemerella sp.[c]	29.1 (–6.3–64.5)	158.2 (76.6–239.8)	0	NC	<56.1	0
Hexagenia spp.	NC	>35600.0	0	NC	1.8 (1.3–2.6)	0
Isonychia bicolor[c]	NC	>9600.0	10	NC	<600.0	10
McCaffertium sp.[c]	NC	>890.0	0	NC	<56.1	0
Neocloeon triangulifer	NC	1.7 (1.2–2.2)	0	NC	1.6 (1.2–2.2)	0
Odonata						
Coenagrion sp.	5439.1 (345.8–10532.3)	24 392.9 (13151.1–35634.7)	10	<5625.0	<5625.0	10
Hemiptera						
Trichocorixa sp.[c,d]	359.9 (97.0–622.8)	1515.2 (758.6–2271.8)	0	NC	63.5 (44.9–89.9)	0
Trichoptera						
Cheumatopsyche sp.[c]	134.8 (47.5–222.1)	403.8 (245.9–561.6)	10	NC	<56.1	10
Coleoptera						
Gyrinus sp.[c]	165.3 (43.2–287.3)	686.5 (311.9–1061.1)	0	NC	88.2 (–35.0–248.7)	0
Stenelmis sp.[c]	152.8 (100.0–205.6)	238.3 (182.2–294.4)	10	63.6 (38.2–88.9)	104.6 (79.3–129.8)	10
Diptera						
Chironomus dilutus	1.6 (1.2–2.0)	2.8 (2.4–3.2)	10	1.4 (1.1–1.8)	2.7 (2.3–3.2)	10
Aedes sp.[c,d]	64.5 (30.0–99.0)	159.6 (106.5–212.8)	11	NC	NC	NC
Crustacea						
Caecidotea sp.[c]	146.7 (–118.3–411.7)	2129.6 (634.0–3625.1)	0	NC	<556.0	0
Ceriodaphnia dubia[d]	NC	>33 500	0	NC	NC	NC
Hyalella azteca	2.3 (1.6–2.9)	4.8 (4.0–5.7)	10	2.0 (1.4–2.6)	4.4 (3.6–5.2)	10
Oligochaeta						
Lumbriculus variegatus	21.6 (19.4–23.8)	26.5 (24.7–28.3)	0	14.6 (11.9–17.4)	15.6 (10.7–20.6)	0
Clothianidin						
Insecta						
Ephemeroptera						
Caenis sp.[c]	52.3 (25.2–79.3)	122.1 (82.9–161.3)	10	NC	NC	40
Cloeon sp.[c]	357.0 (9.1–704.9)	3939.2 (1044.9–6833.5)	0	NC	<16.4	0
Ephemerella sp.[c]	NC	586.9 (415.0–830.0)	0	NC	18.5 (13.3–25.7)	0
Hexagenia spp.	NC	>17 400.0	10	0.9 (0.4–1.5)	5.5 (3.9–7.0)	13
Isonychia bicolor[c]	NC	>17 40.0	0	NC	<108.8	0
McCaffertium sp.[c]	396.0 (120.7–671.3)	1328.3 (653.9–2002.7)	10	NC	<108.8	10
Neocloeon triangulifer	NC	3.5 (2.5–5.0)	0	NC	3.5 (2.5–5)	0
Odonata						
Coenagrion sp.	3336.9 (–197.5–6871.4)	145 56.3 (7632.8–21479.9)	10	<5918.8	<5918.8	10
Plecoptera						
Agnetina, Paragnetina sp.[c]	697.0 (287.0–1107.0)	1714.8 (1105.3–2324.2)	11	NC	<300.5	11
Hemiptera						
Trichocorixa sp.[c,d]	5.5 (–0.3–11.4)	34.8 (17.1–52.5)	0	5.1 (0.5–9.7)	21.3 (11.7–30.9)	0
Trichoptera						
Cheumatopsyche sp.[c]	235.8 (13.0–458.6)	1281.0 (423.1–2138.8)	0	NC	<108.8	0
Coleoptera						
Gyrinus sp.[c]	33.4 (18.8–48.1)	62.6 (45.4–79.8)	0	23.0 (13.6–32.5)	41.2 (30.2–52.1)	0

(*Continued*)

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TABLE 4: *(Continued)*

Species	LC10 ($\mu g\ L^{-1}$)	LC50 ($\mu g\ L^{-1}$)	Control mortality (%)[b]	EC10 ($\mu g\ L^{-1}$)	EC50 ($\mu g\ L^{-1}$)	Control immobilization (%)[b]
Stenelmis sp.[c]	81.2 (32.6–129.7)	208.0 (136.5–279.4)	0	NC	84.9 (60.0–120.0)	0
Diptera						
Chironomus dilutus	0.6 (–0.1–1.2)	11.6 (6.5–16.8)	0	NC	3.4 (2.7–5.5)	0
Aedes sp.[c,d]	10.3 (3.6–17.1)	28.5 (17.5–39.6)	22	NC	NC	NC
Crustacea						
Caecidotea sp.[c]	2368.7 (260.7–4476.8)	16085.8 (2636.6–29534.9)	0	108.5 (–17.8–234.8)	537.2 (248.0–826.3)	0
Ceriodaphnia dubia[d]	NC	>100 000.0	0	NC	NC	NC
Hyalella azteca	3.1 (2.4–3.8)	5.2 (4.4–5.9)	0	2.7 (2.0–3.3)	4.8 (4.1–5.6)	0
Oligochaeta						
Lumbriculus variegatus	NC	177.1 (145.3–207.5)	0	NC	41.7 (34.9–49.8)	0
Dinotefuran						
Insecta						
Ephemeroptera						
Caenis sp.[c]	1524.2 (500.3–2548.1)	5466.5 (3134.4–7798.6)	0	NC	<212.2	0
Cloeon sp.[c]	NC	>13 250.0	0	NC	<25.2	0
Ephemerella sp.[c]	82.0 (–53–217.1)	395.5 (119.4–671.6)	0	NC	<344.3	0
Hexagenia spp.	NC	>27 400.0	20	8.4 (6.0–10.9)	19.0 (15.6–22.5)	0
McCaffertium sp.[c]	NC	>5500.0	0	NC	<344.3	0
Neocloeon triangulifer	3.9 (2.4–5.3)	9.4 (7.1–11.6)	0	3.2 (2.2–4.1)	6.0 (4.8–7.2)	0
Odonata						
Coenagrion sp.	1193.8 (–2284.1–4671.7)	12 347.9 (29.3–24666.5)	20	NC	<5187.5	20
Hemiptera						
Trichocorixa sp.[c,d]	15.8 (7.6–24)	36.9 (25.1–48.8)	20	2.0 (–0.5–4.6)	10.1 (4.3–15.9)	20
Trichoptera						
Cheumatopsyche sp.[c]	NC	>5500.0	10	NC	419.5 (344.3–687.5)	10
Coleoptera						
Gyrinus sp.[c]	54.7 (–66.1–175.5)	1790.6 (306.6–3274.5)	0	NC	<163.0	0
Stenelmis sp.[c]	123.8 (7.8–239.8)	555.6 (299.5–811.8)	10	153.1 (71.1–235.1)	335.6 (230.6–440.7)	10
Diptera						
Chironomus dilutus	6.4 (3.9–8.9)	23.5 (18.0–29.0)	0	5.8 (4.2–7.4)	12.4 (10.2–14.5)	0
Aedes sp.[c,d]	134.8 (21.3–248.3)	741.5 (137.9–1345.1)	0	NC	NC	NC
Crustacea						
Caecidotea sp.[c]	NC	>27 300.0	0	NC	580.5 (426.6–853.1)	0
Ceriodaphnia dubia[d]	NC	>87000.0	0	NC	NC	NC
Hyalella azteca	36.2 (29.9–42.5)	57.8 (52.0–63.7)	0	32.8 (28.1–37.5)	44.5 (40.9–48.2)	0
Oligochaeta						
Lumbriculus variegatus	NC	2404.2 (1700.0–3400.0)	0	NC	601.0 (425.0–850.0)	0
Imidacloprid						
Insecta						
Ephemeroptera						
Caenis sp.[c]	NC	<21.8	0	NC	<21.8	0
Cloeon sp.[c]	126.3 (9.5–243.1)	1152.0 (513.1–1790.8)	0	NC	23.1 (16.2–33.2)	0
Ephemerella sp.[c]	16.1 (2.9–29.4)	68.2 (33.1–103.3)	10	NC	10.6 (7.5–15.0)	10
Hexagenia spp.	427.7 (47.7–807.6)	9320.5 (3757.2–14883.8)	0	NC	NC	20
Isonychia bicolor[c]	113.2 (–18.1–244.4)	715.2 (319.3–1111.0)	0	31.4 (16.9–45.8)	60.4 (43.2–77.7)	0
McCaffertium sp.[c]	738.7 (314.9–1162.5)	1810.2 (1018.2–2602.3)	10	NC	10.6 (7.5–15.0)	0
Neocloeon triangulifer	2.9 (2.0–3.7)	5.2 (4.2–6.2)	5	1.9 (1.4–2.4)	3.1 (2.6–3.7)	5

(Continued)

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TABLE 4: (*Continued*)

Species	LC10 ($\mu g\ L^{-1}$)	LC50 ($\mu g\ L^{-1}$)	Control mortality (%)[b]	EC10 ($\mu g\ L^{-1}$)	EC50 ($\mu g\ L^{-1}$)	Control immobilization (%)[b]
Odonata						
Coenagrion sp.	762.7 (–1835.1–3360.6)	3462.7 (–2046.6–8972.0)	20	NC	<5437.5	20
Hemiptera						
Trichocorixa sp.[c,d]	139.4 (51.4–227.4)	450.4 (274.0–626.7)	0	NC	63.1 (44.6–89.2)	0
Trichoptera						
Cheumatopsyche sp.[c]	48.9 (–3.2–101.0)	324.5 (72.1–576.8)	20	48.6 (12.2–85.1)	176.4 (99.7–253.1)	20
Micrasema sp.[c]	7.0 (3.8–10.1)	14.6 (11.0–18.2)	5	NC	<6.4	5
Coleoptera						
Gyrinus sp.[c]	79.6 (47.4–111.8)	132.2 (99.9–164.5)	0	28.0 (14.9–41.2)	57.5 (40.5–74.5)	0
Stenelmis sp.[c]	35.1 (–20.8–91.1)	365.7 (107.1–624.2)	10	44.6 (17.6–71.7)	99.2 (66.9–131.6)	10
Diptera						
Chironomus dilutes	1.7 (0.7–2.7)	11.8 (8.3–15.4)	0	1.4 (1.1–1.8)	2.5 (2.1–2.8)	0
Aedes sp.[c,d]	18.9 (99.4–28.5)	40.8 (27.9–53.6)	0	NC	NC	NC
Crustacea						
Caecidotea sp.[c]	NC	>15 600.0	0	98.0 (–6.1–202.2)	320.8 (162.0–479.6)	0
Ceriodaphnia dubia[d]	NC	72 124.9 (51 000.0–102 000.0)	0	NC	NC	NC
Daphnia magna[d]	NC	>102 000.0	0	NC	NC	NC
Hyalella azteca	114.0 (73.1–155.0)	363.2 (301.3–425.1)	0	77.2 (51.4–102.9)	176.9 (149.4–204.4)	0
Oligochaeta						
Lumbriculus variegatus	42.6 (9.9–75.4)	45.4 (30.6–60.1)	0	30.4 (8.5–52.3)	32.4 (26.7–38.0)	0
Thiacloprid						
Insecta						
Ephemeroptera						
Caenis sp.[c]	46.4 (1.6–91.3)	231.4 (120.7–342.1)	0	NC	<66.3	10
Cloeon sp.[c]	856.3 (232.7–1479.9)	3882.6 (1948.5–5816.7)	0	NC	23.1 (16.2–33.2)	0
Ephemerella sp.[c]	24.4 (–13.1–61.9)	190.6 (78.3–302.8)	0	NC	<58.0	0
Hexagenia spp.	NC	>9300.0	0	NC	<1.3	0
McCaffertium sp.[c]	NC	>920.0	0	NC	10.6 (7.5–15.0)	0
Neocloeon triangulifer	NC	1.9 (1.4–2.6)	0	NC	1.9 (1.4–2.6)	0
Odonata						
Coenagrion sp.	2377.4 (939.0–3815.9)	5647.2 (3762.42–7531.96)	0	NC	<2500.0	0
Hemiptera						
Trichocorixa sp.[c,d]	45.1 (15.1–75.1)	135.3 (84.7–185.9)	0	NC	<39.7	0
Trichoptera						
Cheumatopsyche sp.[c]	NC	>920.0	0	NC	162.6 (115.0–230.0)	0
Coleoptera						
Gyrinus sp.[c]	NC	180.9 (139.5–279.0)	0	40.8 (23.4–58.3)	72.3 (51.5–93.1)	0
Stenelmis sp.[c]	99.4 (55.5–143.2)	183.6 (133.1–234.1)	0	67.0 (33.8–100.2)	129.0 (92.2–165.9)	0
Diptera						
Chironomus dilutus	0.3 (0.2–0.5)	1.6 (1.2–2.0)	3	0.4 (0.3–0.5)	0.8 (0.7–0.9)	3
Aedes sp.[c,d]	10.0 (0.8–19.2)	53.4 (28.0–78.8)	0	NC	NC	NC
Crustacea						
Caecidotea sp.[c]	NC	NC	0	NC	<136.8	0
Ceriodaphnia dubia[d]	NC	>41 500	0	NC	NC	NC
Hyalella azteca	17.3 (10.9–23.6)	55.0 (43.0–67.0)	0	13.2 (9.6–16.9)	26.9 (22.3–31.5)	0
Oligochaeta						
Lumbriculus variegatus	29.9 (9.2–50.5)	33.8 (23.0–44.6)	0	NC	11.5 (8.1–16.2)	0

(*Continued*)

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TABLE 4: *(Continued)*

Species	LC10 ($\mu g\ L^{-1}$)	LC50 ($\mu g\ L^{-1}$)	Control mortality (%)[b]	EC10 ($\mu g\ L^{-1}$)	EC50 ($\mu g\ L^{-1}$)	Control immobilization (%)[b]
Thiamethoxam						
Insecta						
Ephemeroptera						
Caenis sp.[c]	61.6 (6.2–117.1)	381.9 (185.0–578.8)	0	NC	<23.3	0
Cloeon sp.[c]	815.5 (169.3–1461.7)	4633.6 (1835.8–7431.3)	0	NC	44.1 (31.2–62.4)	0
Ephemerella sp.[c]	45.1 (–13.4–103.8)	334.9 (135.9–533.9)	0	NC	<59.0	0
Hexagenia spp.	NC	>30 800.0	7	1.0 (-0.2–2.2)	35.8 (14.1–57.4)	10
Isonychia bicolor[c]	NC	>7120.0	0	NC	<445.0	0
McCaffertium sp.[c]	NC	>920.0	0	NC	81.7 (58.0–115.0)	0
Neocloeon triangulifer	NC	5.5 (3.9–7.8)	0	NC	5.5 (3.9–7.8)	0
Odonata						
Coenagrion sp.	1290.4 (–2327.2–4908.0)	15 061.8 (619.0–29 504.6)	20	NC	<4187.5	20
Plecoptera						
Agnetina, Paragnetina sp.[c]	NC	>7120.0	0	NC	<445.0	0
Hemiptera						
Trichocorixa sp.[c,d]	126.2 (–18.5–270.9)	1473.1 (176.3–2769.9)	0	NC	56.3 (34.3–68.6)	0
Trichoptera						
Cheumatopsyche sp.[c]	53.1 (–8.7–114.8)	170.1 (78.6–261.6)	14	NC	118.5 (108.8–218.0)	14
Micrasema sp.[c]	23.2 (16.7–29.8)	32.8 (26.4–39.2)	10	NC	18.5 (13.1–26.2)	10
Coleoptera						
Gyrinus sp.[c]	NC	31.0 (21.9–43.8)	0	4.3 (0.6–8.0)	14.0 (7.6–20.4)	0
Stenelmis sp.[c]	86.0 (50.7–121.3)	148.0 (109.6–186.4)	10	86.0 (50.7–121.3)	148 (109.6–186.4)	10
Diptera						
Chironomus dilutus	16.0 (9.5–22.5)	61.9 (45.4–78.4)	0	13.7 (9.3–18.2)	36.8 (29.4–44.3)	0
Aedes sp.[c,d]	23.2 (8.6–37.8)	67.4 (42.2–92.5)	0	NC	NC	NC
Crustacea						
Caecidotea sp.[c]	NC	>35 600	0	1512.0 (566.6–2457.4)	4775.4 (2976.3–6574.6)	10
Ceriodaphnia dubia[d]	NC	>80 000	0	NC	NC	NC
Daphnia magna[d]	NC	>80 000	0	NC	NC	NC
Hyalella azteca	183.8 (105.0–262.7)	801.0 (518.7–1083.3)	7	151.1 (102.4–199.7)	391.0 (312.1–469.9)	7
Oligochaeta						
Lumbriculus variegatus	1762.5 (1406.4–2118.6)	3438.2 (3025.5–3850.9)	0	1908.0 (607.1–3208.8)	2035.1 (1699.7–2370.6)	0

[a]The *x*% lethal concentration (LC*x*) and effect concentration (EC*x*; immobilization) values are given, with 95% confidence intervals in parentheses. All estimates are based on concentrations directly measured or calculated using a correction factor based on the percentage difference between nominal and measured concentrations.
[b]Control mortality/immobilization = percentage of negative control individuals exhibiting mortality/immobilization.
[c]Number of experimental replicates = 1. For all other tested species, replicates ≥ 3.
[d]Test duration 48 h. All other test durations 96 h.
NC = not calculable.

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laboratory-cultured species to be overestimated. Differential binding affinity at the *n*AChR is another potential source of variation. Neonicotinoids are thought to be highly selective in their binding to insect versus mammalian *n*AChRs due to structural differences in the receptor site that lead to changes in binding affinity (Tomizawa and Casida 2003). Within insects, variation in the type and number of *n*AChR subunits leads to differences in the way agonists, such as neonicotinoids, interact with the receptor (Jones et al. 2007). Conversely, different neonicotinoids have been shown to act differently at the same *n*AChR (Brown et al. 2006). For example, imidacloprid has been shown to act as a partial agonist, and clothianidin as a superagonist, on native *Drosophila n*AChRs (Brown et al. 2006).

Among the 7 mayfly species tested, response to neonicotinoids was highly variable, with LC50s spanning 4 orders of magnitude. *Neocloeon triangulifer* was the most sensitive mayfly, whereas the least sensitive in terms of lethality was *Hexagenia* spp., and the field-collected *Cloeon* sp. and *McCaffertium* sp., with LC50s $> 890\,\mu g\,L^{-1}$; however, these species were immobilized at much lower concentrations, with most EC50s $< 100\,\mu g\,L^{-1}$. In general, Ephemeroptera and Diptera exhibited immobility at concentrations much lower than those that caused lethality, as illustrated by the difference between median LC50 and EC50 estimates (Figure 1). An extreme example of this disparity occurred in the mayfly *Hexagenia* spp., which had EC50s of $<40\,\mu g\,L^{-1}$ for all neonicotinoids, but a concentration–response relationship for lethality could not be established even for concentrations 2 to 3 orders of magnitude greater. Although *Hexagenia* spp. was the only sediment-dwelling mayfly studied, all exposures were water-only, which therefore negated the influence of sediment on toxicity. Rubach et al. (2010, 2011) found some freshwater invertebrates that also showed a large sensitivity difference between immobility and lethality for the pesticide chlorpyrifos; through studying uptake and elimination kinetics, they showed that these species had high uptake rates and bioconcentration factors, and suggested differences in biotransformation or compensatory gene-regulation abilities as likely sources of variability (Rubach et al. 2010, 2011). Extrapolating to the present study, *Hexagenia* spp. may have bioconcentrated neonicotinoids to a lesser degree than the other mayflies, or may have been able to biotransform neonicotinoids once accumulated so to keep internal residues low, leading to constant immobility but not lethality.

Macroinvertebrates that become immobile as a result of neonicotinoid exposure may be subject to drift (in a lotic system). Berghahn et al. (2012) showed that repeated 12-h pulses of $12\,\mu g\,L^{-1}$ imidacloprid caused drift of insect larvae and young *Gammarus*. Immobile individuals may also be subject to increased predation due to reduced escape behaviors. Pestana et al. (2009) showed that exposure to a formulation of imidacloprid altered behavior in response to predation in the insects *C. riparius* (Diptera) and *Sericostoma vittatum* (Trichoptera). Both effects could reduce or remove these organisms from the community and make the endpoint of immobilization functionally equivalent to mortality. Endpoints that consider immobility, such as the EC50s in the present study, may therefore be a more relevant measure of real-world toxicity.

Recent studies have shown that neonicotinoid toxicity is influenced by temperature and seasonality. Camp and Buchwalter (2016) found a positive relationship among temperature, imidacloprid uptake, and toxicity for aquatic insects, most notably for mayflies. Van den Brink et al. (2016) also found a slight increase in toxicity at higher temperatures, as well as differences in sensitivity to imidacloprid between summer and overwintering generations of the mayfly *C. dipterum*. When

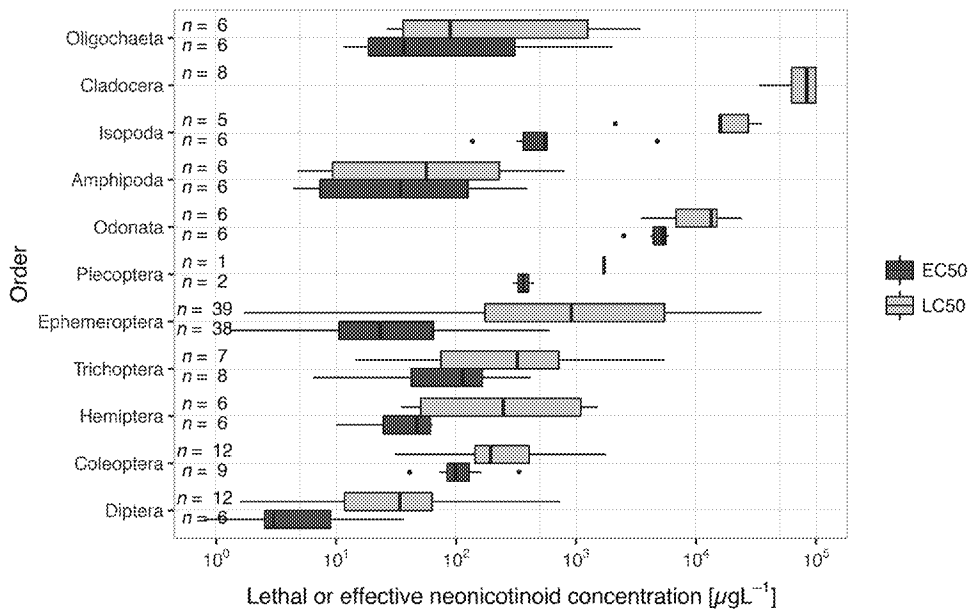

FIGURE 1: Distribution of median lethal (LC50) and effect (EC50) concentrations. Lower and upper threshold data (e.g., $>9600\,\mu g\,L^{-1}$) were treated as-is (e.g., $9600\,\mu g\,L^{-1}$). Data for 6 neonicotinoids are pooled.

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tested at the same temperature, summer generations were more sensitive in terms of immobilization, but not lethality, to imidacloprid than overwintering generations (Van den Brink et al. 2016). The present study used early summer (May) populations for lentic mayflies, and overwintering (November–December) populations for lotic mayflies. Assuming that the seasonal results of Van den Brink et al. (2016) are indicative of other mayfly species, the results for lotic mayflies in the present study could be underprotective. All field-collected organisms were tested at temperatures of 12 to 15 (±2) °C with the goal of mimicking field collection conditions in the laboratory. When compared with laboratory-cultured species, which were cultured and tested at 23 to 25 (±2) °C, field-collected species' toxicity could be underestimated due to lower testing temperatures, and associated lower uptake and toxicity. Additional studies characterizing neonicotinoid toxicity to the most sensitive taxa (e.g., the mayfly *N. triangulifer*) are required to understand how neonicotinoid toxicity is affected by temperature. This is especially needed for spring and early summer temperatures when neonicotinoid exposure is potentially at peak in streams, because transport to, and occurrence in, aquatic systems has been shown to be driven by crop planting and precipitation events (Hladik et al. 2014), both of which occur in spring and early summer.

With few exceptions, the data given in the present study are typically ≤1 order of magnitude compared with neonicotinoid toxicity data in the literature. In general, our median effect and lethal concentrations were greater than those from other studies, which may partially reflect our stricter criteria for the confirmation of lethality and use of technical rather than formulated product. There were some exceptions in which our data indicated toxicity that was >1 order of magnitude compared with published data. These include a reported 96-h LC50 for *C. dipterum* of 26.3 $\mu g\,L^{-1}$ imidacloprid (Roessink et al. 2013), a 44-fold difference compared with our value of 1152 $\mu g\,L^{-1}$ and a reported 48-h LC50 for *C. dubia* of 2.11 $\mu g\,L^{-1}$ (Chen et al. 2010), a 34 000-fold difference compared with our value of 72 124 $\mu g\,L^{-1}$. Both of these studies used formulated products, like many neonicotinoid toxicity studies in the literature (Alexander et al. 2007; Beketov and Liess 2008; Stoughton et al. 2008; Pestana et al. 2009; Roessink et al. 2013); perhaps these authors reasoned that because the formulated product is applied in crop protection it therefore provides a more realistic assessment of possible exposure in the environment. We used technical grade neonicotinoids in our study to facilitate comparison of the relative potency between neonicotinoids without the possible confounding toxicity of formulants. It is unclear whether there is a difference between technical and formulated neonicotinoid products because few studies have compared them. Stoughton et al. (2008) found a 3.75-fold difference in 96-h LC50s between technical imidacloprid and the formulation Admire® for *H. azteca*; however, the LC25s and LOECs were similar. The authors indicated that the difference in toxicity at higher concentrations may have been due to toxicity at higher concentrations of formulants, or combined toxicity between the formulants and the active ingredient (Stoughton et al. 2008). The same experiment with *Chironomus tentans* found no difference in toxicity between the technical and formulated product. In a separate study, a comparison of toxicity to *D. magna* found the commercial liquid formulation Confidor SL 200 (imidacloprid) to be 4× more toxic (by LOEC) than the active ingredient (Jemec et al. 2007). Further studies are needed to better understand the differences between formulated and technical neonicotinoid insecticides.

Hazard assessment

A hazard assessment was conducted to compare acute toxicity data with the maximum or 99th percentile of neonicotinoid surface water detections in Ontario. This assessment conservatively approximates short-term exposures such as pulse exposures. The HC5s determined from both lethality and immobilization-derived SSDs are presented in Table 3. The SSDs (Figure 2) yielded the following order of neonicotinoid toxicity based on the more robust lethality-derived SSDs: acetamiprid ≈ thiacloprid < imidacloprid ≈ clothianidin ≈ dinotefuran ≈ thiamethoxam. The HQs, calculated as the ratio of the HC5 to environmental concentrations for lethality-derived datasets for each neonicotinoid insecticide, indicated no acute hazard (HQ < 0.1) for dinotefuran, thiacloprid, and thiamethoxam, or low acute hazard (0.1 < HQ < 1.0) for acetamiprid, clothianidin, and imidacloprid. Immobilization-derived datasets (see SSDs in the Supplemental Data, Figure S13) indicated low acute hazard for acetamiprid, dinotefuran, thiacloprid, and thiamethoxam, and moderate acute hazard (HQ > 1.1) for imidacloprid. Hazard could not be determined for clothianidin, because none of the immobilization-derived SSDs constructed passed a goodness-of-fit test or visual inspection. Guidelines for interpreting HQs were based on Lemly (1996). When the more robust, but less sensitive endpoint of lethality is considered, this hazard assessment indicates a low or no acute hazard situation for 95% of the species tested exposed to the 99th percentile or the maximum of the Ontario surface water concentrations detected. Alternatively, when the less robust, but more sensitive and perhaps more environmentally relevant endpoint of immobilization is considered, this hazard assessment indicates a moderate hazard for the neonicotinoid imidacloprid. For the other neonicotinoids acetamiprid, dinotefuran, thiacloprid, and thiamethoxam, a conclusion of low or no acute hazard remained; for clothianidin, hazard could not be determined due to ill-fitting species sensitivity distribution models.

The results of this hazard assessment are comparable to those of other recent neonicotinoid hazard or risk assessments for aquatic invertebrates that are based on single-neonicotinoid SSDs. A recent probabilistic ecological risk assessment for imidacloprid by Whitfield-Aslund et al. (2017), which considered both acute lethal and immobility data, derived an acute HC5 of 1.73 μg active ingredient L^{-1}; when compared with exposure distributions for multiple use patterns, they categorized the risk to aquatic invertebrates as de minimis. Similarly, Finnegan et al. (2017) constructed an acute SSD for thiamethoxam and derived HC5s of 5.1 and 22.9 $\mu g\,L^{-1}$ using EC50s and LC50s, respectively. When compared with neonicotinoids in North American surface water samples, the risk was found to be low,

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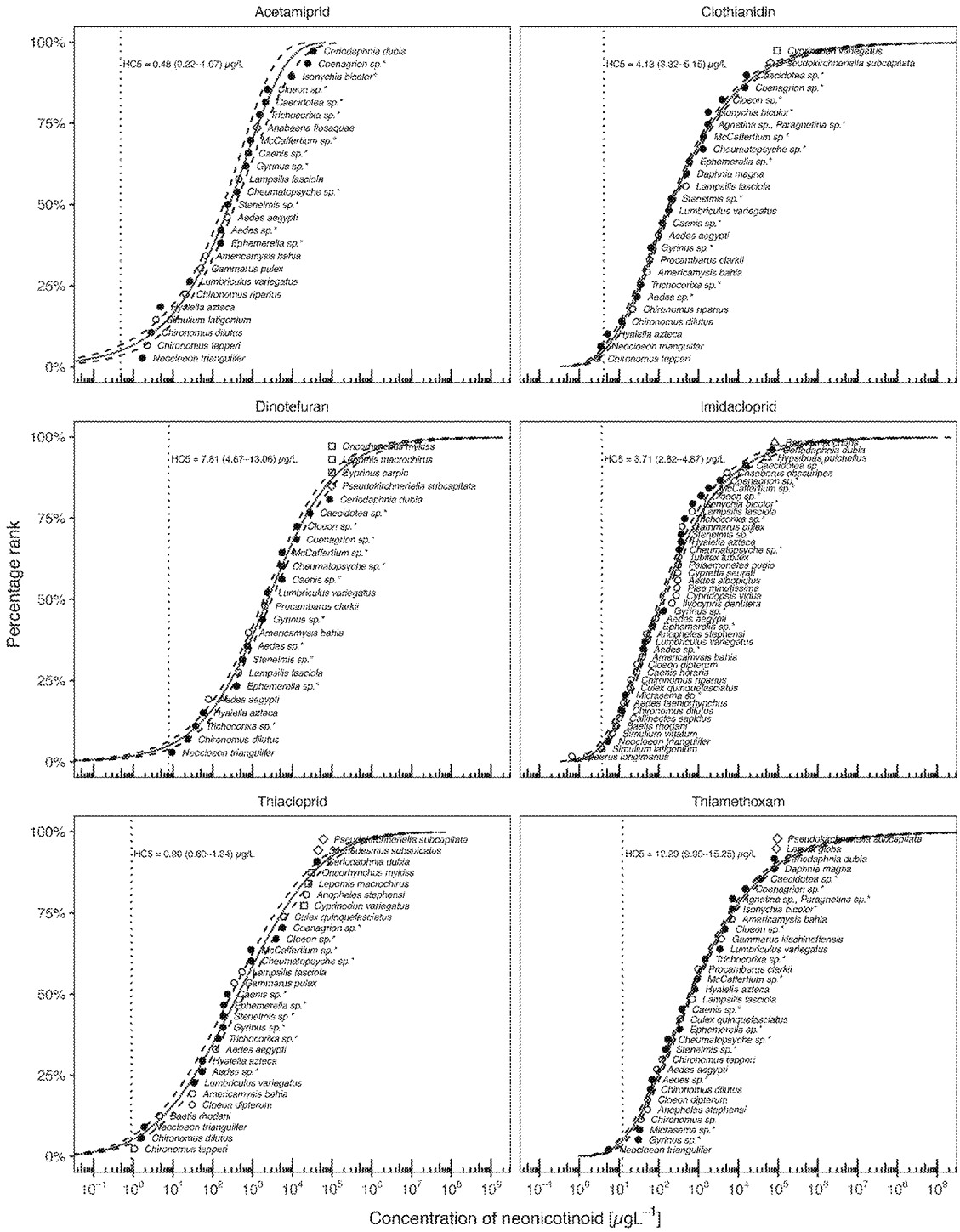

FIGURE 2: Species sensitivity distributions (SSDs) of acute (≤5 d) toxicity data for 6 neonicotinoids constructed using median lethal concentration (LC50) values for aquatic invertebrates generated from the present study and external literature data mined from the ECOTOX database. Closed (black) shapes represent data from the present study (internal), and open (white) shapes represent external data. Circles represent aquatic invertebrates, squares represent fish, triangles represent plants, and diamonds and triangles represent amphibians. For data from the present study, all field-collected species (indicated with an asterisk [*]) were derived from tests with a single replicate; the remaining internal data were laboratory-cultured species tested with a minimum of 3 replicates. Data were fit to regression models (red line) with a 95% confidence band for the regression (dashed line). Data for clothianidin, imidacloprid, and thiamethoxam were fit to Gumbel models; data for acetamiprid were fit to the extreme value model; data for dinotefuran were fit to the logistic model; and data for thiacloprid were fit to the normal model. Data for imidacloprid did not pass the Anderson–Darling goodness-of-fit test for any of the 4 possible models, but did pass visual inspection. The vertical dotted line illustrates the 5th percentile hazardous concentration (HC5), and the 95% confidence intervals are given in parentheses. The SSDs were modeled in CCME V3 Master software and plotted in R with ggplot2. Model parameters are given in the Supplemental Data, Table S10.

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with a likelihood of ~0.5% of exceeding the HC5. In contrast to these studies, an earlier assessment by Morrissey et al. (2015) derived an HC5 of 0.63 $\mu g\ L^{-1}$, which is lower than the results shown in the present study and by others (Finnegan et al. 2017; Whitfield-Aslund et al. 2017).

CONCLUSIONS

The present study contributes novel information to support the growing knowledge of neonicotinoid toxicity to aquatic invertebrates, especially for lesser studied aquatic invertebrates and poorly documented neonicotinoids such as acetamiprid, thiacloprid, and dinotefuran. The acute toxicity data presented in our study are relevant to short-term pulses of neonicotinoids in aquatic environments, which typically occur during heavy precipitation events and during spring freshet. Importantly, our observation of immobilization but not lethality for many species over the exposure periods used in the present study suggests that recovery may be possible following pulse exposure scenarios in the field. Future work in our laboratory will test this hypothesis. Furthermore, we found that sensitivity for many other species was often less than indicated in the literature. The derived HC5s, therefore, are greater than those estimated in previous work and may represent a more realistic assessment of acute neonicotinoid toxicity.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.4088.

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Data Availability—Data pertaining to this manuscript are deposited in figshare at DOI: 10.1002/etc.4088.

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Attention Docket ID No. EPA-HQ-OPP-2019-0185
EPA Proposed Revised Method for National Level Endangered Species Risk Assessment Process for Biological Evaluations of Pesticides

The American Mosquito Control Association (AMCA) is submitting these comments in response to EPA's Proposed Revised Method for National Level Endangered Species Risk Assessment Process for Biological Evaluations of Pesticides Revised Docket # OPP-2019-0185. Our frontline methodologies for controlling mosquito-borne diseases are profoundly impacted by legal restrictions resulting from the Endangered Species Act. Thus, AMCA considers it critical to base modeling assumptions on realistic, albeit conservative, scenarios as proposed in the revised assessment process.

The AMCA is a scientific/educational association of over 1600 vector control professionals that provide mosquito control services to protect both public health and the environment. As a public health association with a profound interest in the safe and effective use of control products, the AMCA recognizes the significance of the risk assessment process in identifying, evaluating, and mitigating potential hazards to humans and the environment from federally registered pesticides, while allowing reasonable and appropriate use of these products in an environmentally compatible manner.

The changes being proposed in the Pre-publication Federal Register Notice are summarized below.

Proposed Revision: The pilot Biological Evaluations relied on extremely conservative usage assumptions from pesticide product labels to represent where the pilot chemicals were likely to be applied. The revised method proposes to incorporate usage data (e.g., survey data, including actual application rates) in the determination of where a pesticide is likely to be applied.

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AMCA Comment: Heretofore, ESA decisions have, by necessity, been primarily based on hazard rather than risk. Without a knowledgeable grasp on application dynamics, EPA has had to rely on the inherent and known toxicity of a pesticide to declare that the application may affect a listed species. It's not that exposures were not taken into account. But without solid data elucidating potential exposures from mosquito control operations, EPA has had to rely on worst-case scenarios that didn't approach comportment with actual product depositions. Oftentimes this resulted in gross overestimations of risk that would never occur during actual operations. It is thus imperative that a listing of species be made in accordance with the most current data sets. It is also incumbent upon the vector control community charged with protecting public health to come up with novel, environmentally compatible control strategies and products, using them strictly in accordance with label specifications.

Proposed Revision: Based on the accuracy of the spatial data utilized and the conservative assumptions related to the action area and potential drift, EPA is interpreting a <1% overlap of listed species' ranges with potential use sites as unreliable and not representative of real exposure potential.
AMCA Comment: Here again, if the goal is protection of listed species, actual usage rates and parameters are critical components. AMCA stands willing to provide the Agency and Services with usage data that it has at its disposal in order to confirm overlap. It should be noted that mosquito control districts do not often spray entire counties. Mosquito populations requiring control are often localized. These affected localities could change over periods of time due to any number of factors including demographics, land development, and changing rainfall patterns.

Proposed Revision: EPA's revised method proposes the use of probabilistic methods to determine the likelihood of a species to be adversely affected by a pesticide. The goal of the probabilistic analysis is to more fully capture and characterize the variability in the range of potential exposures and toxicological effects to listed species and to better inform the biological opinion.
AMCA Comment: Probabilistic risk assessment methodologies are a significantly more accurate measure of actual exposures requiring mitigation than deterministic methods. For this reason, AMCA fully supports their usage in risk assessment. They will ensure protection of listed species while promoting measures that protect human health.

Proposed Revision: The fourth major area of revision is to apply a weight-of-evidence framework to distinguish those listed species that are likely to be adversely affected (LAA) from those that are not likely to be adversely affected (NLAA), based on criteria (e.g., dietary preferences, migration patterns, extent of range potentially exposed) associated with the likelihood that an individual will be exposed and affected.
AMCA Comment: Weight of evidence is a widely used measure of the "strength" of a grouping for separating good and bad risk. In many cases of risk management, there no absolutes involved. Therefore, weight of evidence offers the most precise predictive value. This, too, dovetails nicely with probabilistic methods that take into account the bewildering array of variables that govern exposures.

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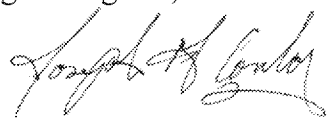
All four changes will assist the Agency in making more informed decisions regarding the risk to listed species posed by mosquito control operations. This is an immensely important and most welcome development and should result in a more scientifically based framework for Endangered Species Act (ESA) enforcement.

This process will become even more important in the coming years as habitat destruction, human encroachment and increasing introduction of exotic mosquito-borne diseases assume a more prominent role in endangered species discussions. Many of these considerations are at odds with each other and will be extremely difficult to reconcile to everyone's satisfaction. Nevertheless, policy decisions will need to reflect an appreciation for both public health and species protections into the foreseeable future.

In conclusion, the AMCA fully supports the proposed revision as part of a thorough, scientifically rigorous risk assessment process. From these revisions, regulators and pesticide users will derive valid and reliable cost/benefit formulae that fully account for environmental impact while allowing appropriate vector-borne disease control. Critical to this process is a full realization of the unique application parameters in mosquito adulticiding operations as a basis for regulatory decisions. The AMCA believes the public's interests are best served when models used to characterize environmental impacts associated with mosquito control product use accurately reflect accepted mosquito control practice. We recognize the efforts made by EPA to appropriately evaluate realistic parameters in their work to date, and we look forward to further collaboration in addressing future risk assessments.

Highest regards,



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August 12, 2019

Tracy Perry
OPP Docket
Environmental Protection Agency Docket Center (EPA/DC) (28221T)
1200 Pennsylvania Avenue NW.
Washington, DC 20460-0001

For Open Comment Period Docket ID
EPA-HQ-OPP-2019-0185
Draft Revised Interim ESA Methodology: Proposed Revised Method for National Level Endangered Species Risk Assessment Process for Biological Evaluations of Pesticides

Dear Ms. Perry:

We appreciate the opportunity to comment on EPA's proposed revised methods for conducting national-level threatened and endangered (listed) species biological evaluations (BEs) for pesticides.

The Xerces Society for Invertebrate Conservation (Xerces Society) is an international nonprofit organization that protects the natural world through the conservation of invertebrates and their habitats. We work to raise awareness about the plight of invertebrates and to gain protection for the most vulnerable species before they decline to a level at which recovery is impossible. Pesticide use is one of the contributing factors to the loss of many invertebrate species. The use of pesticide can also hinder recovery efforts for imperiled species.

Our comments have been informed by the letter submitted by Northwest Center for Alternatives to Pesticides and partners about the revised methodology. We also identify additional information and concerns that are especially relevant to invertebrates listed under the Endangered Species Act (ESA).

1) USAGE DATA

The revised methodology creates a new requirement to rely on estimates of "usage data" to refine pesticide use "Action Areas" used in Step 1, which differentiates "may affect" from "no effect" under consultation procedures.

Since only one state (California) has comprehensive publicly reported usage data available, EPA plans to rely on two proprietary sources as primary sources: Agricultural Market Research Data

(AMRD) (proprietary) and Non-Agricultural Market Research Data (NMRD) (proprietary).[1] These are based on user surveys. On page 10 of the revised methods, EPA states: *"Agricultural crop usage data are summarized at the state level"* and on page 11 *"For non-crop areas, usage data are available at different scales (e.g., state,region or nation)."* Should these sources suggest that no use occurred in a state, under the revised method EPA proposes to exclude all of the label-authorized use sites that would otherwise be included in the "Action Area" even if the state contains one or more listed species that could potentially be affected by pesticide use not contained in these datasets.

a) Reliance on usage data is inconsistent with EPA traditional methods and National Academy of Science recommendations, and contrary to the Endangered Species Act.

Turning to usage data to estimate exposure is in sharp contrast to years of risk assessments. The EPA has traditionally conducted its risk assessments by modeling estimated exposure from maximum **labeled** rates, as these rates are authorized and could occur in any location or time. Using other than authorized rates also appears contrary to the Endangered Species Act. Section 7(a)(2) of the Endangered Species Act requires that *"each federal agency shall, in consultation with and with the assistance of the Secretary, insure that any action **authorized**, funded, or carried out by such agency is not likely to jeopardize the continued existence of any endangered species or threatened species or result in the destruction or adverse modification of habitat of such species which is determined by the Secretary... to be critical."*[2]

Employing usage data to determine the application footprint at Step 1, rather than using label-authorized maximum rates is also directly contrary to the recommendations of the National Academy of Sciences (NAS), which authored authoritative guidance on assessing risks to endangered and threatened species from pesticides.[3] The NAS authors specifically discouraged conducting exposure modeling based on usage data in Step 1, and even in Step 3 cautioned that usage data only be used with statistical description of its spatial and temporal variability (quoted below, p. 70):

> *Steps 1 and 2 of the ESA process (Figure 2-1) should ensure that no potentially unsafe pesticide applications are ignored. Accordingly, an exposure modeler can only assume that a given pesticide is applied at the maximum allowable rate. Furthermore, in Step 3 of the process (Figure 2-1), the Services cannot reasonably be expected to use information that suggests that substantially lower application rates are used unless supporting data are available. Such data must include statistical descriptions of the spatially and temporally distributed application rates. Moreover, some measures would have to be taken to ensure that a use pattern could not dramatically increase in any particular season or locale (for example, because of crop shifts). Only then could exposure modelers use such knowledge to obtain EECs with associated uncertainties. For now, pesticide use is probably an inaccurate input for exposure analysis; registration and labeling are not well suited for solving this exposure-analysis bias.*

It is especially concerning that EPA proposes to rely on usage data in Step 1 of the process to exclude use sites that would otherwise be included and overlaid with species ranges. If a No Effect determination is made incorrectly during Step 1, wildlife agencies are not consulted and no further analysis is completed. Therefore, the species could continue to be harmed.

b) Available usage data are not demonstrably reliable and likely underrepresent seed treatments and urban/developed usage.

To be reliable, usage data must be comprehensive (i.e. required for most or all pesticide applications), reported in a timely manner, supported by realtime records, and publicly available. Usage data used in risk assessment analyses should include important attributes, including date, time, use site (e.g. crop), area treated, amount used (by product) and location (at a meaningful resolution, such as by section, as in the California system).

In contrast, the usage data that EPA proposes to use are deficient in many respects including:

- The proprietary usage data to be used shall be summarized at a coarse, state-wide scale for agricultural data and national scale for non-agricultural data.
- Several of the data sources are incomplete excluding key crops or use sites, states, and active ingredients.
- Some of the data sources are proprietary and there are restrictions on what can be provided to the public.
- The methodologies for the proprietary sources are undisclosed to the general public.

Usage data for seed treatments may be particularly unreliable. For many years, the US Geological Survey has provided coarse maps that display estimated annual agricultural pesticide use.[4] Yet the USGS prominently highlights at its site the following information:

> *Beginning 2015, the provider of the surveyed pesticide data used to derive the county-level use estimates discontinued making estimates for seed treatment application of pesticides because of complexity and uncertainty. Pesticide use estimates prior to 2015 include estimates with seed treatment application.*

It is not clear if this usage data source (Kynetec AgroTrack data) is the one of the primary sources that the EPA will also use. While the NASS Agricultural Chemical Use Program did start surveying for seed treatments in 2015, the survey focuses only on a subset of crops which is rotated by year. It is also not clear if EPA will consider seed treatment as an application that gets quantified and considered in "usage data." It is estimated that approximately 90% of all conventional corn seed and up to 44% of soybean seed is treated with neonicotinoids prior to planting (Douglas and Tooker 2015).[5]

Underestimating the full extent of seed treatment exposure could result in exposure pathways being missed – potentially affecting the analysis for numerous listed invertebrates. For example, terrestrial invertebrates such as the rusty patched bumble bee are likely to be exposed to dust containing pesticide residues when flying as well as when contacting contaminated plants (Krupke et al 2017).[6]

Imidacloprid, a neonicotinoid insecticide that is commonly applied via seed treatment, has been detected at very high concentrations in soils, up to 650 ppb, shortly after planting of treated seed (Donnarumma et al. 2011),[7] compared to typical levels of 1–11 ppb in pollen and nectar. Residues from seed treatment may result in significant exposure pathways for soil-dwelling organisms such as ground beetles and ground-nesting bees (such as the endangered rusty patched bumble bee). Listed, proposed, or candidate ground-dwelling species that may occasionally inhabit crop fields (such as the American burying beetle) or their margins could be directly affected by such omissions. Anderson (2019) simulated the effect of soil exposure on two species of developing non-*Apis* bees and found changes to adult longevity, development speed, and mass in response to increasing concentrations of imidacloprid.[8]

In addition, underestimating seed treatment may also result in an underestimation of risk for many listed aquatic invertebrates. Hladik et al. (2018) found that five neonicotinoid insecticides are prevalent year-round in tributaries to the Great Lakes, with detections of clothianidin and thiamethoxam significantly increasing as the percent of cultivated crops in the basins increased. [9] The authors noted that increases in riverine neonicotinoid concentrations occurred very shortly after planting of treated seeds. This linkage of treated seed with elevated aquatic concentrations underscores the need to include seed treatment in exposure modeling.

Finally, seed treatments may cause off-site contamination to listed species that rely on remnant and weedy habitats on field margins, such as the rusty patched bumble bee. The monarch butterfly (listing decision pending) is another species for which risk could be understated if seed treatment usage data is unreliable.

Usage data sources identified by EPA are especially weak for uses that occur in urban and developed sites. Developed areas are uniquely vulnerable to pesticide contamination, where pesticides frequently enter aquatic systems as runoff across impervious surfaces or through "down-the-drain" uses. These contaminations result in frequent detections and high concentrations of active ingredients in urban streams (Stone et al. 2014; Weston 2005; Carpenter 2016).[10, 11, 12]

c) The coarse spatial unit underlying usage data (state-level) is inappropriate for endangered species analyses.

Much of the usage data, being summarized at very coarse levels, pushes the EPA to use state boundaries as the spatial units for major steps of the analysis process. It is our understanding that at least some of the data sources are available at a finer scale. Will EPA have access to this finer scale data, and if available, will it be used? Within the boundary of many states, we would expect distinct species locations based on habitats and a potentially huge spatial variability in pesticide use.

We do not believe existing usage data can supply the level of information needed to adequately screen out No Effect calls from May Affect calls. We recommend that EPA follow the NAS

guidelines and conduct Step 1 using a simple overlay analysis of use sites with listed species ranges, as was conducted in the pilot BEs.

2) TOXICITY THRESHOLDS

Under the revised method, exposure to listed species in the Action Area can only be considered a may affect to the extent estimated environmental concentrations exceed toxicity thresholds for representative species. Under the revised methodology EPA states that any published study documenting sublethal effects "will not be considered in the BE analyses" unless the study demonstrates a **quantifiable** link to any of the three apical endpoints of survival, growth, and reproduction.

a) **Toxicity threshold data restrictions are contrary to NAS recommendations for Steps 1 and 2, and subvert established ESA consultation steps.**

Disallowing such studies is contrary to NAS recommendations for Step 1 and Step 2 (see p. 93 of the NAS report, quoted below.)

> *Therefore, the committee recommends that EPA in Step 2 (see Figure 2-1) cast a wide net and identify information about sublethal effects of a chemical. If possible, EPA's assessment should include information about responses at various chemical concentrations (a concentration response curve) and, at a minimum, include a qualitative assessment of the relationship between sublethal effects and survival and reproduction. In Step 3 (see Figure 2-1), the Services should show how such effects change demographic measures (survival or reproduction) of a listed species and incorporate such information into the population viability analyses or should state that such relationships are unknown but possible and include a qualitative discussion in the uncertainty section of the biological opinion (BiOp).*

Under the revised methodology, EPA proposes to rely exclusively on studies that show a quantifiable linkage to survival, reproduction, or growth at Step 1, in contrast to the NAS recommendations quoted above. This change in procedure will have the effect of suppressing the studies considered in defining the NOAEC/LOAEC and ultimately the extent of the "Action Area"– all in Step 1. As emphasized in comments submitted by Northwest Center for Alternatives to Pesticides (NCAP), this improperly changes the purpose of Step 1 and undermines the intent and definition of the "may affect" analysis.

b) **Proposed procedures for indirect effects analysis will "lowball" the assessment of pesticide effects on listed species' food items and pollinators.**

On page 13, EPA states: "*When considering indirect effects for listed species that rely on animals (e.g., as prey or pollinators), effects will be focused on mortality endpoints for the taxa relied upon. For generalists, the endpoints will be based on the LD50/LC50 that corresponds to the lower fifth percentile of an SSD (if available) or the most sensitive LD50/LC50 value available for the animal taxa relied upon (using the most sensitive taxon).*"

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No species is an island. It may be obvious that all species need a robust supply of food to survive and thrive. And for many plants, including many listed species, reproduction is not possible without pollinators. But EPA's revised methods will undervalue the loss of prey items (for predators) or plant foods (habitat), or pollinators, by tying the indirect effect calculation to a mortality endpoint for any species that rely on other invertebrates for food or pollination.

Sublethal effects, which are commonly measured at lower concentrations than acute effects (sometimes orders of magnitude differences) are not identified as an endpoint to be considered. Examples of studies that show sublethal effects that could logically be linked to apical endpoints (though the studies themselves did not quantitatively assess growth or reproductive endpoints) include studies that show alterations in feeding behavior (Miles et al. 2017; Yang et al. 2008)[13,14] or navigation to and from food sources (Sappington 2018)[15] or memory involved in foraging (Stanley 2015).[16]

Since sublethal effects can ultimately suppress populations of any taxa, it is a mistake to base the biological evaluation analysis only on mortality endpoints for listed species that rely on animals as prey or pollinators or that are generalist feeders on plants. The same will be true (mortality endpoints used) if the listed species (for example, a butterfly) relies upon plants and is a generalist.

EPA must consider other dependencies in addition to prey, plant food, and pollination. For example, the listed aquatic bivalve known as "snuffbox" (*Epioblasma triquetra*) - as well as other listed mussels in the same genus - requires host fish to complete its reproductive cycle. Such additional dependencies must not be ignored in indirect effects analyses.

c) The revised methods are missing exposure routes and lack clarity on use of studies that incorporate community structure endpoints.

It is unclear what taxon would be considered for indirect effects analysis to the many listed invertebrate filter feeders who consume bacteria, detritus, assimilated organic material, diatoms, phytoplankton, zooplankton, phagotrophic protozoans, and other microorganisms.

It is also not clear how studies that examine community structural dynamics (such as relative abundance and diversity in communities) are to be utilized in EPA's framework and whether they count as "apical endpoint" studies. The EPA should include results from community mesocosm studies that document a significant change in relative abundance for some invertebrates at a certain concentration (as Pestana et al. 2016 documented for imidacloprid

Table 2 appears to exclude certain exposure routes. For example, diet is not included as an exposure route for fish. Similarly the designated exposure routes for aquatic invertebrates is listed as "respiration contact." It is unclear if test results from spiked whole sediment tests or mesocosm studies will be used in evaluating this endpoint.

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The proposed narrow criteria for studies that may be considered in designating toxicity thresholds is likely to further endanger many dwindling species. We believe it is a mistake, with especially grave consequences at Step 1, to require that studies include quantitative linkages to apical endpoints to be considered. We recommend that EPA closely follow the NAS guidance: in Step 1, not exclude studies that were not designed to result in quantitative linkages to survival, growth and reproduction, but rather incorporate the lowest NOAEC/LOAEC levels that are otherwise available and conform to data quality standards. In addition, since indirect effects to pollinators, food sources, hosts and other can result in significant effects to listed species, we recommend that the sublethal endpoints (i.e. NOAEC) be utilized instead to analyze indirect effects of pesticides on listed species' prey, plant habitat, pollinators, hosts and any other species depended upon. In addition, community mesocosm studies should be utilized and incorporated quantitatively.

3) OFF-SITE TRANSPORT

Drift is proposed to be analyzed using AgDrift. EPA proposes in the revised methods only consider off-site transport of pesticides within 2600 feet of the application. EPA justifies this by pointing to AgDRIFT, for which the aerial limit is 2600 feet. EPA states that it will analyze runoff, but proposes to use only models that were designed to estimate concentrations around the immediate edge of a treated field (up to 30 meters), and proposes no method to assess and quantify movement of pesticides in water beyond that distance.

a) The 2600 foot designation ignores abundant empirical evidence of much more extensive transport.

Smalling et al. (2013) documented pesticides in frogs inhabiting numerous remote portions of the Sierra Nevada.[17] Researchers documented current-use pesticides, particularly fungicides, suggesting that the pesticides originated from applications in California's Central Valley and surmised that the pesticides were carried via wind or rain from the Central Valley to the Sierras.

b) The willingness to ignore downstream transport of pesticides creates unacceptable risks for listed aquatic species.

We are deeply concerned that EPA's revised methods ignore downstream transport of pesticides. There are 165 listed, proposed, or candidate invertebrate species that are completely aquatic and many others that occupy aquatic habitats for a portion of their life cycle. This includes clams, mussels, dragonflies, damselflies, and many others. Aquatic listed invertebrates are vulnerable to this significant omission.

Pesticides are frequently found in aquatic habitats and or in sediments of streams, and are not uncommonly included in the listing factors for aquatic invertebrates. Dissolved pesticides can move substantial distances with flowing water, especially when persistent. Some irrigation weed killers are promoted as effective in killing plants 25 miles downstream of the application site.[18]

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EPA justifies turning a blind eye to downstream transport by dismissing its "Downstream Dilution" tool as "*provisional ([i.e., it has not been fully vetted (it has not been made available to the public) or validated]*." But instead of trying to improve the model, EPA is assuming it is safe to ignore downstream transport altogether. This is an unacceptable position that is highly risky for listed aquatic species and undermines the intent of the Endangered Species Act. Research suggests that such a narrow approach to considering off-site transport will disregard downstream exposure particularly for water-soluble pesticides entering the system at higher concentrations (Elias and Bernot 2017).[19] The EPA must ensure that toxicologically significant residues found beyond the 2600 feet range are taken into account.

NAS addressed the issue of fate and transport in estimating exposure. After a discussion of various edge-of-field models in long use (such as GENEEC2, PRZM/EXAMS, and T-REX) the NAS authors include the following text on pages 54-55:

> *Finally, the committee notes that the pesticide fate and transport models do not provide information on the watershed scale; they are intended only to predict pesticide concentrations in bodies of water at the edge of a field on which a pesticide was applied. Different hydrodynamic models are required to predict how pesticide loadings immediately below a field are propagated through a watershed or how inputs from multiple fields (or multiple applications) aggregate throughout a watershed.*
>
> *Watershed-scale models, such as the Soil and Water Assessment Tool (SWAT), have been developed to predict the effects of agronomic practices on water and sediment. SWAT operates on a daily time step and can perform simulations over a long time (30 years) by using physical landscape characteristics (including soil types and topography), data on land cover and land use, weather data, and physical-chemical properties of compounds to simulate processes that dictate routing of water and sediment. The primary routes for chemicals to enter water from a site of application in SWAT are surface runoff and infiltration of applied chemicals into groundwater that can reach surface waters through lateral flow and recharge. Thus, SWAT has an interface with PRZM/EXAMS or the Groundwater Loading Effects of Agricultural Management Systems (GLEAMS) (Leonard et al. 1989; Knisel and Davis 2000) model and can be used to predict chemical concentrations at particular points in a watershed over variable intervals.*

It is not just agricultural uses that we are concerned about, linked as those are to widespread detections of pesticides in streams, rivers, and lakes. Household uses, such as pet shampoos, cause some pesticides to quickly enter the sewage network, showing up in effluent and biosolids at wastewater treatment sites. The revised methodology does not state if and how such exposures (which could be miles downstream from the application site after accounting for the sewage network) will be addressed.

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c) AgDRIFT was never meant to account for volatilization pathways, which can result in unpredictable and long-range transport.

As EPA points out, AgDRIFT is an empirical model based on deposition studies that were conducted in the 1990s. It was never intended to model the fraction of pesticide lost to volatilization, nor the distance that such vapor drift might carry pesticides. Volatility cannot be ignored, as is abundantly clear from the spate of dicamba incidents that affected millions of acres across the Midwest and South over the last several years (Unglesbee, 2019).[20]

EPA is also ignoring recent science which demonstrates that long-range transport is still an issue with certain current-use pesticides (CUPs). Balmer et al. (2018) report that since 2010, at least seven new CUPs have been measured in Arctic air, snow, water and/or biota.[21] The full list of CUPs now reported in these Arctic media includes: chlorothalonil, chlorpyrifos, dacthal, diazinon, dicofol, endosulfan, MCPA, methoxychlor, metribuzin, pendimethalin, pentachloronitrobenzene (PNCB), phosalone, quizalofop ethyl, tefluthrin triallate, and trifluralin. In addition, screening of European and North American databases identified approximately 1200 current-use substances with the potential to reach the Arctic. Presumably deposition via long-range transport is occurring in areas south of the Arctic as well. The EPA's revised methodology ignores this empirical evidence and screening information.

d) EPA's proposed revised methods will also ignore other transport mechanisms.

EPA's revised method says nothing about leaching, subsequent groundwater connections w/ streams, and other known transport mechanisms. Ignoring these transport pathways could be risky, and especially when considering persistent pesticides (such as a relatively new water-soluble systemic insecticide such as chlorantraniliprole).[22] These routes are important to analyze, as some species may be affected by pesticides transported through these mechanisms. For example, most of the larval habitat for the endangered Hine's emerald dragonfly is believed to be fed through groundwater.[23]

EPA should reinstate the use of the Downstream Dilution tool, SWAT, or an adequate substitute, so as not to ignore the transport of pesticides downstream. In addition, EPA must account for developed area usages that result in downstream exposures through the wastewater system, usually much further than 2600 feet from the use site. EPA must also account for documented and potential long-range transport in its methods. The Action Area should be defined as encompassing all areas that may contain pesticide residues at harmful levels – it should not be capped at 2,600 feet.

4) STEP 1 SCREENS

The goal of Step 1 is to determine whether the action "may affect" the listed or proposed species. The revised methodology introduces a number of screens and thresholds for determining a "may affect."

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a) **Screens in Step 1 are contrary to Services' definition of "may affect."**

The use of the thresholds as outlined in the revised methods is not consistent with the Services' definition of "may affect" published in the Service's Consultation Handbook:[24] *"..when a proposed action may pose any effects on listed species or designated critical habitat."* The Services' may affect definition requires no threshold and is clearly worded to include **any** effect.

EPA's use of such thresholds will likely lead to false negatives by ignoring the distribution of the intensity of use relative to the species range or critical habitat and/or the environmental fate and transport of the pesticide in the system.

b) **Screen 1a ("Incomplete Exposure Pathway") incorrectly includes household uses with known capacity to create downstream exposure.**

The revised methodology proposes a No Effect call for "uses with incomplete exposure pathways (e.g., indoor uses; termiticide bait stations; etc.)." Yet, EPA's own 2016 pyrethroid risk assessment identifies down-the-drain as a significant exposure pathway to aquatic species from products such as pet shampoos and drain treatments.[25] The assessment concludes that the use of bifenthrin, cypermethrin, cyfluthrins, deltamethrin, esfenvalerate, cyhalothrins, and permethrin, plus the pyrethrins, in accordance with registered labels, results in acute and/or chronic risk Levels of Concern (LOCs) exceedances for freshwater and estuarine/marine invertebrates, from the indoor down-the-drain exposure to publicly-owned treatment works (POTWs) which in turn result in releases to bodies of water.

c) **Screen 1b ("Species Most Likely Extirpated or Extinct") oversteps EPA's role and undermines ESA protection for species not yet declared extinct.**

EPA proposes to make a No Effect call whenever EPA presumes that a species is most likely extirpated or extinct based on criteria it has set forth to label species likely extirpated or extinct. We disagree that EPA should determine species status. EPA should conduct Biological Evaluations on the list of species provided by the Services, not on an EPA version of that list. By excluding species from Biological Evaluations that EPA presumes extinct or extirpated, EPA runs the risk of further harming (illegal take) a species still extant but so diminished that it may not have been detected for some years. Such species and their habitats should merit enhanced – not diminished – protection from pesticides.

d) **Screen 1c ("1% rule") subverts the Endangered Species Act, ignores spotty source information, species trends, and proximity/intensity considerations.**

This screen would result in a No Effect call where the "Action Area"covers < 1% of a species range. Under the previous process, **any** overlap was deemed a "may affect."

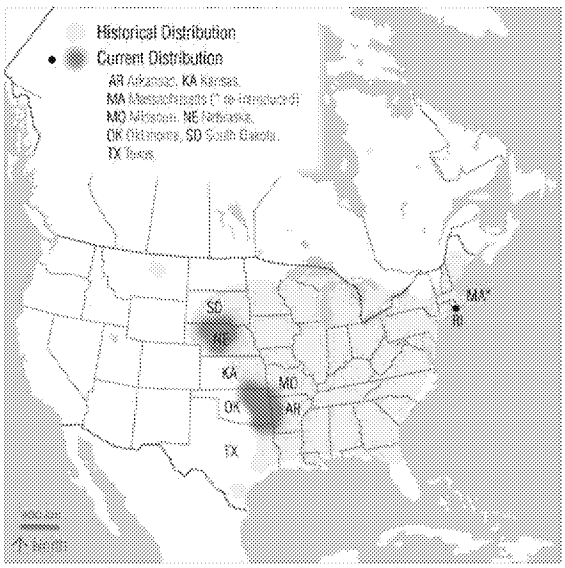
Under the Endangered Species Act, EPA must prepare for and consider worst case scenarios, even if they seem rare, because an unlikely event could be catastrophic to the survival of a species.

A one-percent overlap threshold for a may affect is ill-advised for all species but especially so for small, obscure, or difficult to identify invertebrates. Such species do not necessarily have range maps that can be considered reliable. As described Barry Knisley et al. (2014) summarizing the current status of tiger beetles across America, for invertebrates, the dearth of systematic surveys is a significant problem. Collection records may be fragmentary and present an incomplete or misleading picture of the historic and current range of a taxon.[26]

Frequently, the current distribution of listed invertebrates consists of isolated, disconnected patches (example: Curtis pearlymussel - *Epioblasma florentina curtisii)*. Some, such as the Hay's Spring amphipod (*Stygobromus hayi*), lack recovery plans altogether or have recovery plans that lack objective recoverable criteria to measure progress. Some listed invertebrates lack species status assessments and/or current 5-year reviews documenting progress toward recovery, or their recovery potential is assessed as "Low." Many have no designated critical habitat.

Even those species with recovery plans, designated critical habitat, and population recovery criteria and robust monitoring (for example, the Karner blue butterfly - *Lycaeides melissa samuelis*) may be failing to reach recovery targets. For those many invertebrate species without these advantages, a grim story is available by reading the 5-year reviews; in some cases, no representative of the species has been located in years.

Frequently, listed invertebrates have current distributions or populations already much reduced from their historical range. To illustrate, the map below compares the current vs. the historical distribution of the endangered American burying beetle (*Nicrophorus americanus*).



Source: https://www.canada.ca/en/environment-climate-change/services/species-risk-public-registry/cosewic-assessments-status-reports/american-burying-beetle-2011.html

Screen 1c ignores important considerations such as proximity of the application to the species' habitats and whether the individuals of the species are evenly distributed across the mapped range. A one percent overlap, even if made with highly accurate maps, does not equate to a 1% exposure across the population or a 1% chance of impact.

Screen 1c also ignores important considerations about migration and dispersal habitat, which are important for genetic flow between disjunct populations. See for example, the recovery plan for Hine's emerald dragonfly.[27]

Further, because the pesticide analyses are being conducted "one-by-one," an overlay of a species range with the use sites for a single pesticide could easily ignore cumulative effects related to the use of multiple different pesticides but each on a small area compared to the full species range.

Using the species range maps in a quantitative analysis to create No Effect calls in Step 1 is unwise and under-protective. We strongly urge the EPA to go back to the procedure contained in the interim methods – in concert with the Consultation Handbook definition of "may affect." Any overlap of an Action Area with a species range map should be considered a "may affect" and advanced to Step 2.

e) Screen 1d (Federal lands rule) is inappropriate.

Screen 1d would make an automatic determination of No Effect if the species range overlaps >99% with federal lands. EPA reasons that since Federal agencies must consult on their actions, existing consultation documents will apply.

But, the federal actions in question under the revised methodology are pesticide registration decisions. The federal actions that are at issue on federal lands are not registration decisions. The registration action is unique to the EPA and should be comprehensive in nature – in other words, encompass all areas that include use sites listed on the label.

In addition, it is unlikely that federal biologists conducting the BEs on actions that might include pesticide use have 1) access to the same expertise in pesticide toxicology as the EPA, 2) an awareness of data sources and literature for determining lethal and sublethal effects, and 3) ability to accurately estimate Estimated Environmental Concentrations (EECs) on and offsite associated with any use of pesticides affecting federal lands.

5) STEP 2 SCREENS

Time Frame Must Encompass 15-Year registration authorization. Step 2a, 2b, and 2c screens are phrased : "*...on any given year.*" Since the federal action is the registration of the pesticide, with has a 15-year time frame, the EPA must ensure that these screens cover the potential for risk to an individual **at any time during the 15-year time frame** – not just in any "given" year.

a) Screen 2a (Likelihood of Exposure) is risky for invertebrates.

Under Screen 2a, EPA proposes to further constrain the area it estimates is subject to drift by using the estimated percent of crop area that has been treated (PCT). EPA proposes to use the PCT to reduce the area of "off-site transport" within the overlap area to conform to the PCT by "scaling" the number of acres impacted by off-site drift and subsequently lowering the total predicted overlap with a species range due to drift.

Yet, EPA acknowledges that, for the purposes of finding data on PCT, some uses are not surveyed at all and some uses are only surveyed in some states.

Even within a state, PCT may vary significantly due to varying pest pressure or other reasons (for example, some pesticides are not permitted for use on certain soil types). As a result, overall PCT calculated at a state level may ignore large variations, and those variations may have significance for listed species.

For many, if not most pests, users have multiple pesticide tools from which to choose. Changing product availabilities, costs, pest pressures and other factors may play into varying PCTs, especially over the 15-year time frame of the consultation. These make the PCT a particularly unstable metric to incorporate into Step 2. A calculation using PCT to further reduce the Action Area is an incautious approach based on data that may easily change from year to year or location to location.

After completing the PCT calculation to refine the Action area acres, EPA proposes a deterministic calculation to separate NLAA from LAA: a quotient of acres treated per state divided by acres of species range per state multiplied by the best available population size estimate to determine the "number of individuals potentially exposed".

If we were confident of population sizes and actual distributions for all listed, proposed, and candidate invertebrates, the use of the deterministic procedure outlined might be reasonable. But we don't have reliable ranges (see earlier discussions). And many listed invertebrates have imprecise population estimates. Many also have small populations. Small population size confers its own set of issues for species conservation, including genetic decline and the Allee effect. To use population size in an equation as though it is a precise, known quantity for each species is risky.

b) Screen 2b (Additional Exclusion Criteria) discounts risk inappropriately.

EPA proposes including several other factors to prevent species from receiving an LAA call.

For example, EPA proposes to assume that species which are dormant, or absent from the area due to migration, will be protected from exposure, thus justifying an NLAA call. We urge the EPA to drop this dangerous assumption. Any persistent or systemic pesticides may pose a risk for months to come, regardless of whether the species is active or present at the time of the

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application or not. It is helpful to remember that invertebrate species possess an enormous array of life history strategies, including dormancy that can occur during any life stage and multiple seasons. For example, some invertebrates produce multiple generations per year and may have dormant states at different points in the growing season.

The monarch butterfly (listing status pending) is migratory, lays its eggs on milkweed, and nectars on a variety of plants, including those growing adjacent to crop fields. A recent study by Olaya-Arenas and Kaplan (2019) measured pesticide residues in leaf tissue of milkweed plants growing adjacent to agricultural fields, and in soil, at seven sites in northwest Indiana. [28] Fourteen pesticides were detected (four insecticides, four herbicides and six fungicides) in milkweed tissue over the two years of the study. Seven of the fourteen pesticides were also detected in soil at the site, and one of these during all seasons. This study demonstrated that crop applications do affect adjacent milkweed plants and that at least one of the pesticides, persists for months in soil. This study reinforces our concern about the wisdom of adopting Screen 2b.

Krichik et al. (2015) also demonstrated the potential for risk to monarchs and other invertebrates when application timing does not overlap with species presence. The researchers tested translocation of soil-applied imidacloprid at horticultural rates for greenhouse and nursery ornamental plants to nectar and pollen of milkweed flowers.[29] Translocation of imidacloprid from soil (300 mg AI) to flowers of *Asclepias curassavica* resulted in 6,030 ppb - 10,400 ppb in flowers. A second imidacloprid soil application 7 months later resulted in 21,000 ppb - 45,000 ppb. These levels measured 793 to 1,368 times higher concentration compared to an imidacloprid seed treatment (7.6 ppb pollen in seed- treated canola), where most research has focused. This study shows that levels of imidacloprid in ornamental plants from label-authorized rates may result in concentrations many times lethal exposure. Degradation rates may not be rapid enough to avoid harm to migratory species, even if those species are not present during the time of application.

In addition, EPA proposes to examine the precision of the species data range, assigning NLAA to species that it deems protected due to their use of federal or "protected" areas. But many federal and "protected" areas are used for commercial purposes, so "protection" may be an illusion. Moreover, if exposure is happening in proximity to the species (in space or time) land jurisdiction is irrelevant. In addition, species found on federal lands may depend on other species that might be affected in wider-ranging areas.

And, EPA proposes to assume that pesticide applications in developed areas will not include any applications to impervious surfaces. But structural pest control companies routinely spray impervious surfaces for exterior ant treatments. This particular method of application has been discussed at length as major pathway to surface water.

The EPA also proposes to only look at the "preferred" dietary item when identifying harm. Given the limitations of toxicity data, as well as the fact that so many of the habitats of listed,

proposed, and candidate species are themselves threatened, it seems imprudent to rely on preferred dietary items as a screen.

We recommend that Screen 2b be dropped due to its many erroneous assumptions that would put listed species at risk.

6) ADDITIONAL COMMENTS

a) It is not clear if EPA will consider proposed and candidate species under the revised methods.

In footnote 3 of the revised methods, EPA references the interim methods as outlined in the NAS report and says "These assessments will also consider those species that are currently proposed or candidates for listing." However, this is the only reference in the document to candidate species and it is not clear if EPA is saying that under the interim methods proposed and candidate species were assessed, but they might not be under the revised methods.

We urge the EPA to continue to include proposed and candidate species in the suite of species analyzed, as in the interim methods. Not doing so is contrary to conservation principles and could cause harm to declining (but not yet listed) species, which is when we have the greatest window to improve species' status.

5) Key definitions used in consultation process are misrepresented or redefined.

We agree with NCAP that EPA misrepresents key consultation terms, substituting alternative unpublished definitions for key terms, including "May Affect" "Likely to Adversely Affect" "Direct Effects" and "Indirect Effects." This is an egregious inconsistency that needs to be righted.

CONCLUSION

At the outset of the revised methods, EPA states that its objective is to *"produce both a sustainable and scientifically defensible risk assessment process to prepare BEs."*

The direction EPA is headed does not appear to be scientifically defensible. EPA has ignored important direction in the Endangered Species Act. EPA has ignored recommendations from the NAS report. EPA has tried to redefine consultation terms and steps without regard to regulation or to honoring the definitions contained in the longstanding Consultation handbook. EPA has made assumptions about lack of risk that are not borne out by a closer look at pesticide fate and species life histories. And EPA has inserted numerous procedures clearly **designed** to reduce the quantity of Likely to Adversely Affect calls, rather than using an objective process for understanding and determining effects based on longstanding guidance. In short, EPA's proposed revised methodology undermines the intent of the Endangered Species Act.

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EPA appears convinced that its authorized labels are not borne out in reality. If this is the case, EPA should revisit the rates, sites, and number of applications allowed per year; reissue labels, and consult on these.

Finally, insects comprise 59% of all described U.S. animal species, but less than one percent are listed as endangered or threatened. Recent attention to the "insect apocalypse" underscores how many threats invertebrates face in the modern world. Many insect and other invertebrate species are considered imperiled, but lack ESA protection altogether. EPA must take care not to diminish the protections afforded those few on the list.

Thank you for the opportunity to comment.

Sincerely,

Aimee Code
Pesticide Program Director

Sharon Selvaggio
Pesticide Program Specialist

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[1] See EPA's June 10, 2019 presentation, posted at the docket.

[2] 16 U.S.C. § 1536(a)(2)

[3] National Research Council of the National Academies (NRC) (2013). Assessing Risks to Endangered and Threatened Species from Pesticides. The National Academies Press. Washington, DC. Pp. 175.

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ED_002896_00040456-00018

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Florida Department of Agriculture and Consumer Services

Commissioner Nicole "Nikki" Fried

Public Comment Submission

DATE: August 8th, 2019

TO: Tracy Perry, Office of Pesticide Programs, Docket ID EPA-HQ-OPP-2019-0185

THROUGH: Dr. James Cooper, Environmental Administrator, Scientific Evaluation Section, Bureau of Scientific Evaluation and Technical Assistance

FROM: Mr. Harris Walters, Environmental Consultant, Scientific Evaluation Section, Bureau of Scientific Evaluation and Technical Assistance

Upon review of the *Draft EPA Proposed Revised Method for National Level Endangered Species Risk Assessment Process for Biological Evaluations of Pesticides* I have these comments to submit.

I am concerned with the proposed switch from assessing all potential use sites and application rates listed on the legally defendable pesticide label, to the reliance on unpublished, unregulated, and self-reported "Usage Data". The accuracy of the data appears to have no external checks and looks likely to be taken at face value with little to no vetting from the Agency. It is my opinion, that such data holds much higher potential to underrepresent use and will not be able to fully account for changes in use rates and patterns (at field level) over time. Agriculture is not a static system, new pest pressures, changes in market, and weather variance all have the potential to impact use patterns. Look no further than the impacts observed in the Florida citrus industry as an example, the last ten years have seen drastic reductions to yields (we are at around 25% of peak) and large increases in pesticide use in attempts to fight off previously unknown pest pressures. Does the Agency plan to recalculate and incorporate new "Usage Data" as it becomes available (e.g. when new survey data has been collected), and how much time will be deemed necessary before "Usage Data" is considered out of date (annually, bi-annually, every decade)? Additionally, how will the agency account for urban uses? Who will be the source of "Usage Data" in this sector? Will the surveys be sent to pest control operators? How will the survey's account for use by individual home owners? While, in theory, the "Usage Data" should allow the assessments to be more catered to real world use patterns, I believe a switch to this method will introduce more uncertainty and is more likely to inadvertently underrepresent risk to threatened and endangered species.

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I also fear that the proposal to assign a "No Effect" determination for pesticides with "Action Areas" of less than 1% of a species total range. I do not believe this to be an adequate safe guard for all species. Organism utilization of range is highly variable between different species and temporally within species. Certain times of the year, fish spawning events for instance, may concentrate a large portion of a population in a relatively small area, proportionate to that species' range. It is possible that the spawning location could be less than 1% of an organisms' range and impact a high percentage of the population in question. I fear that a change to the "No Effect" based on impacts to less than 1% of a species range may not be an appropriate fit for all situations and organisms; any impacts within a species range should still be considered a "May Effect" and should trigger further screening.

The removal of certain sublethal effects, which cannot be quantitatively linked to apical endpoints, for consideration in the assessment is shortsighted. While I understand some sublethal effects will be more impactful than others, wholescale abandonment of sublethal effects not tied to growth, reproduction, or survival will not fully account for indirect and direct impacts. Sublethal effects to mobility, olfaction, behavior, etc. can be as impactful in the long term as the more easily quantifiable sublethal effects.

It would be prudent to include both the US Fish and Wildlife Service and the National Marine Fisheries Service in the revision process. As the final determination, in the form of Biological Opinions, ultimately falls to those agencies, their inclusion on the Biological Evaluation revision would be ideal. The biological evaluation process, as I understand it, was initiated on the grounds of failed communication between the EPA and the Services. Their exclusion from this revision process is vexing.

The Florida Department of Agriculture and Consumer Services appreciates being afforded the opportunity to participate in this ongoing revision.

Target journal: STOTEN
Running title: Modeling insecticide impacts on ecosystem services with AQUATOX
Title: Predicting impacts of chemicals from organisms to ecosystem service delivery: A case study of insecticide impacts on a freshwater lake.

Nika Galic[1], Chris J. Salice[2], Bjorn Birnir[3], Randy J.F. Bruins[4], Virginie Ducrot[5], Kristina Garber[6], Henriette I. Jager[7], Andrew Kanarek[6], Robert Pastorok[8], Richard Rebarber[9], Pernille Thorbek[10], Valery E. Forbes[11]

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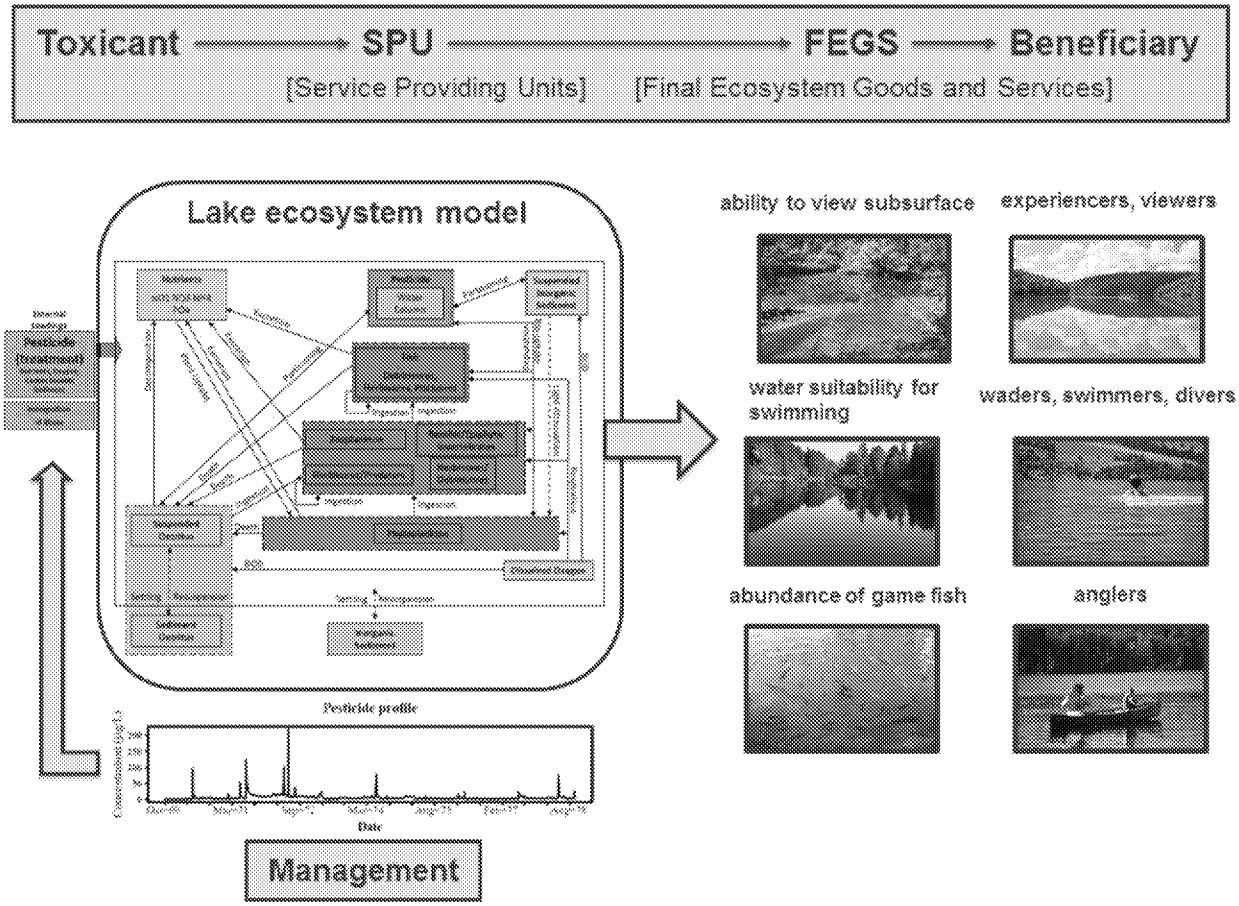
Graphical abstract



Highlights (3-5 bullets of max 85 characters each; submit as separate file)

- Impacts of a hypothetical insecticide on ecosystem services provided by a lake were modeled
- Complex response of fishing services due to non-linear feedbacks in the lake food web
- Water clarity increased with reduced insecticide use -> increase in value by waders and swimmers
- Models can generalize to meaningful endpoints and facilitate quantitative scenario comparison

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Abstract

Assessing and managing risks of anthropogenic activities to ecological systems is necessary to ensure sustained delivery of ecosystem services for future generations. Ecological models provide a means of quantitatively linking measured risk assessment endpoints with protection goals, by integrating potential chemical effects with species life history, ecological interactions, environmental drivers and other potential stressors. The aim of this study was to demonstrate how an ecosystem modeling approach can be used to quantify insecticide-induced impacts on ecosystem services provided by a lake from toxicity data for organism-level endpoints. We used a publicly available aquatic ecosystem model AQUATOX that integrates environmental fate of chemicals and their impacts on food webs in aquatic environments. By simulating a range of exposure patterns, we illustrated how exposure to a hypothetical insecticide could affect aquatic species populations (e.g., recreational fish abundance) and environmental properties (e.g., water clarity) that would in turn affect delivery of ecosystem services. Different results were observed for different species of fish, thus the decision to manage the use of the insecticide for ecosystem services derived by anglers depends upon the favored species of fish. In our hypothetical shallow reservoir, water clarity was mostly driven by changes in food web dynamics, specifically the presence of zooplankton. In contrast to the complex response by fishing value, water clarity increased with reduced insecticide use, which produced a monotonic increase in value by waders and swimmers. Our study clearly showed the importance of considering nonlinear ecosystem feedbacks where the presence of some level of insecticide changed the modeled food-web dynamics in unexpected ways. Our study highlights one of the main advantages of using ecological models for risk assessment, namely the ability to generalize to meaningful levels of organization and to facilitate quantitative comparisons among alternative scenarios and potential trade-offs among them while explicitly accounting for different groups of beneficiaries .

Keywords

ecosystem services, aquatic ecosystem model, ecological valuation, fishers, swimmers, trophic cascade, ecological risk assessment

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1. Introduction

Ecosystems provide various goods and services to human beneficiaries. These include food and water, flood protection, climate regulation, and recreational opportunities [ADDIN EN.CITE <EndNote><Cite><Author>Fisher</Author><Year>2009</Year><RecNum>5</RecNum><DisplayText>(Fisher et al., 2009)</DisplayText><record><rec-number>5</rec-number><foreign-keys><key app="EN" db-id="tp2saazphvawt6evpr7pw0de9t0dfs9p02dx">5</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Fisher, B.</author><author>Turner, R. K.</author><author>Morling, P.</author></authors></contributors><auth-address>Centre for Social and Economic Research on the Global Environment, University of East Anglia, Norwich, NR4 7TJ, United Kingdom
Royal Society for the Protection of Birds, The Lodge, Sandy, Bedfordshire, United Kingdom</auth-address><titles><title>Defining and classifying ecosystem services for decision making</title><secondary-title>Ecological Economics</secondary-title></titles><periodical><full-title>Ecological Economics</full-title></periodical><pages>643-653</pages><volume>68</volume><number>3</number><keywords><keyword>Ecosystem benefits</keyword><keyword>Ecosystem services</keyword><keyword>Environmental decision making</keyword><keyword>Human welfare</keyword><keyword>Millennium Assessment</keyword><keyword>classification</keyword><keyword>decision making</keyword><keyword>ecosystem function</keyword><keyword>ecosystem service</keyword><keyword>environmental management</keyword><keyword>welfare provision</keyword></keywords><dates><year>2009</year></dates><isbn>09218009 (ISSN)</isbn><urls><related-urls><url>http://www.scopus.com/inward/record.url?eid=2-s2.0-57149107777&partnerID=40&md5=01d9fa6f9524d2753bbbc4594ec92341</url></related-urls></urls></record></Cite></EndNote>]. Processes at the population, community, and ecosystem scale contribute to various degrees to the delivery of ecosystem services [ADDIN EN.CITE <EndNote><Cite><Author>Luck</Author><Year>2003</Year><RecNum>65</RecNum><DisplayText>(Luck et al., 2003)</DisplayText><record><rec-number>65</rec-number><foreign-keys><key app="EN" db-id="tp2saazphvawt6evpr7pw0de9t0dfs9p02dx">65</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Luck, Gary W.</author><author>Daily, Gretchen C.</author><author>Ehrlich, Paul R.</author></authors></contributors><titles><title>Population diversity and ecosystem services</title><secondary-title>Trends in Ecology & Evolution</secondary-title></titles><periodical><full-title>Trends in Ecology & Evolution</full-title></periodical><pages>331-336</pages><volume>18</volume><number>7</number><dates><year>2003</year></dates><isbn>0169-5347</isbn><urls><related-urls><url>http://www.sciencedirect.com/science/article/B6VJ1-48H3WYH-2/2/79a09e62668791853bf5040d0f82620c</url></related-urls></urls></record></Cite></EndNote>]. At the same time, anthropogenic activities can potentially affect different ecosystems and components adversely, thus interrupting service delivery [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Assessing and managing risks of anthropogenic activities to ecological systems is necessary to ensure sustained delivery of ecosystem services for future generations.

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Ecological risk assessment quantifies potential impacts on different receptors (e.g. species), and typically focuses on a small set of representative species [ADDIN EN.CITE <EndNote><Cite><Author>Hommen</Author><Year>2010</Year><RecNum>2</RecNum><DisplayText>(Hommen et al., 2010)</DisplayText><record><rec-number>2</rec-number><foreign-keys><key app="EN" db-id="dsrt9xx57vtsxgetsfm5watyaprrattxr2e0">2</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Hommen, U.</author><author>Baveco, J. M.</author><author>Galic, N.</author><author>Van Den Brink, P. J.</author></authors></contributors><auth-address>Fraunhofer IME, P.O. Box 1260, 57377 Schmallenberg, Germany
Alterra, Wageningen University and Research Center, P.O. Box 47, 6700 AA Wageningen, Netherlands
Wageningen University, Department of Aquatic Ecology and Water Quality Management, Wageningen University and Research Center, P.O. Box 47, 6700 AA Wageningen, Netherlands</auth-address><titles><title>Potential application of ecological models in the european environmental risk assessment of chemicals I: Review of protection goals in EU directives and regulations</title><secondary-title>Integrated Environmental Assessment and Management</secondary-title></titles><periodical><full-title>Integrated Environmental Assessment and Management</full-title></periodical><pages>325-337</pages><volume>6</volume><number>3</number><keywords><keyword>Chemical risk assessment</keyword><keyword>Data requirements</keyword><keyword>Ecological model</keyword><keyword>Protection goals</keyword></keywords><dates><year>2010</year></dates><isbn>15513793 (ISSN)</isbn><urls><related-urls><url>http://www.scopus.com/inward/record.url?eid=2-s2.0-77954716314&partnerID=40&md5=fffeb18eaf68e8038b545172061cb1ec</url></related-urls></urls></record></Cite></EndNote>]. However, when assessing potential environmental risks from chemicals, we are rarely interested in effects on individual organisms belonging to a handful of species, chosen as they are sensitive to chemicals and easy to culture in laboratory settings. Rather, we are interested in how chemicals might affect higher levels of organization, such as populations [ADDIN EN.CITE <EndNote><Cite><Author>Efroymson</Author><Year>2004</Year><RecNum>21</RecNum><DisplayText>(Efroymson et al., 2004)</DisplayText><record><rec-number>21</rec-number><foreign-keys><key app="EN" db-id="pzaw5xrt5s0z25ewefrx2wspta2fvdts2wd5">21</key></foreign-keys><ref-type name="Book Section">5</ref-type><contributors><authors><author>Efroymson, Rebecca A.</author><author>Carlsen, Tina M.</author><author>Jager, Henriette I.</author><author>Kostova, Tanya</author><author>Carr, Eric A.</author><author>Hargrove, William W.</author><author>Kercher, James</author><author>Ashwood, Tom L.</author></authors></contributors><titles><title>Toward an ecological framework for assessing risk to vertebrate populations from brine and petroleum spills at exploration and production sites</title><secondary-title>Landscape Ecology and Wildlife Habitat Evaluation: Critical Information for Ecological Risk Assessment, Land-Use Management Activities, and Biodiversity Enhancement</secondary-title></titles><dates><year>2004</year></dates><publisher>ASTM International</publisher><urls></urls></record></Cite></EndNote>], habitats [ADDIN EN.CITE

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<EndNote><Cite><Author>Efroymson</Author><Year>2010</Year><RecNum>25</RecNum><DisplayText>(Efroymson et al., 2010)</DisplayText><record><rec-number>25</rec-number><foreign-keys><key app="EN" db-id="pzaw5xrt5s0z25ewefrx2wspta2fvdts2wd5">25</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Efroymson, Rebecca A.</author><author>Jager, Henriette I.</author><author>Hargrove, William W.</author></authors></contributors><titles><title>Valuing wildlands</title><secondary-title>In: Kapustka, L.; Landis, W. eds. Environmental Risk Assessment and Management from a Landscape Perspective. John Wiley & Sons. 156-185.</secondary-title></titles><periodical><full-title>In: Kapustka, L.; Landis, W. eds. Environmental Risk Assessment and Management from a Landscape Perspective. John Wiley & Sons. 156-185.</full-title></periodical><pages>156-185</pages><dates><year>2010</year></dates><urls></urls></record></Cite></EndNote>], ecological communities [ADDIN EN.CITE <EndNote><Cite><Author>Suter</Author><Year>1999</Year><RecNum>24</RecNum><DisplayText>(Suter et al., 1999)</DisplayText><record><rec-number>24</rec-number><foreign-keys><key app="EN" db-id="pzaw5xrt5s0z25ewefrx2wspta2fvdts2wd5">24</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Suter, Glenn W.</author><author>Barnthouse, Lawrence W.</author><author>Efroymson, Rebecca A.</author><author>Jager, Henriette</author></authors></contributors><titles><title>Ecological risk assessment in a large river-reservoir: 2. Fish community</title><secondary-title>Environmental toxicology and chemistry</secondary-title></titles><periodical><full-title>Environmental toxicology and chemistry</full-title></periodical><pages>589-598</pages><volume>18</volume><number>4</number><dates><year>1999</year></dates><isbn>1552-8618</isbn><urls></urls></record></Cite></EndNote>], ecosystems and the services that they provide [ADDIN EN.CITE <EndNote><Cite><Author>Nienstedt</Author><Year>2012</Year><RecNum>145</RecNum><DisplayText>(Nienstedt et al., 2012)</DisplayText><record><rec-number>145</rec-number><foreign-keys><key app="EN" db-id="tp2saazphvawt6evpr7pw0de9t0dfs9p02dx">145</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Nienstedt, Karin M.</author><author>Brock, Theo C. M.</author><author>van Wensem, Joke</author><author>Montforts, Mark</author><author>Hart, Andy</author><author>Aagaard, Alf</author><author>Alix, Anne</author><author>Boesten, Jos</author><author>Bopp, Stephanie K.</author><author>Brown, Colin</author><author>Capri, Ettore</author><author>Forbes, Valery</author><author>Köpp, Herbert</author><author>Liess, Matthias</author><author>Luttik, Robert</author><author>Maltby, Lorraine</author><author>Sousa, José P.</author><author>Streissl, Franz</author><author>Hardy, Anthony R.</author></authors></contributors><titles><title>Development of a framework based on an ecosystem services approach for deriving specific protection goals for environmental risk assessment of pesticides</title><secondary-title>Science of The Total Environment</secondary-title></titles><periodical><full-title>Science of The Total Environment</full-title></periodical><pages>31-38</pages><volume>415</volume><keywords><keyword>Protection

goals</keyword><keyword>Ecosystem services</keyword><keyword>Environmental risk assessment</keyword><keyword>Pesticides</keyword><keyword>Guidance documents</keyword><keyword>Ecotoxicology</keyword></keywords><dates><year>2012</year><pub-dates><date>2012/01/15/</date></pub-dates></dates><isbn>0048-9697</isbn><urls><related-urls><url>http://www.sciencedirect.com/science/article/pii/S0048969711005821</url></related-urls></urls><electronic-resource-num>https://doi.org/10.1016/j.scitotenv.2011.05.057</electronic-resource-num></record></Cite></EndNote>].

When considering ecosystem services, relevant assessment endpoints may be at the population or community level. Standard toxicity testing, the basis for chemical risk assessments, relies on testing a handful of species and focuses on effects at the individual level [ADDIN EN.CITE <EndNote><Cite><Author>Forbes</Author><Year>2016</Year><RecNum>48</RecNum><DisplayText>(Forbes and Galic, 2016)</DisplayText><record><rec-number>48</rec-number><foreign-keys><key app="EN" db-id="dsrt9xx57vtsxgetsfm5watyaprrattxr2e0">48</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Forbes, Valery E.</author><author>Galic, Nika</author></authors></contributors><titles><title>Next-generation ecological risk assessment: Predicting risk from molecular initiation to ecosystem service delivery</title><secondary-title>Environment International</secondary-title></titles><periodical><full-title>Environment International</full-title></periodical><pages>215-219</pages><volume>91</volume><keywords><keyword>Adverse outcome pathways</keyword><keyword>Ecological protection goals</keyword><keyword>Ecosystem services</keyword><keyword>Environmental management</keyword><keyword>Mechanistic effect models</keyword><keyword>Predictive systems models</keyword></keywords><dates><year>2016</year></dates><isbn>0160-4120</isbn><urls><related-urls><url>http://www.sciencedirect.com/science/article/pii/S0160412016300824</url></related-urls></urls><electronic-resource-num>http://dx.doi.org/10.1016/j.envint.2016.03.002</electronic-resource-num></record></Cite></EndNote>]. For example, in freshwater ecosystems phytoplankton, invertebrates and fish are often used as test organisms [ADDIN EN.CITE <EndNote><Cite><Author>Hommen</Author><Year>2010</Year><RecNum>2</RecNum><DisplayText>(Hommen et al., 2010)</DisplayText><record><rec-number>2</rec-number><foreign-keys><key app="EN" db-id="dsrt9xx57vtsxgetsfm5watyaprrattxr2e0">2</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Hommen, U.</author><author>Baveco, J. M.</author><author>Galic, N.</author><author>Van Den Brink, P. J.</author></authors></contributors><auth-address>Fraunhofer IME, P.O. Box 1260, 57377 Schmallenberg, Germany
Alterra, Wageningen University and Research Center, P.O. Box 47, 6700 AA Wageningen, Netherlands
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Review of protection goals in EU directives and regulations</title><secondary-title>Integrated Environmental Assessment and Management</secondary-title></titles><periodical><full-title>Integrated Environmental Assessment and Management</full-title></periodical><pages>325-337</pages><volume>6</volume><number>3</number><keywords><keyword>Chemical risk assessment</keyword><keyword>Data requirements</keyword><keyword>Ecological model</keyword><keyword>Protection goals</keyword></keywords><dates><year>2010</year></dates><isbn>15513793 (ISSN)</isbn><urls><related-urls><url>http://www.scopus.com/inward/record.url?eid=2-s2.0-77954716314&partnerID=40&md5=fffeb18eaf68e8038b545172061cb1ec</url></related-urls></urls></record></Cite></EndNote>]. Even though impacts on their survival, growth, and reproduction are rigorously quantified and are assumed to be protective, these organisms and corresponding endpoints do not directly represent services that are valued by beneficiaries. Rather, beneficiaries may care about clean drinking water, clean water for recreation (swimming and boating), harvestable fish populations, and simply the aesthetic enjoyment of experiencing freshwater ecosystems [ADDIN EN.CITE ADDIN EN.CITE.DATA].

Ecological models provide a means of quantitatively linking measured risk assessment endpoints with protection goals [ADDIN EN.CITE ADDIN EN.CITE.DATA]. By integrating potential chemical effects with species life history, ecological interactions, environmental drivers and other potential stressors, we can predict long-term risks to ecological entities that deliver valued ecosystem services [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Furthermore, we can easily and inexpensively evaluate multiple management options and quantify trade-offs among multiple services [ADDIN EN.CITE <EndNote><Cite><Author>Bennett</Author><Year>2009</Year><RecNum>43</RecNum><DisplayText>(Bennett et al., 2009)</DisplayText><record><rec-number>43</rec-number><foreign-keys><key app="EN" db-id="tp2saazphvawt6evpr7pw0de9t0dfs9p02dx">43</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Bennett, Elena M.</author><author>Peterson, Garry D.</author><author>Gordon, Line J.</author></authors></contributors><titles><title>Understanding relationships among multiple ecosystem services</title><secondary-title>Ecology Letters</secondary-title></titles><periodical><full-title>Ecology Letters</full-title></periodical><pages>1394-1404</pages><volume>12</volume><number>12</number><keywords><keyword>ecosystem management</keyword><keyword>Ecosystem services</keyword><keyword>Resilience</keyword><keyword>social-ecological systems</keyword></keywords><dates><year>2009</year></dates><isbn>1461-0248</isbn><urls><related-urls><url>http://dx.doi.org/10.1111/j.1461-0248.2009.01387.x</url></related-urls></urls></record></Cite></EndNote>].

The aim of this study was to demonstrate how an ecosystem modeling approach can be used to quantify insecticide-induced impacts on ecosystem services from organism-level toxicity data. The case study was developed as one of the outputs of a National Institute of Mathematical and Biological Synthesis (NIMBioS) working group hosted by the University of Tennessee in Knoxville ([HYPERLINK "http://www.nimbios.org/workinggroups/WG_o2e" \h]). It represents an example of a recently-developed framework for extrapolating impacts of chemicals from individual organisms to ecosystem services (Forbes et al. 2017).

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The approach taken here was to quantify the benefits derived from a Midwestern US lake relative to potential effects of a hypothetical insecticide across a range of exposure scenarios, based on possible mitigation measures. We purposefully applied exposure scenarios that ensured a large disruption to the lake ecosystem to observe and quantify impacts on ecosystem services and possible trade-offs among them. A well-tested lake ecosystem model was used to simulate the effects of a hypothetical insecticide on sensitive aquatic crustaceans and fish species. The main beneficiaries of ecosystem services were identified and provided by a generic lake in the Midwestern US as recreational fishers, swimmers and boaters. We focused on final ecosystem goods and services (FEGS), i.e., those directly used or enjoyed by beneficiaries [ADDIN EN.CITE ADDIN EN.CITE.DATA]. A distinction is made between final (direct) and intermediate (indirect) ecosystem services. For example, most supporting services such as primary production or nutrient cycling are intermediate because, although they are not directly valued by the public, they support populations of game fishes that are directly valued.

By simulating a range of exposure patterns, we illustrated how risks from exposure to this hypothetical insecticide could affect aquatic species populations (e.g., recreational fish abundance) and environmental properties (e.g., water clarity) that would in turn affect delivery of ecosystem services. We used a publicly available aquatic ecosystem model AQUATOX (Park et al. 2008), supported by the US Environmental Protection Agency. This model integrates environmental fate of chemicals and their impacts on generic and specific food webs in aquatic environments. It may be used for assessing impacts on aquatic ecosystems [ADDIN EN.CITE <EndNote><Cite><Author>Clough</Author><Year>2017</Year><RecNum>3</RecNum><DisplayText>(Clough et al., 2017; Park et al., 2008)</DisplayText><record><rec-number>3</rec-number><foreign-keys><key app="EN" db-id="pzaw5xrt5s0z25ewefrx2wspta2fvdts2wd5">3</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Clough, Jonathan S</author><author>Blancher II, Eldon C</author><author>Park, Richard A</author><author>Milroy, Scott P</author><author>Graham, W Monty</author><author>Rakocinski, Chet F</author><author>Hendon, J Read</author><author>Wiggert, Jerry D</author><author>Leaf, Robert</author></authors></contributors><titles><title>Establishing nearshore marine injuries for the Deepwater Horizon natural resource damage assessment using AQUATOX</title><secondary-title>Ecological Modelling</secondary-title></titles><periodical><full-title>Ecological Modelling</full-title></periodical><pages>258-268</pages><volume>359</volume><dates><year>2017</year></dates><isbn>0304-3800</isbn><urls></urls></record></Cite><Cite><Author>Park</Author><Year>2008</Year><RecNum>32</RecNum><record><rec-number>32</rec-number><foreign-keys><key app="EN" db-id="pzaw5xrt5s0z25ewefrx2wspta2fvdts2wd5">32</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Park, Richard A</author><author>Clough, Jonathan S</author><author>Wellman, Marjorie Coombs</author></authors></contributors><titles><title>AQUATOX: Modeling environmental fate and ecological effects in aquatic ecosystems</title><secondary-title>Ecological Modelling</secondary-title></titles><periodical><full-title>Ecological Modelling</full-title></periodical><pages>1-15</pages><volume>213</volume><number>1</number><dates><year>2008</year></dates>

<isbn>0304-3800</isbn><urls></urls></record></Cite></EndNote>], and a number of site- and condition-specific scenarios exist that are readily available to use.

Impacts were translated on ecological entities to changes in services delivered to beneficiaries, and benefits transfer methods were used to assign economic values to them [ADDIN EN.CITE <EndNote><Cite><Author>Richardson</Author><Year>2015</Year><RecNum>35</RecNum><DisplayText>(Richardson et al., 2015)</DisplayText><record><rec-number>35</rec-number><foreign-keys><key app="EN" db-id="pzaw5xrt5s0z25ewefrx2wspta2fvdts2wd5">35</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Richardson, Leslie</author><author>Loomis, John</author><author>Kroeger, Timm</author><author>Casey, Frank</author></authors></contributors><titles><title>The role of benefit transfer in ecosystem service valuation</title><secondary-title>Ecological Economics</secondary-title></titles><periodical><full-title>Ecological Economics</full-title></periodical><pages>51-58</pages><volume>115</volume><dates><year>2015</year></dates><isbn>0921-8009</isbn><urls></urls></record></Cite></EndNote>]. We further assumed that certain mitigation measures (e.g., changes in insecticide application rate or frequency or presence of buffers) would change the insecticide loading that reaches the lake food web. We compared several management options to demonstrate how applying a biophysical model can advance our capabilities beyond risk assessment, i.e., to inform decision-making to improve the management of ecosystems facing various pressures from anthropogenic activities.

2. Methods

2.1. AQUATOX Model

AQUATOX is an aquatic ecosystem simulation model, which was developed by the US Environmental Protection Agency, that links the physical or abiotic environment (i.e., water quality) and aquatic communities [ADDIN EN.CITE <EndNote><Cite><Author>Park</Author><Year>2008</Year><RecNum>32</RecNum><DisplayText>(Park et al., 2008)</DisplayText><record><rec-number>32</rec-number><foreign-keys><key app="EN" db-id="pzaw5xrt5s0z25ewefrx2wspta2fvdts2wd5">32</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Park, Richard A</author><author>Clough, Jonathan S</author><author>Wellman, Marjorie Coombs</author></authors></contributors><titles><title>AQUATOX: Modeling environmental fate and ecological effects in aquatic ecosystems</title><secondary-title>Ecological Modelling</secondary-title></titles><periodical><full-title>Ecological Modelling</full-title></periodical><pages>1-15</pages><volume>213</volume><number>1</number><dates><year>2008</year></dates><isbn>0304-3800</isbn><urls></urls></record></Cite></EndNote>]. In this process-based model, different communities are represented as compartments of biomass that change based on the gains and losses defined for each group. In a series of linked differential equations, biomass is gained through growth, reproduction and external inflow, whereas biomass is lost through natural mortality, respiration, predation, exposure to toxicants, and emigration. The model also simulates cycling of all major nutrients, oxygen dynamics, and different fate processes for

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organic toxicants (Figure 1). By simultaneously computing important chemical and biological processes over time, the model helps to identify cause-effect relationships between chemical water quality, the physical environment, and aquatic communities.
AQUATOX version 3.1 has been applied to simulate multiple environmental stressors (including nutrients, organic loadings, sediments, chemicals, and temperature) and their effects on algal, macrophyte, invertebrate, and fish communities. Various model applications have represented different aquatic ecosystems, including vertically stratified lakes, reservoirs and ponds, rivers and streams, and estuaries [ADDIN EN.CITE
<EndNote><Cite><Author>Clough</Author><Year>2017</Year><RecNum>3</RecNum><DisplayText>(Clough et al., 2017)</DisplayText><record><rec-number>3</rec-number><foreign-keys><key app="EN" db-id="pzaw5xrt5s0z25ewefrx2wspta2fvdts2wd5">3</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Clough, Jonathan S</author><author>Blancher II, Eldon C</author><author>Park, Richard A</author><author>Milroy, Scott P</author><author>Graham, W Monty</author><author>Rakocinski, Chet F</author><author>Hendon, J Read</author><author>Wiggert, Jerry D</author><author>Leaf, Robert</author></authors></contributors><titles><title>Establishing nearshore marine injuries for the Deepwater Horizon natural resource damage assessment using AQUATOX</title><secondary-title>Ecological Modelling</secondary-title></titles><periodical><full-title>Ecological Modelling</full-title></periodical><pages>258-268</pages><volume>359</volume><dates><year>2017</year></dates><isbn>0304-3800</isbn><urls></urls></record></Cite></EndNote>].

2.2. Case study description

We chose to model the aquatic ecosystem of a generic reservoir in the Midwestern US for this case study because such lake systems are popular recreation destinations and therefore offer an opportunity to evaluate potential tradeoffs among ecosystem services (Figure 2). As a starting point, we focused on a well-studied system, but modified it to represent a clear reservoir that would increase its attractiveness to the public. We identified Coralville Reservoir (CR), which we used as a template to build a simulation model for a generic shallow reservoir in Johnston County, Iowa (USA). Coralville Reservoir is a run-of-river reservoir that is influenced by agricultural runoff [ADDIN EN.CITE
<EndNote><Cite><Author>Schnoor</Author><Year>1981</Year><RecNum>33</RecNum><DisplayText>(Schnoor, 1981)</DisplayText><record><rec-number>33</rec-number><foreign-keys><key app="EN" db-id="pzaw5xrt5s0z25ewefrx2wspta2fvdts2wd5">33</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Schnoor, Jerald L</author></authors></contributors><titles><title>Fate and transport of dieldrin in Coralville Reservoir: Residues in fish and water following a pesticide ban</title><secondary-title>Science</secondary-title></titles><periodical><full-title>Science</full-title></periodical><pages>840-842</pages><volume>211</volume><number>4484</number><dates><year>1981</year></dates><isbn>0036-8075</isbn><urls></urls></record></Cite></EndNote>]. As a result, the reservoir often experiences high turbidity that substantially limits phytoplankton growth. Simulations of toxicant impacts on zooplankton with original CR parameters did not cause

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substantial changes in biomass of phytoplankton or in turbidity because the influence of inorganic solids (i.e., silt from agricultural lands) was so dominant. Therefore, we restricted that influence to represent a reservoir/lake without substantial riverine inputs of suspended inorganic solids.

The approach to parameterization included an iterative process of adjusting a few parameters for site characteristics and biota to achieve a model reservoir in which phytoplankton dominate over submerged aquatic macrophytes, and an impact on herbivorous zooplankton leads to higher biomass of phytoplankton. The food web used in our lake model included four species of forage fish (bluegill, shad, catfish, and buffalofish), two piscivorous fish species (walleye and largemouth bass), four zooplankton functional taxa (daphnids, rotifers, copepods, and predatory zooplankton), three benthic/epiphytic functional taxa (chironomids, Tubifex, and sphaerids), and four phytoplankton taxa (diatoms, dinoflagellates, and green and blue-green algae) (Forbes et al. 2017; Figure 3). This is a realistic food-web dynamic in small lakes and reservoirs [ADDIN EN.CITE
<EndNote><Cite><Author>Carpenter</Author><Year>2010</Year><RecNum>11</RecNum><DisplayText>(Carpenter et al., 2010; Carpenter et al., 1985)</DisplayText><record><rec-number>11</rec-number><foreign-keys><key app="EN" db-id="pzaw5xrt5s0z25ewefrx2wspta2fvdts2wd5">11</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Carpenter, Stephen R</author><author>Cole, Jonathan J</author><author>Kitchell, James F</author><author>Pace, Michael L</author></authors></contributors><titles><title>Trophic cascades in lakes: lessons and prospects</title><secondary-title>Trophic Cascades: Predators, Prey and the Changing Dynamics of Nature</secondary-title></titles><periodical><full-title>Trophic Cascades: Predators, Prey and the Changing Dynamics of Nature</full-title></periodical><pages>55-69</pages><dates><year>2010</year></dates><urls></urls></record></Cite><Cite><Author>Carpenter</Author><Year>1985</Year><RecNum>10</RecNum><record><rec-number>10</rec-number><foreign-keys><key app="EN" db-id="pzaw5xrt5s0z25ewefrx2wspta2fvdts2wd5">10</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Carpenter, Stephen R</author><author>Kitchell, James F</author><author>Hodgson, James R</author></authors></contributors><titles><title>Cascading trophic interactions and lake productivity</title><secondary-title>BioScience</secondary-title></titles><periodical><full-title>BioScience</full-title></periodical><pages>634-639</pages><volume>35</volume><number>10</number><dates><year>1985</year></dates><isbn>0006-3568</isbn><urls></urls></record></Cite></EndNote>] and provides an opportunity to examine tradeoffs among ecosystem services. A more detailed description of these modifications can be found in the Supplementary Information. Due to our modifications, in the following we refer to this model system as the "case study reservoir".

For this case study, we assumed that the reservoir is exposed to a hypothetical organophosphate insecticide. For simulation purposes, we used available toxicity data for a representative organophosphate insecticide that is registered for use on corn, which is the predominant crop in the watershed of the Coralville Reservoir. The case study chemical was slightly mobile in soil (K_{oc} = 6040 L / kg organic C) and moderately persistent (aerobic soil metabolism $t_{1/2}$ = 170 d). The most sensitive taxa to this insecticide include zooplankton, especially cladocerans (i.e. *Daphnia* sp.), and pan fish, such as bluegill, followed by walleye and

largemouth bass. Catfish and buffalo fish were the least sensitive. We used surrogate species for those for which toxicity data were lacking; a table with all toxicity parameters used in this case study can be found in the Supplementary Information. Insecticide loading was modeled using Pesticide Root Zone Model (PRZM) ([HYPERLINK "https://www.epa.gov/exposure-assessment-models/przm-version-index" \h]), by assuming label-rate insecticide application to all corn acreage (applied at 1.68 kg active ingredient / ha, twice per year) in the Coralville Reservoir watershed. We made the conservative assumption that all agricultural land in the watershed was used for growing corn, i.e., ~57% of total watershed area ([HYPERLINK "https://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcs142p2_006352.pdf"]).

Historical simulations were conducted for the period between October 1969 and September 1978 – the choice of the simulation period was driven by the availability of weather profiles in the original AQUATOX template. We further simulated several management scenarios (MS) by reducing insecticide loading by different amounts. We simulated the following management scenarios: no reduction (MS 0), a 5x reduction (MS 5), a 10x reduction (MS 10), a 25x reduction (MS 25), a 100x reduction (MS 100), and a complete absence of the insecticide (MS No pesticide). Management scenarios represent a reduction in total loading of the insecticide into the watershed through a combination of relevant mitigation measures. Conceptually, a given management scenario may be achieved through a combination of actions, including reduced application rates, fewer applications per field or use of riparian buffers; however, this analysis does not quantify impacts of specific actions on loading.

2.3. Selection and valuation of ecosystem services

For this case study, ecosystem services were identified using U.S. EPA's Final Ecosystem Goods and Services Classification System, or FEGS-CS [ADDIN EN.CITE <EndNote><Cite><Author>Landers</Author><Year>2013</Year><RecNum>2</RecNum><DisplayText>(Landers and Nahlik, 2013)</DisplayText><record><rec-number>2</rec-number><foreign-keys><key app="EN" db-id="pzaw5xrt5s0z25ewefrx2wspta2fvdts2wd5">2</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Landers, Dixon H</author><author>Nahlik, Amanda M</author></authors></contributors><titles><title>Final ecosystem goods and services classification system (FEGS-CS)</title><secondary-title>Anonymous EPA United States Environmental Protection Agency. Report Number EPA/600/R-13/ORD-004914</secondary-title></titles><periodical><full-title>Anonymous EPA United States Environmental Protection Agency. Report Number EPA/600/R-13/ORD-004914</full-title></periodical><dates><year>2013</year></dates><urls></urls></record></Cite></EndNote>]. The FEGS-CS classifies environments and the corresponding ecological components that are directly enjoyed by different classes of beneficiaries.

Ecosystem services relevant to Midwestern reservoirs included the ability to view the lake bottom in wading areas, abundance of game fishes, and water suitability for swimming (Table 1). We then identified corresponding metrics that could be estimated using AQUATOX outputs (Table 1). Finally, we selected abundance of game fishes and water suitability for recreational purposes for economic valuation which was conducted using the benefits transfer method [ADDIN EN.CITE <EndNote><Cite><Author>Richardson</Author><Year>2015</Year><RecNum>35</RecNum

><DisplayText>(Richardson et al., 2015)</DisplayText><record><rec-number>35</rec-number><foreign-keys><key app="EN" db-id="pzaw5xrt5s0z25ewefrx2wspta2fvdts2wd5">35</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Richardson, Leslie</author><author>Loomis, John</author><author>Kroeger, Timm</author><author>Casey, Frank</author></authors></contributors><titles><title>The role of benefit transfer in ecosystem service valuation</title><secondary-title>Ecological Economics</secondary-title></titles><periodical><full-title>Ecological Economics</full-title></periodical><pages>51-58</pages><volume>115</volume><dates><year>2015</year></dates><isbn>0921-8009</isbn><urls></urls></record></Cite></EndNote>]. This required us to assume that the transferred values pertain to this case study and that the demographics of beneficiaries are similar. Below we describe valuation methods for abundance of game fishes and the suitability of a water body for wading or swimming.

2.3.1. Abundance of game fishes

To illustrate valuation of this ecosystem service, fishing in the reservoir was translated from fish biomass (ρ) generated by AQUATOX to value (V) in dollars of a specific MS (i) of the insecticide loading (i.e., 25x decrease, 10x decrease, no insecticide use in the watershed). The focus of this analysis was on an annual change in the value of fishing that could be attributed to a specific type of fish (i.e., bluegill, bass, walleye or catfish). An annual time step (t) seemed most realistic for the time frame in which watershed and reservoir managers could collect data (e.g., crop practices, number of anglers visiting the reservoir, etc.) and make decisions.

For each type of fish, daily biomass values were generated by AQUATOX (dry weight) for the baseline scenario (i.e., insecticide use as on label) and for the three management scenarios. These values were then converted from a dry to a wet weight [ADDIN EN.CITE <EndNote><Cite><Author>U.S. Environmental Protection Agency</Author><Year>1993</Year><RecNum>7</RecNum><DisplayText>(U.S. Environmental Protection Agency, 1993)</DisplayText><record><rec-number>7</rec-number><foreign-keys><key app="EN" db-id="pzaw5xrt5s0z25ewefrx2wspta2fvdts2wd5">7</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>U.S. Environmental Protection Agency,</author></authors></contributors><titles><title>Wildlife Exposure Factors Handbook</title></titles><number>EPA/600/R-13/187a</number><dates><year>1993</year></dates><pub-location>Washington, DC.</pub-location><publisher>Office of Research and Development</publisher><urls></urls></record></Cite></EndNote>]. The daily change in biomass attributed to a management scenario was calculated as the difference between the biomass in the management and baseline scenarios. For each of the simulated full years (1970-1977), an average daily change in biomass ρ was calculated (Equation 1) for each year t and type of fish i:

[EMBED Equation.DSMT4] (1)

For each year and each type of fish, the value of an insecticide management scenario ($; V_i; equation 2) was calculated by multiplying the daily change in density by willingness of fishers to pay (*WTP*) for a change in fish biomass and the number of visits to the lake per year (*N*)

[EMBED Equation.DSMT4] (2)

There were 187,382 visits to the reservoir in 2012 for fishing [ADDIN EN.CITE <EndNote><Cite><Author>U.S. Army Corps of Engineers</Author><Year>2014</Year><RecNum>6</RecNum><DisplayText>(U.S. Army Corps of Engineers, 2014)</DisplayText><record><rec-number>6</rec-number><foreign-keys><key app="EN" db-id="pzaw5xrt5s0z25ewefrx2wspta2fvdts2wd5">6</key></foreign-keys><ref-type name="Government Document">46</ref-type><contributors><authors><author>U.S. Army Corps of Engineers,</author></authors></contributors><titles><title>Coralville Lake Fact Sheet.</title></titles><dates><year>2014</year></dates><urls><related-urls><url>http://www.mvr.usace.army.mil/Portals/48/docs/CC/FactSheets/ODC/Coralville%20Lake%20-%20Fact%20Sheet.pdf</url></related-urls></urls></record></Cite></EndNote>], and we used 187, 000 as the value for all simulation years in further analyses. *WTP* data used in this analysis were expressed as average cost ($) per trip per kg/ha increase in a given type of fish. We assumed that all trips were attributed to a single species and that there were no species preferences, i.e., trips were not taken to fish for a specific species. This allowed us to weigh all species equally for comparison. We used the following WTP: $1.55 for bluegill (type of panfish), $1.57 for largemouth bass and $4.03 for walleye [ADDIN EN.CITE <EndNote><Cite><Author>Melstrom</Author><Year>2015</Year><RecNum>1</RecNum><DisplayText>(Melstrom et al., 2015)</DisplayText><record><rec-number>1</rec-number><foreign-keys><key app="EN" db-id="pzaw5xrt5s0z25ewefrx2wspta2fvdts2wd5">1</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Melstrom, Richard T</author><author>Lupi, Frank</author><author>Esselman, Peter C</author><author>Stevenson, R Jan</author></authors></contributors><titles><title>Valuing recreational fishing quality at rivers and streams</title><secondary-title>Water Resources Research</secondary-title></titles><periodical><full-title>Water Resources Research</full-title></periodical><pages>140-150</pages><volume>51</volume><number>1</number><dates><year>2015</year></dates><isbn>1944-7973</isbn><urls></urls></record></Cite></EndNote>]. Comparable *WTP* data for the Midwest were not found for catfish; however, some sources suggest that catfish may be valued at a similar level as panfish and bass and half of the value of walleye [ADDIN EN.CITE <EndNote><Cite><Author>Charbonneau</Author><Year>1978</Year><RecNum>148</RecNum><DisplayText>(Charbonneau and Hay, 1978)</DisplayText><record><rec-number>148</rec-number><foreign-keys><key app="EN" db-id="tp2saazphvawt6evpr7pw0de9t0dfs9p02dx">148</key></foreign-keys><ref-type name="Conference Proceedings">10</ref-type><contributors><authors><author>Charbonneau, John J</author><author>Hay, Michael J</author></authors></contributors><titles><title>Determinants and economic values of hunting and fishing</title><secondary-title>Transactions of the Forty-third North American Wildlife and Natural Resources Conference, March 18-22 Phoenix, Arizona.</secondary-

title></titles><pages>391-403</pages><dates><year>1978</year></dates><publisher>Wildlife Management Institute.</publisher><urls></urls></record></Cite></EndNote>]. We, therefore, used the cost per trip value of $1.55, which corresponds to bluegill, as a surrogate for catfish.

2.3.2 Water suitability for wading or swimming

The goal of this analysis was to illustrate a valuation of improved water clarity in the simulated reservoir. Water clarity is an important ecosystem service that is valued by different types of beneficiary (Figure 2), including swimmers, boaters, and property owners; it has been identified as the best measure of high-quality water [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Surveys show that clear water, measured by how many meters beneath the surface of the water a Secchi disk is visible, is highly valued by the general public [ADDIN EN.CITE <EndNote><Cite><Author>Schuetz</Author><Year>2001</Year><RecNum>3897</RecNum><DisplayText>(Schuetz et al., 2001)</DisplayText><record><rec-number>3897</rec-number><foreign-keys><key app="EN" db-id="zev9ra0zprssfpeev2lvwwr75xww9swpp9zz" timestamp="1519770925">3897</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>Schuetz, J.F., </author><author>K.J. Boyle,</author><author>R. Bouchard</author></authors></contributors><titles><title>The effects of water clarity on economic values and economic impacts of recreational uses of Maine's great ponds</title></titles><pages>25</pages><number> Miscellaneous Report 421</number><dates><year>2001</year></dates><publisher>Maine Agricultural & Forest Experiment Station</publisher><urls></urls></record></Cite></EndNote>]. This is because the public has an intuitive understanding of what it means, and that it is a single aggregated measure reflecting a range of watershed impairments leading to eutrophication or high suspended sediment concentrations. A positive hyperbolic relationship exists between water clarity and an index of water quality (based on several parameters, incl. dissolved oxygen, pH, total phosphorus, nitrates, turbidity) [ADDIN EN.CITE <EndNote><Cite><Author>Ge</Author><Year>2013</Year><RecNum>4183</RecNum><DisplayText>(Ge et al., 2013)</DisplayText><record><rec-number>4183</rec-number><foreign-keys><key app="EN" db-id="zev9ra0zprssfpeev2lvwwr75xww9swpp9zz" timestamp="1519786087">4183</key><key app="ENWeb" db-id="">0</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>Ge, J</author><author>CL Kling</author><author>JA Herriges</author></authors></contributors><titles><title><style face="normal" font="default" size="10">How much is clean water worth? Valuing water quality improvement using a meta analysis</style></title><secondary-title>Economics Working Papers (2002–2016)</secondary-title></titles><pages>38 </pages><number>51</number><dates><year>2013</year></dates><urls></urls></record></Cite></EndNote>]. There is a threshold increase at relatively low Secchi depths (<1 m), which means that small increases in Secchi depths will yield large increases in perceived water quality. Secchi depths were generated in AQUATOX simulations for the scenarios described in the Case Study Description (2.2). Our valuation considered the impact of management scenarios on the hypothetical insecticide . We examined the literature to determine what expressions of Secchi depth (SD) should be used to evaluate FEGS for recreational users of the modeled lake.

We relied on a water clarity valuation model developed using 2002 water quality data and a survey of Iowa households to quantify recreational visits to Iowa lakes in the same year [

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<EndNote><Cite><Author>Egan</Author><Year>2009</Year><RecNum>3848</RecNum><DisplayText>(Egan et al., 2009)</DisplayText><record><rec-number>3848</rec-number><foreign-keys><key app="EN" db-id="zev9ra0zprssfpeev2lvwwr75xww9swpp9zz" timestamp="1519590937">3848</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Egan, K. J.</author><author>Herriges, J. A.</author><author>Kling, C. L.</author><author>Downing, J. A.</author></authors></contributors><titles><title>Valuing water quality as a function of water quality measures</title><secondary-title>American Journal of Agricultural Economics</secondary-title></titles><periodical><full-title>American Journal of Agricultural Economics</full-title></periodical><pages>106-123</pages><volume>91</volume><number>1</number><dates><year>2009</year><pub-dates><date>Feb</date></pub-dates></dates><isbn>0002-9092</isbn><accession-num>WOS:000262346000007</accession-num><urls><related-urls><url><Go to ISI>://WOS:000262346000007</url></related-urls></urls><electronic-resource-num>10.1111/j.1467-8276.2008.01182.x</electronic-resource-num></record></Cite></EndNote>]. The resulting logistic model linked two responses: 1) the change in number of visits to lakes and 2) willingness-to-pay (*WTP*, 2009$US/household) for increasing water clarity associated with a set of unimpaired lakes (annual median Secchi depth = 1.27 m) compared to impaired lakes (annual median Secchi depth = 0.81 m). From this, we estimated a marginal value (per-meter increase in Secchi depth). We then summarized our simulated data for April – October, which is assumed to be the time period representative of the recreational season for swimmers and boaters. From that, we calculated median Secchi depths for each simulated year and scenario. From changes in clarity (differences in median Secchi depth between impaired and non-impaired lakes) we estimated changes in the number of trips and value per household. Water clarity is appreciated regionally, so the above estimates of value per household were expanded by estimating the number of households likely to visit the reservoir. The 2009 US Census estimated 55,967 households for Johnston County (where Coralville Reservoir is located), representing 4.2% of Iowa households ([HYPERLINK "http://www.census.gov/2010census/popmap/ipmtext.php?fl=19"]). The WTP data based on annual Secchi depth values were the only data available to us, and using them for the valuation of our seasonal patterns is a clear limitation of this analysis. Ideally, we would prefer to use a value representative of the recreational period (e.g., median values representing Secchi depts from April-October).

3. Results

3.1. Ecosystem dynamics under exposure to a hypothetical insecticide

Exposure to insecticide concentrations resulting from loadings provided by PRZM had substantial impacts on several taxa in the food web/ecosystem (relative to a scenario with no pesticide; Figure 3). We show only a subset of compartments that AQUATOX simulated. Under the no management scenario (MS 0), biomass of daphnids, bluegill sunfish, and largemouth bass were kept low. Conversely, biomasses of phytoplankton and catfish benefitted from insecticide exposure and both increased. By applying different management scenarios that reduced exposure concentrations, we found concentration–response relationships that differed among taxa. For the

majority of taxa, a 25x reduction in insecticide exposure (MS 25) yielded biomass values that did not visibly differ from those in which no insecticide was assumed (MS No pesticide). Bluegill biomass was reduced even at 100x reduction in exposure (MS 100, as compared with MS No pesticide), whereas catfish biomass declined as insecticide exposure decreased. In further analyses, we show only scenarios with no management (MS 0), with 10x reduction (MS 10), with 25x reduction (MS 25) and the scenario with no insecticide (MS No pesticide).

Catfish, bluegill sunfish, and largemouth bass experienced very different dynamics due to exposure in the four management scenarios (Figure 4). We compared biomass in different scenarios to the scenario without any exposure (MS No pesticide). Bluegill biomass declined almost instantly after exposure to the insecticide and did not recover in the three management scenarios with partial reduction of insecticide loading. Biomass of largemouth bass was almost zero in the no management scenario and dropped substantially in MS 10 after 3 years of exposure, with a subsequent recovery after 3 additional years. Biomass dynamics of both largemouth bass and walleye were similar and consistently higher through the simulation period in MS 25 than in MS No pesticide (Figure 4). Biomass of catfish consistently declined with reductions in exposure (MS 0 > MS 10 > MS 25 > MS No pesticide).

Secchi depths varied across years and management scenarios (Figure 5). Nevertheless, there was a clear impact of the insecticide. In the years 1971 - 1972, when simulated concentrations were higher than in other years (Figure 4), Secchi depths for all insecticide-use scenarios were smaller than in other years. Across all simulation years, mean water clarity in the recreational season was 0.88 m ($\pm$ 0.51 SD) in the no management scenario, 1.5 ($\pm$ 0.8 SD) in the MS 10, 1.8 m ($\pm$ 0.77 SD) in the MS 25, and 2.02 m ($\pm$ 0.6 SD) in the scenario with no insecticide use.

3.2. Ecosystem Services Valuation

3.2.1. Abundance of game fishes

Valuation of the ecosystem service of abundance of game fishes for anglers estimated the marginal values of management scenarios considered here over the baseline (MS 0) to increase between $300,000 and $500,000 for bass and walleye (Figure 6), with overlapping confidence intervals. As walleye had the same responses as largemouth bass, values for both species were increased in the MS 25 when compared to MS No pesticide. Bluegill did not increase in value, except in the MS No pesticide scenario, in which the value was increased by $276,000. Catfish decreased in value for all scenarios presented here. Decreases ranged from $500,000 to $800,000 over the nine year simulation period.

3.2.2. Water suitability for swimming

We estimated a marginal change in water clarity value of $0.21m^{-1} household^{-1} lake^{-1} y^{-1} based on [ADDIN EN.CITE <EndNote><Cite><Author>Egan</Author><Year>2009</Year><RecNum>3848</RecNum><DisplayText>(Egan et al., 2009)</DisplayText><record><rec-number>3848</rec-number><foreign-keys><key app="EN" db-id="zev9ra0zprssfpeev2lvwwr75xww9swpp9zz" timestamp="1519590937">3848</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Egan, K. J.</author><author>Herriges, J. A.</author><author>Kling, C. L.</author><author>Downing, J. A.</author></authors></contributors><titles><title>Valuing water quality as a function of water

quality measures</title><secondary-title>American Journal of Agricultural Economics</secondary-title></titles><periodical><full-title>American Journal of Agricultural Economics</full-title></periodical><pages>106-123</pages><volume>91</volume><number>1</number><dates><year>2009</year><pub-dates><date>Feb</date></pub-dates></dates><isbn>0002-9092</isbn><accession-num>WOS:000262346000007</accession-num><urls><related-urls><url><Go to ISI>://WOS:000262346000007</url></related-urls></urls><electronic-resource-num>10.1111/j.1467-8276.2008.01182.x</electronic-resource-num></record></Cite></EndNote>]. The number of reservoir visits per household increased from a median below 0.1 for MS 10 to a median of 0.18 as exposure to the insecticide decreased (Figure 7). This increase in visits was associated with a higher willingness to pay on a per-household basis for increased water clarity (results not shown). When expanded to the number of households in Johnston County, we estimated a regional economic benefit realized by waders and swimmers of between $6,000 (MS 10) and $15,000 (MS No pesticide) (Figure 7). Furthermore, the variability in values declined as the level of insecticide decreased in the simulated lake.

4. Discussion

The goal of this study was to use an ecological model to quantify changes in the provisioning of ecosystem services associated with alternative management scenarios. We applied a well-established aquatic ecosystem model to quantify the impacts of a hypothetical insecticide on several ecosystem services provided by a generic reservoir lake. Our case study illustrates a modeling framework that can prove useful in evaluating management approaches and resolving tradeoffs in ecosystem services. Although the results are purely dependent on the assumptions and data for this hypothetical case study, they nonetheless show that the best management approach may depend on which services we aim to protect. We have shown that ecosystem services can respond to the presence of an insecticide differently and that careful consideration of service beneficiaries and their values needs to be incorporated into the risk assessment process if ecosystem service delivery is a protection goal.

4.1. Abundance of game fishes

Hypothetical exposure to the insecticide affected fish species differently. Differences depended on the species' trophic status and interactions within the food web, as well as species sensitivity to the toxicant. Both bluegills and their main prey (daphnids) were highly sensitive to the modeled insecticide. Consequently, management scenarios that resulted in large insecticide runoff to the reservoir resulted in extirpation of both populations. Largemouth bass and walleye experienced similar dynamics (data for walleye not shown because they are the same as bass). Both populations reached very low levels in scenarios with higher insecticide concentrations. However, biomass of both species was highest in a low-insecticide scenario, MS 25, suggesting that they benefitted from low concentrations of the modeled insecticide in the system. A closer inspection of the trophic matrix (Supplementary Information) suggested that a potential cause could be increased detrital biomass which then impacts bass and walleye via the detrital food web. Detritivores, such as chironomids and *Tubifex*, represent the main food resource for largemouth bass juveniles. However, the prey base for walleye included mostly other fish species such as shad and especially buffalofish, which is relatively insensitive to the modeled insecticide

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and feeds largely on detritivores. So even though biomass increases of both species were driven by the detrital food web, the effect on bass was direct, whereas increased resources for walleye were mediated by intermediary prey species.

On the other end of fish responses was the three-fold increase in catfish biomass in the baseline scenario which could not be explained merely by low sensitivity to the modeled insecticide. The trophic matrix details revealed that an insecticide-induced decline in walleye released juvenile catfish from predation and also led to an increase in chironomid biomass which forms the base of catfish diet. In other words, we observed a trophic cascade [ADDIN EN.CITE <EndNote><Cite><Author>Carpenter</Author><Year>1985</Year><RecNum>10</RecNum><DisplayText>(Carpenter et al., 1985)</DisplayText><record><rec-number>10</rec-number><foreign-keys><key app="EN" db-id="pzaw5xrt5s0z25ewefrx2wspta2fvdts2wd5">10</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Carpenter, Stephen R</author><author>Kitchell, James F</author><author>Hodgson, James R</author></authors></contributors><titles><title>Cascading trophic interactions and lake productivity</title><secondary-title>BioScience</secondary-title></titles><periodical><full-title>BioScience</full-title></periodical><pages>634-639</pages><volume>35</volume><number>10</number><dates><year>1985</year></dates><isbn>0006-3568</isbn><urls></urls></record></Cite></EndNote>]. A combination of low sensitivity to the insecticide, diminished predation pressure and an increase in prey biomass resulted in overall positive impacts of the modeled insecticide for this species.

These differences in fish biomass dynamics translated into different economic benefits and losses among scenarios, which varied substantially across years. Variability in economic value was expressed as the difference across years attributed to differences in biomass output of AQUATOX. This difference was likely driven by simulated weather that influenced insecticide loading to the reservoir, e.g., increased rainfall leads to increased runoff from treated fields, and by temperature impacts on the simulated community. Year-to-year variability in number of trips or WTP was not accounted for, as these data were not available.

These results illustrate how tradeoffs among ecosystem services may influence management decisions. Because different results were observed for different species of fish, the decision to manage the use of the insecticide for the ecosystem service of angling will depend upon the favored species of fish. If beneficiaries value walleye and bass fishing above fishing for bluegill, any management that reduces insecticide exposure (from MS 10 to MS 100) will be sufficient for improving the ecosystem service of interest. On the other hand, if anglers prefer to fish for bluegill, then only no use of the insecticide in the watershed will be sufficient. Counterintuitively, any management of the insecticide would result in economic losses if anglers prefer to fish for catfish.

4.2. Water clarity

We simulated the impacts of an insecticide on grazing zooplankton, which resulted in increased phytoplankton biomass and decreased water clarity in the reservoir. Our case study showed that waders and swimmers experienced a monotonic increase in this ecosystem service in response to reduced insecticide use. Water clarity, as measured by Secchi depth, varied substantially across years and simulated scenarios, possibly because phytoplankton biomass is driven by a multitude of factors [ADDIN EN.CITE <EndNote><Cite><Author>Boyce</Author><Year>2010</Year><RecNum>14</RecNum><Di

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splayText>(Boyce et al., 2010; Carpenter et al., 1998b)</DisplayText><record><rec-number>14</rec-number><foreign-keys><key app="EN" db-id="pzaw5xrt5s0z25ewefrx2wspta2fvdts2wd5">14</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Boyce, Daniel G</author><author>Lewis, Marlon R</author><author>Worm, Boris</author></authors></contributors><titles><title>Global phytoplankton decline over the past century</title><secondary-title>Nature</secondary-title></titles><periodical><full-title>Nature</full-title></periodical><pages>591</pages><volume>466</volume><number>7306</number><dates><year>2010</year></dates><isbn>1476-4687</isbn><urls></urls></record></Cite><Cite><Author>Carpenter</Author><Year>1998</Year><RecNum>13</RecNum><record><rec-number>13</rec-number><foreign-keys><key app="EN" db-id="pzaw5xrt5s0z25ewefrx2wspta2fvdts2wd5">13</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Carpenter, Stephen R</author><author>Cole, Jonathan J</author><author>Kitchell, James F</author><author>Pace, Michael L</author></authors></contributors><titles><title>Impact of dissolved organic carbon, phosphorus, and grazing on phytoplankton biomass and production in experimental lakes</title><secondary-title>Limnology and Oceanography</secondary-title></titles><periodical><full-title>Limnology and Oceanography</full-title></periodical><pages>73-80</pages><volume>43</volume><number>1</number><dates><year>1998</year></dates><isbn>0024-3590</isbn><urls></urls></record></Cite></EndNote>]. Water clarity is affected by ecosystem properties, such as fish community composition and the relative abundance of macrophytes versus algae. In real systems, other drivers also contribute to water clarity, such as suspended sediment [ADDIN EN.CITE <EndNote><Cite><Author>Davies-Colley</Author><Year>2001</Year><RecNum>149</RecNum><DisplayText>(Davies-Colley and Smith, 2001)</DisplayText><record><rec-number>149</rec-number><foreign-keys><key app="EN" db-id="tp2saazphvawt6evpr7pw0de9t0dfs9p02dx">149</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Davies-Colley, RJ</author><author>Smith, DG</author></authors></contributors><titles><title>Turbidity, Suspended Sediment, and Water Clarity: a Review</title><secondary-title>JAWRA Journal of the American Water Resources Association</secondary-title></titles><periodical><full-title>JAWRA Journal of the American Water Resources Association</full-title></periodical><pages>1085-1101</pages><volume>37</volume><number>5</number><dates><year>2001</year></dates><isbn>1752-1688</isbn><urls></urls></record></Cite></EndNote>], dissolved organic matter [ADDIN EN.CITE <EndNote><Cite><Author>Solomon</Author><Year>2015</Year><RecNum>151</RecNum><DisplayText>(Solomon et al., 2015)</DisplayText><record><rec-number>151</rec-number><foreign-keys><key app="EN" db-id="tp2saazphvawt6evpr7pw0de9t0dfs9p02dx">151</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Solomon, Christopher T</author><author>Jones, Stuart E</author><author>Weidel, Brian C</author><author>Buffam, Ishi</author><author>Fork, Megan L</author><author>Karlsson, Jan</author><author>Larsen, Søren</author><author>Lennon, Jay T

Steven</author></authors></contributors><titles><title>Ecosystem consequences of changing inputs of terrestrial dissolved organic matter to lakes: current knowledge and future challenges</title><secondary-title>Ecosystems</secondary-title></titles><periodical><full-title>Ecosystems</full-title></periodical><pages>376-389</pages><volume>18</volume><number>3</number><dates><year>2015</year></dates><isbn>1432-9840</isbn><urls></urls></record></Cite></EndNote>] or water chemistry, such as presence of tannins [ADDIN EN.CITE <EndNote><Cite><Author>Stumm</Author><Year>1970</Year><RecNum>152</RecNum><DisplayText>(Stumm and Morgan, 1970)</DisplayText><record><rec-number>152</rec-number><foreign-keys><key app="EN" db-id="tp2saazphvawt6evpr7pw0de9t0dfs9p02dx">152</key></foreign-keys><ref-type name="Book">6</ref-type><contributors><authors><author>Stumm, Werner</author><author>Morgan, James J</author></authors></contributors><titles><title>Aquatic chemistry; an introduction emphasizing chemical equilibria in natural waters</title></titles><dates><year>1970</year></dates><isbn>0471834955</isbn><urls></urls></record></Cite></EndNote>]. In our hypothetical shallow reservoir, water clarity was mostly driven by changes in food web dynamics, specifically the presence of zooplankton, which is common in shallow lakes [ADDIN EN.CITE <EndNote><Cite><Author>Jeppesen</Author><Year>1999</Year><RecNum>150</RecNum><DisplayText>(Jeppesen et al., 1999)</DisplayText><record><rec-number>150</rec-number><foreign-keys><key app="EN" db-id="tp2saazphvawt6evpr7pw0de9t0dfs9p02dx">150</key></foreign-keys><ref-type name="Book Section">5</ref-type><contributors><authors><author>Jeppesen, Erik</author><author>Jensen, Jens Peder</author><author>Søndergaard, Martin</author><author>Lauridsen, Torben</author></authors></contributors><titles><title>Trophic dynamics in turbid and clearwater lakes with special emphasis on the role of zooplankton for water clarity</title><secondary-title>Shallow lakes' 98</secondary-title></titles><pages>217-231</pages><dates><year>1999</year></dates><publisher>Springer</publisher><urls></urls></record></Cite></EndNote>].

The increase in water clarity with reduced insecticide use described above was accompanied by a monotonic increase in value. A significant, linear relationship was reported between Secchi depth and distance traveled to visit lakes in Iowa and Minnesota, as determined by photo-visitation [ADDIN EN.CITE <EndNote><Cite><Author>Keeler</Author><Year>2015</Year><RecNum>129</RecNum><DisplayText>(Keeler et al., 2015)</DisplayText><record><rec-number>129</rec-number><foreign-keys><key app="EN" db-id="tp2saazphvawt6evpr7pw0de9t0dfs9p02dx">129</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Keeler, Bonnie L.</author><author>Wood, Spencer A.</author><author>Polasky, Stephen</author><author>Kling, Catherine</author><author>Filstrup, Christopher T.</author><author>Downing, John A.</author></authors></contributors><titles><title>Recreational demand for clean water: evidence from geotagged photographs by visitors to lakes</title><secondary-title>Frontiers in

Ecology and the Environment</secondary-title></titles><periodical><full-title>Frontiers in Ecology and the Environment</full-title></periodical><pages>76-81</pages><volume>13</volume><number>2</number><dates><year>2015</year><pub-dates><date>2015/03/01</date></pub-dates></dates><publisher>Ecological Society of America</publisher><isbn>1540-9295</isbn><urls><related-urls><url>http://dx.doi.org/10.1890/140124</url></related-urls></urls><electronic-resource-num>10.1890/140124</electronic-resource-num><access-date>2015/09/16</access-date></record></Cite></EndNote>]. The relationship was significant for Iowa lakes alone, where mean Secchi depth was 1.1 m, but not for Minnesota lakes alone, which have a mean Secchi depth of 2.7 m. This may suggest that differences in clarity become less important as clarity improves, a finding that was not possible to consider in our case study. Furthermore, the public benefits more from paying to improve at least one lake to a higher level of clarity than to increase the clarity of all lakes by a smaller amount [ADDIN EN.CITE <EndNote><Cite><Author>Egan</Author><Year>2009</Year><RecNum>3848</RecNum><DisplayText>(Egan et al., 2009)</DisplayText><record><rec-number>3848</rec-number><foreign-keys><key app="EN" db-id="zev9ra0zprssfpeev2lvwwr75xww9swpp9zz" timestamp="1519590937">3848</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Egan, K. J.</author><author>Herriges, J. A.</author><author>Kling, C. L.</author><author>Downing, J. A.</author></authors></contributors><titles><title>Valuing water quality as a function of water quality measures</title><secondary-title>American Journal of Agricultural Economics</secondary-title></titles><periodical><full-title>American Journal of Agricultural Economics</full-title></periodical><pages>106-123</pages><volume>91</volume><number>1</number><dates><year>2009</year><pub-dates><date>Feb</date></pub-dates></dates><isbn>0002-9092</isbn><accession-num>WOS:000262346000007</accession-num><urls><related-urls><url><Go to ISI>://WOS:000262346000007</url></related-urls></urls><electronic-resource-num>10.1111/j.1467-8276.2008.01182.x</electronic-resource-num></record></Cite></EndNote>]. This is, in part, because clarity improvements smaller than 0.5 meters are visually imperceptible [ADDIN EN.CITE <EndNote><Cite><Author>Smeltzer</Author><Year>1990</Year><RecNum>3898</RecNum><DisplayText>(Smeltzer and Heiskary, 1990)</DisplayText><record><rec-number>3898</rec-number><foreign-keys><key app="EN" db-id="zev9ra0zprssfpeev2lvwwr75xww9swpp9zz" timestamp="1519773166">3898</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Smeltzer, E.</author><author>S.A. Heiskary</author></authors></contributors><titles><title>Analysis and applications of lake user survey data</title><secondary-title>Lake and Reservoir Management</secondary-title></titles><periodical><full-title>Lake and Reservoir Management</full-title></periodical><pages>109-118</pages><volume>6</volume><number>1</number><dates><year>1990</year></dates><urls></urls></record></Cite></EndNote>]. In our study, changes in Secchi depth between scenarios with no insecticides and those in which different management strategies were implemented ranged from 0.7 (MS 0 to MS 10) to 1.1 m (MS 0 to MS No pesticide). This suggests that improvements would be visible to, and presumably valued by the public.

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Here, we have demonstrated the implications for management of taking different beneficiary preferences into account. If walleye or largemouth bass are preferred over other fish, then reducing insecticide loading by a factor of 10 would markedly increase service delivery compared to the baseline (no management) scenario. At the same time, catfish would incur a modest reduction in biomass, and there would be a substantial increase in water clarity. In monetary terms, the MS 10 would yield a net benefit of ~ $670,000 which is the sum of the mean increased value of largemouth bass, walleye biomass, and bluegill and water clarity. If we were to add the losses associated with reduced catfish, then a net benefit of $32,000 would be expected. For comparison, removing insecticide use from the region would yield a benefit of ~ $1,100,000; this is the sum of the mean increase in biomass of largemouth bass, walleye, and bluegill and increased water clarity. Subtracting the losses from reduced catfish biomass results in a net value of $270,000 over the whole simulation period.

It is worth noting that we did not account for services derived from agriculture in the watershed surrounding this reservoir. Furthermore, we did not account for direct or indirect costs of different management scenarios on agriculture. We assumed that insecticide loading in the watershed could be reduced with different mitigation measures, such as reduced application rates and frequencies or use of buffer strips, or wetlands [ADDIN EN.CITE <EndNote><Cite><Author>Reichenberger</Author><Year>2007</Year><RecNum>16</RecNum><DisplayText>(Reichenberger et al., 2007)</DisplayText><record><rec-number>16</rec-number><foreign-keys><key app="EN" db-id="pzaw5xrt5s0z25ewefrx2wspta2fvdts2wd5">16</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Reichenberger, Stefan</author><author>Bach, Martin</author><author>Skitschak, Adrian</author><author>Frede, Hans-Georg</author></authors></contributors><titles><title>Mitigation strategies to reduce pesticide inputs into ground- and surface water and their effectiveness; A review</title><secondary-title>Science of The Total Environment</secondary-title></titles><periodical><full-title>Science of The Total Environment</full-title></periodical><pages>1-35</pages><volume>384</volume><number>1</number><keywords><keyword>Pesticides</keyword><keyword>Risk</keyword><keyword>Mitigation measures</keyword><keyword>Diffuse sources</keyword><keyword>Point sources</keyword><keyword>Effectiveness</keyword><keyword>Practicability</keyword></keywords><dates><year>2007</year><pub-dates><date>2007/10/01/</date></pub-dates></dates><isbn>0048-9697</isbn><urls><related-urls><url>http://www.sciencedirect.com/science/article/pii/S004896970700513X</url></related-urls></urls><electronic-resource-num>https://doi.org/10.1016/j.scitotenv.2007.04.046</electronic-resource-num></record></Cite></EndNote>]; however, these mitigation measures may come at a cost to growers. For example, constructing wetlands may be costly whereas using the right insecticide application nozzle size is less so [ADDIN EN.CITE <EndNote><Cite><Author>Reichenberger</Author><Year>2007</Year><RecNum>16</RecNum><DisplayText>(Reichenberger et al., 2007)</DisplayText><record><rec-number>16</rec-number><foreign-keys><key app="EN" db-id="pzaw5xrt5s0z25ewefrx2wspta2fvdts2wd5">16</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Reichenberger, Stefan</author><author>Bach, Martin</author><author>Skitschak,

Adrian</author><author>Frede, Hans-Georg</author></authors></contributors><titles><title>Mitigation strategies to reduce pesticide inputs into ground- and surface water and their effectiveness; A review</title><secondary-title>Science of The Total Environment</secondary-title></titles><periodical><full-title>Science of The Total Environment</full-title></periodical><pages>1-35</pages><volume>384</volume><number>1</number><keywords><keyword>Pesticides</keyword><keyword>Risk</keyword><keyword>Mitigation measures</keyword><keyword>Diffuse sources</keyword><keyword>Point sources</keyword><keyword>Effectiveness</keyword><keyword>Practicability</keyword></keywords><dates><year>2007</year><pub-dates><date>2007/10/01/</date></pub-dates></dates><isbn>0048-9697</isbn><urls><related-urls><url>http://www.sciencedirect.com/science/article/pii/S004896970700513X</url></related-urls></urls><electronic-resource-num>https://doi.org/10.1016/j.scitotenv.2007.04.046</electronic-resource-num></record></Cite></EndNote>]. In some cases, mitigation can also generate income for farmers. For example, three-stage buffers from which perennial grasses, willow and poplar are harvested for biomass energy [ADDIN EN.CITE <EndNote><Cite><Author>Jager</Author><Year>2017</Year><RecNum>31</RecNum><DisplayText>(Jager and Efroymson, 2017; Maringanti et al., 2011)</DisplayText><record><rec-number>31</rec-number><foreign-keys><key app="EN" db-id="pzaw5xrt5s0z25ewefrx2wspta2fvdts2wd5">31</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Jager, Henriette I</author><author>Efroymson, Rebecca A</author></authors></contributors><titles><title>Can upstream biofuel production increase the flow of downstream ecosystem goods and services?</title><secondary-title>Biomass and Bioenergy</secondary-title></titles><periodical><full-title>Biomass and Bioenergy</full-title></periodical><dates><year>2017</year></dates><isbn>0961-9534</isbn><urls></urls></record></Cite><Cite><Author>Maringanti</Author><Year>2011</Year><RecNum>30</RecNum><record><rec-number>30</rec-number><foreign-keys><key app="EN" db-id="pzaw5xrt5s0z25ewefrx2wspta2fvdts2wd5">30</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Maringanti, Chetan</author><author>Chaubey, Indrajeet</author><author>Arabi, Mazdak</author><author>Engel, Bernard</author></authors></contributors><titles><title>Application of a multi-objective optimization method to provide least cost alternatives for NPS pollution control</title><secondary-title>Environmental management</secondary-title></titles><periodical><full-title>Environmental management</full-title></periodical><pages>448-461</pages><volume>48</volume><number>3</number><dates><year>2011</year></dates><isbn>0364-152X</isbn><urls></urls></record></Cite></EndNote>]. In order to consider additional impacts of possible management options, it may be useful to include a quantitative analysis of the costs or benefits associated with actions that would serve to implement the possible management scenarios.

Furthermore, our hypothetical example was designed to illustrate the worst-case insecticide effects using available models and data expected to be available for an insecticide. The model we used to derive exposure concentrations is conservative in its choice of parameters and

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assumptions, yielding worst-case exposure concentrations. There are other factors that may impact pesticide loading, such as implementation of agricultural best management practices, use of lower rates (than allowed on the label) and a lower proportion of fields treated within the watershed (i.e., we assumed all of the agricultural acres in the watershed were treated). Analysis of water samples from reservoirs in the Northern Great Plains reveals that for the period of 2003-2005, the maximum detected concentration of the organophosphate chlorpyrifos was 0.02 μg/L [ADDIN EN.CITE <EndNote><Cite><Author>Donald</Author><Year>2007</Year><RecNum>39</RecNum><DisplayText>(Donald et al., 2007)</DisplayText><record><rec-number>39</rec-number><foreign-keys><key app="EN" db-id="pzaw5xrt5s0z25ewefrx2wspta2fvdts2wd5">39</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Donald, David B.</author><author>Cessna, Allan J.</author><author>Sverko, Ed</author><author>Glozier, Nancy E.</author></authors></contributors><titles><title>Pesticides in Surface Drinking-Water Supplies of the Northern Great Plains</title><secondary-title>Environmental Health Perspectives</secondary-title></titles><periodical><full-title>Environmental Health Perspectives</full-title></periodical><pages>1183-1191</pages><volume>115</volume><number>8</number><dates><year>2007</year></dates><publisher>National Institute of Environmental Health Sciences</publisher><isbn>0091-6765
1552-9924</isbn><accession-num>PMC1940079</accession-num><urls><related-urls><url>http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1940079/</url></related-urls></urls><electronic-resource-num>10.1289/ehp.9435</electronic-resource-num><remote-database-name>Pmc</remote-database-name></record></Cite></EndNote>]. Observations that empirical concentrations are orders of magnitude lower than modeled estimates (mean simulated concentration was 5 μg/L), support the assumption that exposure estimated in this analysis is conservative. Moreover, other consequences of agricultural practices were also omitted from this study. Nutrient run-off may cause substantial disruption to aquatic ecosystems, including algal blooms, hypoxic events or shifts in the whole food web [ADDIN EN.CITE ADDIN EN.CITE.DATA]. However, our aim was not to perform an actual risk assessment, but instead to provide an example of how provision of ecosystem services to different beneficiaries can be simulated under different insecticide management scenarios to facilitate decision-making in ecological risk assessment and management [ADDIN EN.CITE ADDIN EN.CITE.DATA]. To clearly demonstrate this, we used a wide range of exposures that ensured a larger disruption to the ecosystem and possible ecosystem service trade-offs.

Our study clearly showed the importance of considering nonlinear ecosystem feedbacks when attempting to assess risk from chemicals and other stressors [ADDIN EN.CITE <EndNote><Cite><Author>De Laender</Author><Year>2013</Year><RecNum>20</RecNum><DisplayText>(De Laender and Janssen, 2013)</DisplayText><record><rec-number>20</rec-number><foreign-keys><key app="EN" db-id="pzaw5xrt5s0z25ewefrx2wspta2fvdts2wd5">20</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>De Laender, Frederik</author><author>Janssen, Colin R</author></authors></contributors><titles><title>Brief communication: The ecosystem perspective in ecotoxicology as a way forward for the ecological risk assessment of chemicals</title><secondary-title>Integrated Environmental Assessment and

Management</secondary-title></titles><periodical><full-title>Integrated Environmental Assessment and Management</full-title></periodical><pages>e34-e38</pages><volume>9</volume><number>3</number><dates><year>2013</year></dates><urls><related-urls><url>https://setac.onlinelibrary.wiley.com/doi/abs/10.1002/ieam.1428</url></related-urls></urls><electronic-resource-num>doi:10.1002/ieam.1428</electronic-resource-num></record></Cite></EndNote>]. The presence of some level of insecticide changed the modeled food-web dynamics in non-linear ways, resulting in increased catfish biomass in all scenarios, and walleye and largemouth bass in low insecticide scenarios (MS 25). It is highly unlikely that such responses would have been detected without the use of quantitative models that integrate relevant ecological information. Our study highlights one of the main advantages of using ecological models for risk assessment, namely the ability to generalize to meaningful levels of organization that matter to society, rather than individual-level toxicological sensitivity of a handful of species [ADDIN EN.CITE ADDIN EN.CITE.DATA]. The models also facilitate quantitative comparisons among alternative scenarios while explicitly accounting for different groups of beneficiaries and potential trade-offs among them. This approach has the potential to enhance the transparency and consistency of risk assessments by enlisting and representing different stakeholder communities, and may thereby improve environmental management decisions.

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Tables

Table 1. Framework for economic valuation of changes in final ecosystem goods and services (FEGS) across case study scenarios. Abbreviations are: Final ecosystem goods & services (FEGS), FEGS Classification system (FEGS-CS), and willingness-to-pay (WTP).

FEGS		Beneficiary		Available Benefit Transfer Data	Corresponding model endpoint
From FEGS-CS	In case study	From FEGS-CS	In case study		
Presence of the environment/ Water suitability for swimming	Mean seasonal Secchi depth (m)[1]	Recreational/ Waders, Swimmers, and Divers	Recreational users of the case study reservoir	WTP (Iowans' travel to Iowa lakes) for change in water clarity = $\$0.21 m^{-1}$ $household^{-1}$ $lake^{-1}$ y^{-1} (Egan et al. 2009)	Change in seasonal Secchi depth (m) in different management scenarios
Presence of the environment/ Ability to view subsurface environment	Mean seasonal Secchi depth (m)[1]	Recreational/ Experiencers and Viewers	General public and property owners at the case study reservoir	This FEGS was assumed to overlap with the previous one	Change in Secchi depth (m)
Fish/ Abundances of game fishes	Biomass of several fish species	Recreational/ Anglers	Recreational anglers of the case study reservoir	WTP (Michigan anglers' travel to Michigan rivers, streams, impoundments) per additional fish caught = $1.55 for panfish, $1.57 for largemouth bass, $4.03 for walleye (Melstrom et al. 2015)	Change in biomass of the four species of interest for angling: largemouth bass, walleye, bluegill, catfish

[1] Secchi depths are defined as the number of meters beneath the surface of the water where a Secchi disk is visible

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Figures

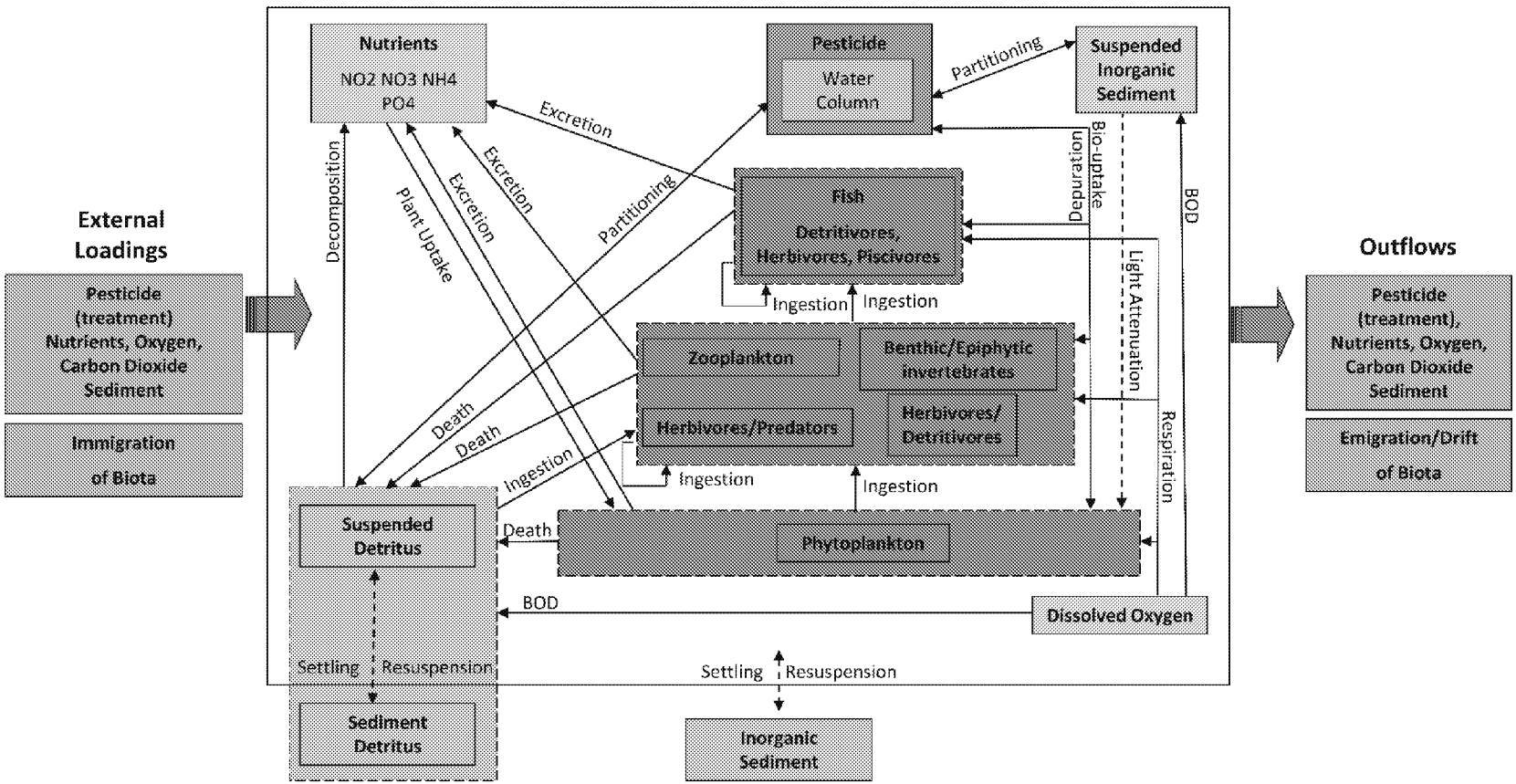

Figure 1. Conceptual representation of different compartments represented in AQUATOX.

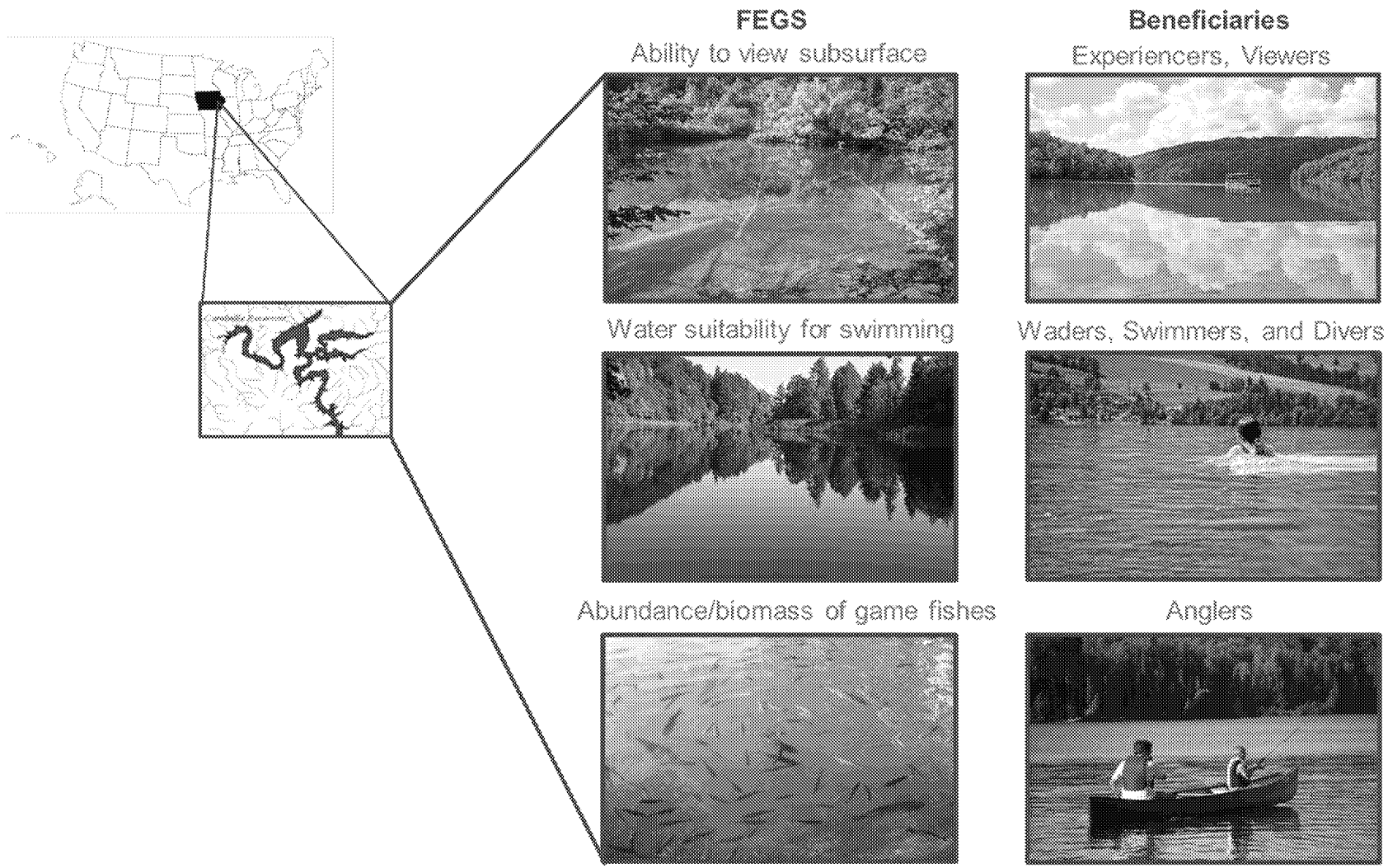

Figure 2. Final ecosystem goods and services (FEGS) provided by a reservoir lake, representative of Midwestern lakes, and their beneficiaries.

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[EMBED AcroExch.Document.DC]

Figure 3. Biomass of different compartments in the lake ecosystem, under management scenarios (MS) described in the legend. Boxplots represent biomass across the whole duration of the simulations. The boxes enclose the 25th and 75th percentile, the horizontal center line denotes median values, and boxplot whiskers extend to the minimum and maximum values.

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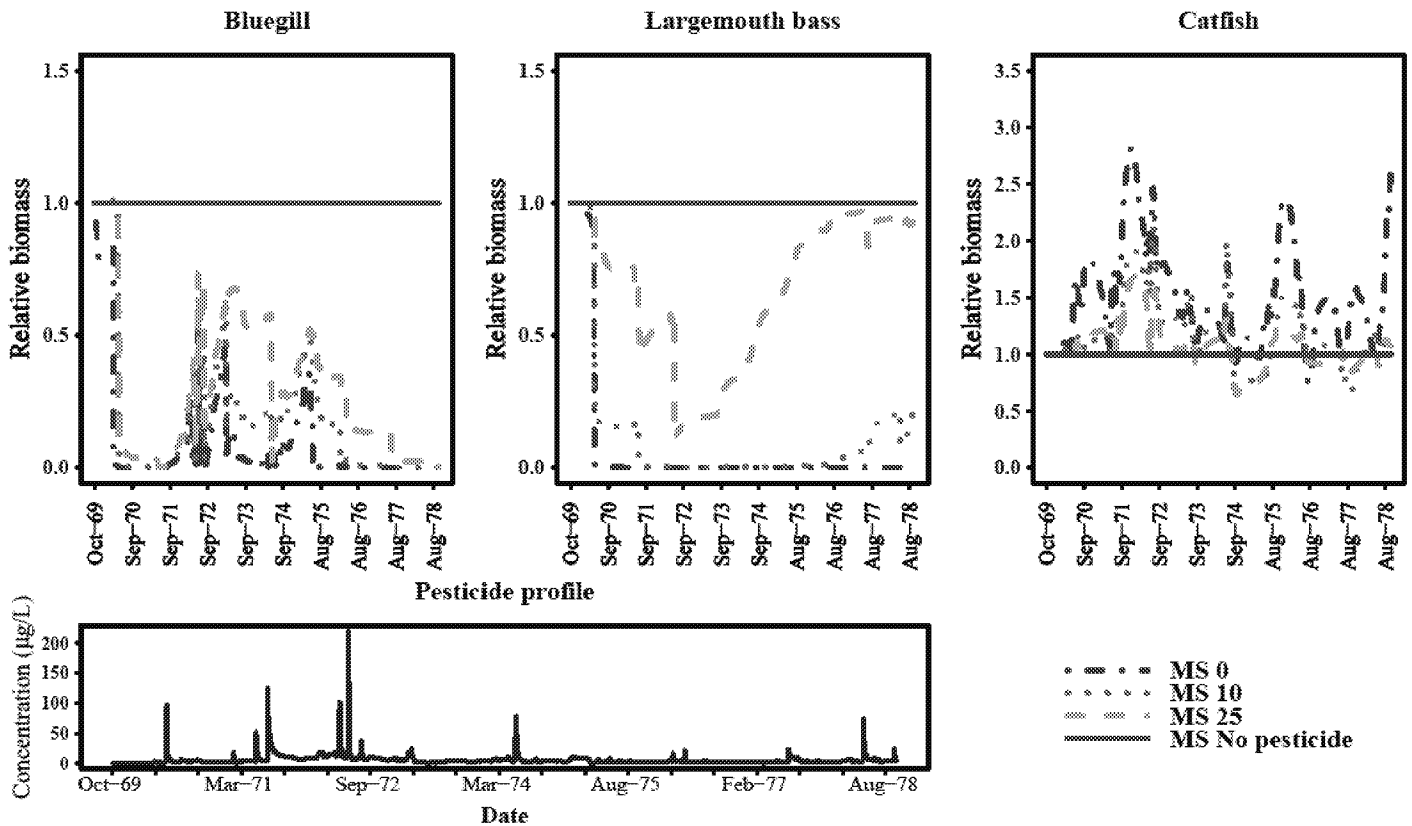

Figure 4. Time series of bluegill, largemouth bass, and catfish biomass in four management scenarios; biomass is represented as relative to the management scenario with no insecticide, i.e. MS No pesticide. The insecticide exposure profile was provided by the Insecticide Root Zone Model (PRZM). MS 0 is the scenario without any management, MS 10 is a scenario with 10x reduction in exposure, MS 25 is a scenario with a 25X reduction in exposure and MS No pesticide represents a scenario in which the insecticide was not used.

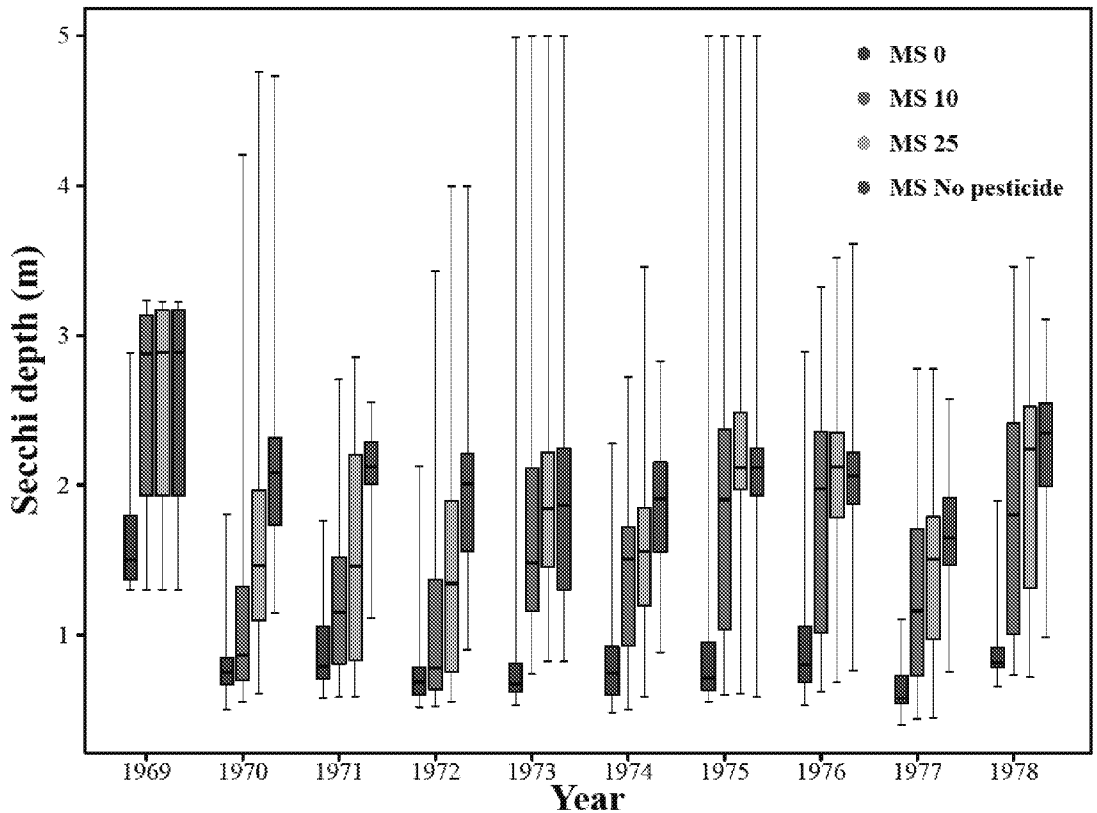

Figure 5. Simulated Secchi depths (m) during the full recreational season from April 1st to October 31st, which is a total of 214 days for years 1970 to 1977, 31 d in 1969 and 183 d 1978, under different management scenarios (MS). MS 0 is the scenario without any management, MS 10 is a scenario with 10x reduction in insecticide exposure, MS 25 is a scenario with a 25X reduction in exposure and MS No pesticide represents a scenario in which the insecticide was not used. Boxplots represent values in one year of the simulation, with denoted median values, whereas boxplot whiskers extend to the full range of values.

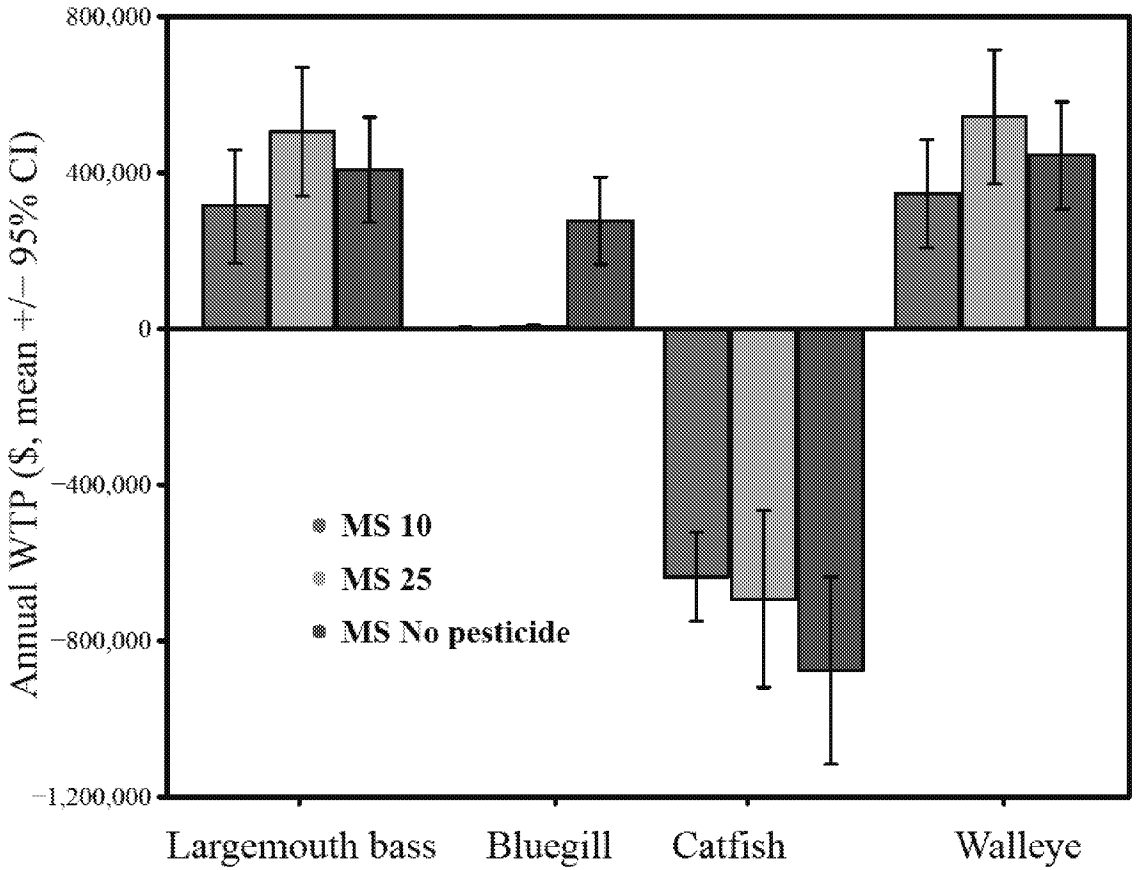

Figure 6. Change in value associated with the ecosystem service of recreational fishing, estimated by willingness to pay (WTP), on four species in different management scenarios. Error bars represent 95% confidence intervals around annual means. MS 10 is a scenario with 10x reduction in exposure, MS 25 is a scenario with a 25X reduction in exposure and MS No pesticide represents a scenario in which the insecticide was not used. WTP is the willingness-to-pay for recreational fishing.

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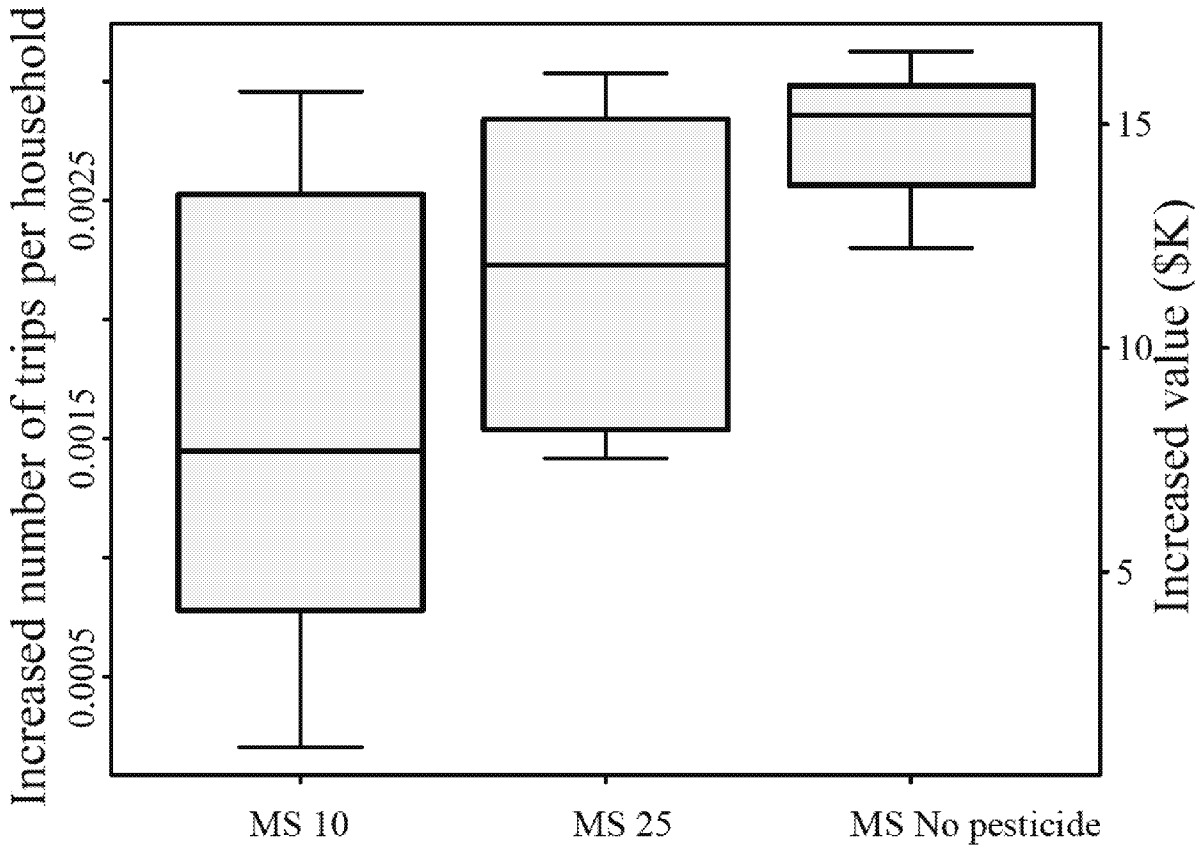

Figure 7. Increased recreational use (left axis) and total value (right axis) across the county for Iowa swimmers. MS 10 is a scenario with 10x reduction in insecticide exposure, MS 25 is a scenario with a 25X reduction in exposure and MS No pesticide represents a scenario in which the insecticide was not used. The boxes enclose the 25th and 75th percentile, the horizontal center line denotes median values, and boxplot whiskers extend to the minimum and maximum values.

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References

[ADDIN EN.REFLIST]

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Message

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To: Lennartz, Steven [Lennartz.Steven@epa.gov]; Garber, Kristina [Garber.Kristina@epa.gov]; Blankinship, Amy [Blankinship.Amy@epa.gov]; Donovan, Elizabeth [Donovan.Elizabeth@epa.gov]; Connolly, Jennifer [Connolly.Jennifer@epa.gov]
Subject: RE: malathion uses (question about developed)
Attachments: malathion QUA.pdf

Deliberative Process / Ex. 5

From: Lennartz, Steven
Sent: Tuesday, May 22, 2018 10:35 AM
To: Eckel, William <Eckel.William@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>; Blankinship, Amy <Blankinship.Amy@epa.gov>; Donovan, Elizabeth <Donovan.Elizabeth@epa.gov>; Connolly, Jennifer <Connolly.Jennifer@epa.gov>
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Deliberative Process / Ex. 5

From: Eckel, William
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To: Garber, Kristina <Garber.Kristina@epa.gov>; Blankinship, Amy <Blankinship.Amy@epa.gov>; Donovan, Elizabeth <Donovan.Elizabeth@epa.gov>; Connolly, Jennifer <Connolly.Jennifer@epa.gov>; Lennartz, Steven <Lennartz.Steven@epa.gov>
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Deliberative Process / Ex. 5 ?

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It is my understanding t Deliberative Process / Ex. 5
Could you please confirm?

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Armed Forces Pest Management Board

TECHNICAL GUIDE NO. 13

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DISPERSAL OF ULTRA LOW VOLUME (ULV) INSECTICIDES BY COLD AEROSOL AND THERMAL FOG GROUND APPLICATION EQUIPMENT

****

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TABLE OF CONTENTS

ACKNOWLEDGMENTS iv

DISCLAIMER iv

FOREWORD v

SECTION

1. Introduction and Purpose 1
2. Definition of Aerosol and ULV Insecticide Dispersal 1
3. Advantages and Disadvantages of Cold Aerosol and Thermal Fob Application Methods 2
4. Droplet Size Considerations for Aerosol Applications 3
5. Measurement of Droplets for Analysis of ULV Performance 4
6. Dispersal Methods and Equipment 5
7. Equipment for Military Use 6
8. Equipment Calibration 12
9. ULV Insecticides 13
10. Application Parameters for ULV Insecticides 17
11. Practical Considerations for ULV Applications 19
12. Equipment Maintenance and Repair 30

APPENDIX A. Literature Cited 32

APPENDIX B. Instructions for Determining Flow Rate 35

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APPENDIX C. Equipment Calibration Data Sheet 37

APPENDIX D. Testing Results of Spray Equipment Used in Vector Control 38

APPENDIX E. Directions for Determining Particle Size of Aerosols and Fine Sprays 47

APPENDIX F. Sources of Supply for ULV Aerosol Insecticide Equipment and Nozzles 61

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DISCLAIMER

Trade names are used in this TG to provide specific information and do not imply endorsements of the products named or criticism of similar ones not mentioned. Mention of trade names does not constitute a guarantee or warranty of the products by the AFPMB, the Military Services, or the Department of Defense.

AFPMB TECHNICAL GUIDES

This is one of a series of Technical Guides (TGs) published by the Information Services Division (ISD), Armed Forces Pest Management Board (AFPMB). The AFPMB is a directorate within the Office of the Deputy Under Secretary of Defense (Installations and Environment) that recommends policies and procedures, provides guidance, and coordinates the exchange of information related to pest management throughout the Department of Defense (DoD).

TGs are not policy documents; rather, they provide technical guidance for the use of the DoD pest management community and others. TGs are a contributing part of the overarching DoD approach to integrated pest management. Each TG should not be considered as stand-alone guidance and should not be construed or referenced as policy. DoD pest management policies may be found in DoD Directive 4715.1E, "Environment, Safety, and Occupational Health (ESOH)" DoD Instruction 4150.07, "DoD Pest Management Program," other DoD directives and instructions, and implementing component directives/instructions/regulations.

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FOREWORD

This Technical Guide is designed to give specific information on DoD policy and current practices for ground application in outdoor situations. Aerial application policies and procedures are not considered, nor are indoor applications. Competency in performance of aerial application requires separate certification by DoD, or an appropriate state agency. Given the sensitivity of the subject, it is imperative that the activity considering outdoor ULV application take time to consider undesired side effects and the opinions of others in the pesticide use debate. As a weapon in the arsenal of integrated pest management, ULV application should be included in the context of reducing the risk of transmission of vector-borne disease through full implementation of as many techniques as are practical given the military, political, or social conditions existing in the area of operations. Protecting the health of service members, family members, and other civilians from excessive exposure to pesticides must be fully incorporated into the treatment plan.

As practices and pest management materiel change, information in this TG may need to be updated. Your constructive comments are most welcome and will be given full consideration in further revisions of this document. Comments should be sent to Armed Forces Pest Management Board, ATTN: Equipment Committee Ex-Officio, Forest Glen Section, Walter Reed Army Medical Center, Forest Glen Annex, Bldg. 172 6900 Georgia Avenue NW, Washington, DC 20307-5001. Phone: 301.295.7476. Fax: 301.295.7473. Email: http://www.acq.osd.mil/eie/afpmb/contactUs.html.

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1. INTRODUCTION AND PURPOSE

a. Disease vector and nuisance pest control for routine and contingency military operations requires continual modification and updating of techniques and equipment. Coupled with changing pesticide requirements due to resistance and/or environmental considerations, the military utilizes state-of-the-art techniques to ensure protection of its forces. The use of ultra low volume (ULV) insecticides and employment of aerosol generating equipment is one example of the methods introduced since 1960 in an effort to improve flying insect control. Primary emphasis is placed on ground control techniques; however, aerial dispersal equipment may be used successfully for vector-borne disease outbreaks where access or terrain curtail the use of ground equipment and vector suppression is immediately required.

b. This guide provides information concerning ULV pesticides, ground dispersal techniques, equipment, operation and maintenance considerations, and methods for required equipment evaluation. Equipment and techniques utilized for indoor or aerial dispersal of ULV insecticides are not included. Please see Mount et. al (1998) for a review of aerial dispersal methods and insecticides.

2. DEFINITION OF AEROSOL AND ULV INSECTICIDE DISPERSAL

a. Aerosol sprays consist of particles ranging in diameter from 0.1 to 50 microns (micrometers = μm) with most (80%) of the particles between 0.1 and 30 microns. According to the U. S. Environmental Protection Agency (EPA 1998) ULV as used in common agricultural practice refers to a total volume of 0.5 gal or less per acre (1.89 L or less per hectare) broadcast. For the control of public health vectors and pests, formulations are dispersed in concentrations of 10-90% at flow rates up to 18 fluid ounces per minute. It is critical that insecticides be delivered within certain droplet size parameters to be effective. For agricultural applications, the suitable droplet size range is between 70-90 μm VMD (Mathews 1993; volume median diameter – see VMD section below for details). The optimum droplet size for effective (≥90% mortality) mosquito control by space spraying via ground application equipment is 8–15 μm (Mount 1998), while a slightly larger droplet size (5-25 μm VMD) is most effective when applied by aerial methods (Mount et al. 1996). For controlling larger flying insects such as filth flies or tsetse flies, VMDs closer to 30 μm are recommended (WHO 2003). Mosquito control efficacy declines rapidly either as the droplet size falls below 5 μm or rises above 25 μm VMD. Generating the correct droplet size range is therefore paramount to successfully controlling flying insects. The volume of material delivered per unit area is a second important parameter that will help determine and measure the correct application rate. Technical grade ULV application rates run from 0.13-13 fl. oz. per acre using cold foggers, whereas thermal foggers deliver in excess of 25 fl. oz/acre. With the appropriate insecticides, droplet sizes and application rates, cold aerosol generators and thermal foggers have effectively controlled mosquitoes for decades and are therefore very capable of delivering insecticides within the effective droplet ranges needed to control flying insects of public health importance.

b. The term "ULV" in this TG is used to indicate an insecticide aerosol applied by either cold or thermal fog application equipment and delivered within the defined parameters for effective flying insect control (i. e., VMD <25 μm and where 90% of the spray volume is < 50 μm in

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diameter). Cold-ULV, cold aerosol and ULV insecticides are therefore synonymous in this TG, whereas thermal fog aerosol droplets are usually smaller and occur at higher density, but they still fall within these parameters.

3. ADVANTAGES AND DISADVANTAGES OF COLD AEROSOL AND THERMAL FOG APPLICATION METHODS

a. The following table from the Allman Equipment internet web site summarizes the advantages and disadvantages of each aerosol application method.

Table 1. Advantages and disadvantages of cold aerosol and thermal fog application methods. (Adapted from http://www.allman-sprayers.co.uk/fogging/pubhealth.html)

Thermal fog generators	Cold fog generators
Advantages	
Shorter application time due to higher flow rate (liter/hour)	No traffic hazards because fog cloud is nearly invisible
Dense, visible fog, therefore perfect observation of fog distribution and fog drift	Little or no quantities of carrier substances resulting in lower application cost
Lower concentration of active ingredient (lower hazard)	Reduced volume of output (liter/hectare) (but not of active ingredient)
Positive psychological effect on people (something is being done)	Little or no smell caused by carrier substances
People can escape direct contact with the fog cloud	Lower noise level
Better dispersion and higher density of droplets increases chances of impinging on flying insects	Some formulations are ready to use, thereby reducing operator exposure
Disadvantages	
Higher cost of carrier substances	Requires longer application time
Strong smell and staining of oily carrier substances	Fog is hardly visible, therefore observation of fog distribution and fog drift is difficult
Possible traffic hazards through dense fog	People cannot easily avoid the fog cloud
High noise level of the machines	Less psychological effect (nothing can be seen)
Smaller droplets are more likely to drift and be affected by atmospheric factors (i.e., thermals, turbulence)	Higher concentration of active ingredient (greater hazard)
	Higher technical skills and regular calibration are required for efficient operation of equipment

b. The advantages listed above also apply to contingency or emergency operations. Development of lightweight cold aerosol dispersal units using concentrated insecticide without additives has significantly reduced weight and space requirements for deployment blocks. An

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additional advantage to the applicator of thermal fogging is that the spray cloud is clearly visible, showing exactly where active ingredient is dispersed. Observations of air currents, inversions and penetrations into vegetation where vectors may be resting are therefore easier. Thus, reasons for success or failure of control operations will be more evident when the spray cloud is observed directly. Additionally, although thermal fogging methods may be more expensive, they may be more effective. Recent evaluations of ULV and thermal fog application effectiveness against mosquitoes in different environments have been reported by Britch et al. (2010). It was shown that even if the dosage of active ingredient per acre is similar or even higher for cold aerosols, the mortality achieved using a thermal fog was higher. The reason for this was because a much higher density of thermal fog particles was present and mixed within the air column, making thermal fog droplets 100 times more likely to contact a mosquito and cause mortality than cold aerosol droplets. Such characteristics of droplets within thermal fog clouds indicate the potential that more effective control may be possible for phlebotomine sand flies in the Middle East and elsewhere. In these situations, host-seeking females fly under relatively calm conditions throughout the night, and if an aerosol cloud were released consisting of small droplets at high density, that cloud would disperse more uniformly and many droplets would remain air-borne in the sprayed area much longer, greatly increasing the chances of droplet impingement on vectors present. This hypothesis remains to be field-tested, however.

4. DROPLET SIZE CONSIDERATIONS FOR AEROSOL APPLICATIONS

a. Because ULV methods employ only a small amount of insecticide, knowledge of the characteristics of droplets and their ultimate destination and deposition becomes of paramount importance if ULV dispersal is to be accomplished effectively and safely. This is especially important when low flow rates such as one fluid ounce per minute are used.

b. Adherence to optimal droplet size is essential so that the appropriate amount of insecticide is available in the droplet. It is generally accepted by the mosquito control industry that optimum droplet size for mosquito control is in the range of 5-25 microns in diameter. Insecticidal efficiency decreases rapidly for sizes smaller than 6 and larger than 18 microns. A minimum of 65%, preferably 80% of the total spray must consist of droplets not exceeding 25 microns in size, but little difference in efficiency is noted for a range from about 7 to 22 microns. Generating droplets larger than 25 microns cause these droplets to fall to the ground quickly, reducing insect exposure time and increasing potential hazards to the environment or personnel and is therefore wasteful or at best inefficient for ground dispersal application. Furthermore, droplets exceeding sizes stated in label specifications are capable of causing damage to automobile finishes or other painted surfaces. On the other hand, smaller droplets are more susceptible to drift and droplets that are too small may not impinge on the flying insect to deliver a lethal dose.

c. Understanding Droplet Size Distributions for ULV Dispersal.

(1) Understanding label specifications and droplet distribution in an aerosol cloud requires knowledge of the term mass (or volume) median diameter (MMD or VMD) expressed in microns. This is the closest description that can be employed to characterize a wide distribution of droplet sizes. There are several methods currently used to determine droplet size. Three popular for field use in mosquito control are: PCDC-3 (KLD Labs, Inc., Huntington Station, NY), Teflon® slides,

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and silicone slides. The PCDC-3 is a 'hot-wire' probe that is inserted into an aerosol cloud, and conductance change is measured when droplet impingement occurs. This measurement is converted to an MMD and printed out automatically. The distance at which the probe must be held is dependent on the 'airblast' from the sprayer and will differ from machine to machine. However, the probe must be held where the air velocity is 5-7 m/sec. Teflon® and silicone slides are used in the following manner to characterize droplet size distribution and can be employed to characterize a wide distribution of droplet sizes. The total volume of a droplet sample collected on a coated microscope slide (200 droplets on some labels) is calculated and divided into two equal volumes. The division line is the VMD, which means that half of the volume will have droplets smaller than the VMD number, and half the volume will have droplets larger than the VMD number. The measurement is expressed in microns. Some current ULV labels still use the term MMD, although most recent literature uses the term VMD or $V_{0.5}$. These terms are synonymous and interchangeable. Likewise, the $V_{0.1}$ and $V_{0.9}$ are the droplet diameters below which contain 10% and 90% of the spray volume, respectively.

(2) Droplet sizes should show a reasonable distribution around the VMD, and an aerosol for ULV dispersal will generally fall between 5 and 25 microns. A large number of very small or large droplets outside the 5-25 micron range indicate incorrect atomization. Usually some adjustment to the flow rate or other machine operating characteristics will be required to establish the correct range. If a large number of droplets, five microns or less, are produced and they constitute a small percentage (less than 10%) of the volume (mass), the remaining droplets will normally produce effective control. However, the final guidance, and the law, is the insecticide label. Labels vary in their requirement of VMD and selected ones are listed below in Table 4 of Section 5, ULV insecticides.

5. MEASUREMENT OF DROPLETS FOR ANALYSIS OF ULV PERFORMANCE

a. General Considerations. Droplet size determination is necessary at frequent intervals to determine particle size and confirm adherence to application requirements. This should be accomplished at the beginning of each spray season and for every 50-100 hours of operation thereafter. If a machine remains idle for a month or more, recalibration and droplet evaluation are advisable.

b. Specialized equipment and training of personnel are required to properly determine and evaluate droplet production. With instructions provided in this TG, the operator can collect droplets from a machine and submit them to a laboratory for analysis. When a slide sample is analyzed, factors such as the distribution of droplets by number (numerical median diameter - NMD), percentage of volume in each droplet size category, and other factors related to label specifications are considered. The knowledge of VMD size, percentage of volume within a certain range, and the largest droplets generated by the machine will usually be sufficient to determine if the equipment is operating properly. Contact the appropriate pest management professional for information concerning services that can assist in making droplet measurements. ***NOTE*: *see Appendix E for additional information, equipment and supplies needed, and specific guidelines for all aspects of procedures required.***

c. Measurement Techniques.

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(1) General. As outlined above, critical considerations must be met for droplet size in ULV applications, and the methods of analysis are as important as the insecticides and equipment employed to disperse them. Techniques such as automatic particle size counters, electronic scanning photography, lasers, chemically treated paper, "roto-rods", Cascade Impactor, magnesium oxide slides, settling chambers, carbon black and several types of gels may be used to determine droplet size. Several methods measure too coarsely or may fall into the realm of research techniques. Each method measures an optimum droplet size range for which it is selective and requires careful preparation techniques (Rathburn, 1970).

(2) For practical routine work, microscopic techniques are adequate to perform droplet analysis. The only equipment required is a good quality compound microscope, and an eyepiece micrometer for measurement of droplet diameters. Accurate calibration of the microscope is essential and can be easily accomplished by an experienced laboratory worker.

(3) *Silicone-coated and magnesium oxide-coated slides are accepted as being the method of choice for water-based formulations* (Rathburn, 1970).

(4) *Teflon-coated microscope slides are recommended for collecting droplets where oil is used as a diluent*, such as Fyfanon™ and pyrethroid insecticides. Silicone coatings are miscible with oil formulations and will produce erroneous results.

(5) Routine Field Collection Procedure for Insecticide Aerosol Droplets. Samples of droplets may be collected by waving a coated slide through the aerosol cloud at the required label distance. For each pesticide labeled for ULV, the collection procedure is described and the sampling distance is specified (see examples in Section 9, ULV Insecticides). The general procedures for droplet collection and measurement can be found in Appendix E, along with a list of materials and potential sources of supplies to make a ULV droplet analysis kit.

6. DISPERSAL METHODS AND EQUIPMENT

a. Basic Considerations. Effectiveness of ULV ground dispersal for adult flying insect control is dependent upon many important factors such as the inherent toxicity of the insecticide, insecticide concentration and flow rate, proper equipment calibration and operation, vehicle speed, activity of the target population, meteorological conditions, topography, and vegetation height and density to name a few. Droplet size, droplet distribution and application rate have added significance because of the very small volume of insecticide applied to an area to achieve control.

b. Equipment. ULV ground equipment, whether produced for commercial or military use, must meet certain requirements. Components coming in contact with the insecticide merit special attention; these are: tubing carrying the insecticide from the reservoir to the atomizing head, flow control valves, pump assemblies, and exterior portions of the dispersal unit subject to liquid contamination. Equipment intended for use over rough terrain should be designed to withstand hard usage without breakdown. Electronic components should be heavy duty to withstand the effects of heat, solvents, and vibration. A review of ULV ground dispersal equipment indicates that many

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manufacturers use common components. Therefore, several generalizations concerning design (e.g. FMI pumps, Roots blowers) and operation may be made.

c. Methods of Generating ULV Droplets.

(1) Air-liquid mixing chambers (turbulence chamber) (mechanical methods). Insecticide and air are mixed together under pressure in a chamber to produce atomization. The mixture is delivered to the outside air through single or multiple nozzles. Pressure generated by a blower or compressor may deliver air at high volume and low pressure, or high pressure and low volume, depending on equipment design. Dispersal units are powered by electric or gasoline engines. These devices have the greatest versatility in the selection of air and liquid pressures or flow rates to facilitate liquid breakup and control particle size.

(2) Thermal Fogger (thermal methods). Two basic types of thermal foggers are manufactured: rotating disk and heat chamber (e.g., pulse jet). In the rotating disk type, insecticide is drawn between rotating disks, heated and shunted into the exhaust system. The insecticide-fuel oil mixture is vaporized at a relatively high temperature (generally $\geq 250^{\circ}$ F) in the exhaust port and forced into a relatively low atmospheric temperature where condensation (= fog) occurs. The heat chamber type also disperses an insecticide fuel oil mixture. However, a standard insecticide pump is used to shunt the material into a "heating chamber" where vaporization occurs. A Roots blower is used to force the vaporized material into the adjacent atmosphere, which drives the condensation.

(3) Rotating sleeves (mechanical). Insecticide under pump pressure is introduced into a rotating porous cylindrical sleeve, forcing the liquid through minute openings to produce atomization. Droplet size may be controlled by the porosity of the sleeve and by varying the insecticide pressure and flow rate. Breakup of the liquid may be further enhanced by air delivered from a fan producing a shearing action on the droplets. These units are usually electrically powered by 12-volt storage battery systems; sleeves and pumps are driven by electric motors.

(4) Rotating discs (mechanical). Insecticide breakup is accomplished by introducing the liquid by gravity or pressure onto a motor-driven, cylindrically shaped disc rotated at high speed. The insecticide impinging on the disc is propelled outward to provide initial breakup. Shear produced by a motor-operated fan produces atomization. These devices may be hand-held or portable and are powered by batteries.

(5) There are several brands/models available for each method described above. Generally, each equipment company develops its own proprietary nozzle design to ensure the company's uniqueness. There are many references comparing proprietary nozzles. The label requirement for droplet spectra, however, is what determines whether a nozzle meets standards.

7. EQUIPMENT FOR MILITARY USE

a. General Considerations. Since the initial acceptance of the ULV method for insect control, design and construction of commercial and military dispersal devices have been in states of constant change. The first generation of ULV dispersal units required application methods based on rather strict insecticide temperature conditions and vehicle speeds. Although it is beyond the scope

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of this guide to describe the history of ULV equipment development, it should be noted that the military has been instrumental in promoting significant improvements. Current military specifications reflect these developments for large engine-operated and hand-held equipment. Commercial competition and field requirements generated by civilian and military agencies have stimulated innovations in equipment design that have simplified control operations and allowed greater latitude in application methods independent of speed and temperature requirements.

b. Tables 2 and 3 at the end of this section summarize the equipment specifications used for cold aerosol and thermal fog applications (stock and non-stock). Appendix D includes summaries of current testing and evaluation results for cold aerosol and thermal fog application equipment using a variety of ULV insecticides that are oil-, diesel- and water-based.

c. Equipment for Contingency Operations. Adulticiding equipment for use by military units in contingency or combat operations requires unique features. Equipment must be simple to operate and maintain, lightweight and portable, generate low noise levels, utilize a variety of pesticide formulations, and atomize effectively. Battery/electric-powered units often fulfill these requirements and have been accepted by the mosquito control community in the last decade. Both hand-held and truck-mounted units are available. Consult the DoD Pest Management Material Other Than Pesticides list on the AFPMB website for further information and images of the below listed items.

d. Standard Stock ULV Generators.

(1) Dispensers procured for standard stock consumption are supplied as complete units with operating manuals and are ready for use. Spare parts kits are generally included with the sprayer, but may be procured by military or open purchase procedures if needed. Operating and maintenance procedures should be thoroughly studied before attempting operations. Units dependent on liquid temperature should be initially calibrated for the range of their expected use in five-degree increments, usually 60 to 100°F. To ensure maximum effectiveness and minimize paint damage, droplet samples should be taken and validated prior to machine use.

(2) ***Vehicle-transported*** **electric, gasoline or diesel engine powered equipment**.

(a) *Fog Generator, Skid Mounted, gasoline engine driven,* ***Grizzly PDS*** (NSN 3740-00-375-9154). 53" lg. X 31" wi X 37" ht, wt 456 lb. empty, 18 horsepower engine, positive displacement blower w/6 psi max pressure, 5 gallon polystyrene pesticide tank. View Product Image and Specifications.

(b) *Sprayer, Pesticide, Skid Mounted,* ***London Fog ULV XKD*** *Diesel Sprayer* (NSN 3740-01-525-7453). HP 7, Flow Rate0-10 oz/min, Wt: 271 lbs. View Product Image and Specifications.

(c) *Sprayer, Pesticide, Skid Mounted,* ***London Fog ULV XKE*** (NSN 3740-01-076-1341). Gas Sprayer 8 HP, Flow Rate 0 to 18 oz/min, Wt: 280 lbs. View Product Image and Specifications.

(d) *Sprayer, Pesticide, Skid Mounted, Model* ***Pro Mist, ULV*** **15MP** (NSN 3740-01-206-9635). Atomization is accomplished by a motor-driven porous polyethylene sleeve and blower

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combination. Insecticide is supplied to the sleeve by a motor-driven constant volume pump delivering a maximum of 6 fluid ounces per minute. A remote module controls all dispersal and flow system cleaning functions through a series of switches. Vehicle wiring kit (PN A4006), 12V DC powered. Wt: 95 lbs. View Product Image and Specifications.

(e) *Insecticidal Aerosol Generator, Ultra Low Volume,* ***AGULVE****, Sprayer, Pesticide, Skid Mounted, Model Pro Mist* (NSN 3740-01-445-8380). With aluminum frame, ULV, DC driven, wt 95 lb., includes vehicle wiring installation kit (PN A4006), and repair parts kit (PN A0532.2). ***Note****: This is an older Beecomist model still in the DoD inventory.* View Product Image and Specifications.

(f) *Aerosol Generator, Pesticide, Skid Mounted, Curtis Dyna Model* ***MAXI-PRO 4P*** (NSN 3740-01-141-2557). 18 HP, Flow rate: 0-40 oz/min, 15 gallon tank, 48" X 41" X 35", wt 495 lbs. View Product Image and Specifications.

(g) *Sprayer, Pesticide, Skid Mounted, London Fog,* ***M.A.G.*** (Medium Area Generator) (NSN 3740-01-548-9102). HP 3.5 HP, Flow Rate 0-10 oz/min, Wt: 110 lbs. View Product Image and Specifications.

(h) Sprayer, Pesticide, Skid Mounted, Curtis Dyna-Fog **Dynajet L-30** (NSN 01-495-0914). Wt 105 lb, includes spare parts kits and battery. View Product Image and Specifications.

(i) *Sprayer, Pesticide, Skid Mounted, RD* ***Terminator Diesel*** *ULV Sprayer* (NSN 3740-01-525-7600). HP 4.7, Flow Rate 3-6 oz/min, Wt: 125 lbs. View Product Image and Specifications.

(j) *Fog Generator, Insecticidal,* ***Silver Cloud*** *Model 2650* (NSN 3740-01-535-8884). ***Thermal fogger*** with twin pulse engines. Attachments are provided for connecting to a suitable drum such as a 55-gal (2001) drum, which is not provided. Remote Control box included with Model 2650. L: 68" X 245" wide x 22" high, wt 106 lbs empty. View Product Image and Specifications.

(k) *Aerosol Generator, Pesticide, Skid Mounted, Curtis Dyna-Fog* ***Maxi Pro4***. (There is no NSN for this ULV machine but it may still be available in some Air Force deployable team kits). This heavy-duty unit employs a positive displacement pump. Operation can be controlled from the truck cab with a remote device that provides flow control, flushing control, and a fault indicator. 18 HP, Flow Rate 0-20 oz/min, Wt: 556 lbs.

(3) ***Hand-held*** **Standard Stocked ULV Generators for Contingency Operations**. Several units have been accepted for use by the Services for contingency operations and are currently listed on some or all of the military services authorized materials allowance list. These are:

(a) *Fog Generator, Manually Carried, Curtis Dyna Model 2610* ***Golden Eagle*** (NSN 3740-00-818-6648). ***Thermal fogger.*** Gasoline engine driven, thermal fog, provides indoor/outdoor thermal fog for mosquito and fly control, 52" X 9.5" X 25.5", wt 19 lbs., 1

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gallon pesticide tank. Flow rate - 5 GPH at 6 psi, adjustable shoulder strap included. View Product Image and Specifications.

(b) *Fogger, Hand Held, Gasoline Engine Driven, thermal fogger, London Fog* ***Eliminator*** (NSN 3740-01-456-2625). ***Thermal fogger*** PN# 8100, 25" long X 20 ½" high X 10 ½" wide, 24 lbs. empty wt. Flow rate: 5-6 GPH. View Product Image and Specifications.

(c) *Fogger, Hand Held, Gasoline Engine Driven, ULV, London Aire* ***Colt*** (NSN 3740-01-456-2622). PN# 8675, 14" long X 11" wide X 12" high. Flow rate: 0-4 oz/min, Wt: 19 lbs. View Product Image and Specifications.

(d) *Fogger, Hand Held, Gasoline Engine Driven, ULV, Clarke* ***P-1*** (NSN 3740-01-456-2623). Flow rate: 0-4 oz/min, Wt: 17 lbs. PN# L7800-001. ***NOTE***: *Hoffman et al. (2001) found this sprayer to produce droplet sizes outside the true ULV range and should be used only if other more effective sprayers are not available.* View Product Image and Specifications.

(e) *Sprayer Manually Carried, DC powered (rechargeable battery),* ***Ulvafan****, Dram Model MK2* (NSN 3740-01-206-9636). Flow rate: 60 ml/min at 3 PSIG. Wt: 3.25 lbs. This electric powered hand-held device is configured to disperse aerosols and mists. It is powered by a rechargeable 12-volt Gel Cell™ shoulder-carried battery pack. A spinning disc dispersal head and fan combination provide pesticide atomization. Flow rate is controlled by interchangeable gravity feed nozzles. ***NOTE:*** *Hoffman et al. (2001) found this sprayer to produce droplet sizes outside the true ULV range and should be used only if other more effective sprayers are not available*). *This model no longer has an NSN but is still available in some equipment blocks.*

(4) **Backpack Dispersal Units**. Most of the currently manufactured backpacks can disperse liquid, dust and/or granular formulations with accessory attachments. ***NOTE:*** *liquid dispersal rates and droplet sizes exceed those needed for ULV insect control (Hoffman et al. 2001), therefore these devices will provide only limited flying insect control and should therefore be used primarily for residual treatments*. One unit has been accepted for use by the military services for contingency operations and is currently authorized for use in the military services materials allowance list. This backpack sprayer is:

Sprayer-Duster, Pesticide, Backpack, ***STIHL Model SR450*** (NSN 3740-01-463-0147). Gasoline engine driven, includes granular spreader. ULV nozzle only if ordered with pressure pump kit. Discharges mist 38 ft vertical/40 ft horizontal Comes with field parts kit. Tank size – 3.5 gal., 24.6" high X 18.9" wide X 11" deep, Wt: 24 lbs. View Product Image and Specifications

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Table 2: Summary characteristics of ULV cold aerosol sprayers (Stock and Non-Stock)

Sprayer	Power Source		Nozzle type	Air Flow rate, CFM	Liquid Flow rate, oz/min		Tank Capacity gal	Fuel Capacity gal	Dimensions inch			Weight lbs
	Type	Size, hp			Min	Max			L	W	H	
Hand Held Colt ULV	2-Cycle gasoline	?	Air shear		0	4	0.25	0.25	14	11	12	19
ADAPCO Guardian 190 ES	Gasoline	19	Air shear	350	0	20	15	8.30	45	37	30	510
AST Terminator	Diesel	4.7	Air shear	12	3	6	2.5	0.65	32	34	28	125
B&G Phoenix Fogger 680 MD	Diesel	6.8	Air shear	220	1	18	15	1.25	40	34	33	605
Clark Grizzly	Gasoline	18	Air shear	350	0	18	15	9	53	31	37	475
CDF Dyna-Jet L-30	Electric	12 V DC	Rotary	1350	0	20	15	NA	44	29	39	105
CDF Maxi-Pro 4	Gasoline	18	Air shear	400	0	20	15	12.5	44	37	30	426
CDF Minilite	2-Cycle gasoline	1.8	Air shear	95	0	17	1.25	0.26	15	15	28	29
London Fog 18-20	Gasoline	18	Air shear	356	0	20	15	7.5	37	46	39	445
LF Medium Area Generator	Gasoline	6.0	Air shear		0	10	2	0.5	29	18	19	110
BS Pro-Mist ULV 15 MP	Electric	12 V DC	Rotary	500	0	6	15	NA	42	28	37	101
WM Chemical Dispersal Unit, Model G-4	Gasoline	5	Air Shear		0	6	Both	0.18 – 0.54				79

ED_002896_00040479-00015

Table 3: Summary characteristics of thermal foggers (Stock and Non-Stock).

Sprayer	Power Source		Water-based Products	Fog rate 1000 CFM	Liquid Flow rate, oz/min		Tank Capacity gal	Fuel Capacity gal	Dimensions inch			Weight lbs
	Type	Size, hp			Min	Max			L	W	H	
Hand Held												
CDF Golden Eagle	Gasoline	30	No	57	0	19.2	1.1	0.25	52	9.5	25.5	19
CDF Trailblazer	Gasoline	24	Yes	31.7	0	10.7	1.0	0.21	29	10	18	25
LF Eliminator	2 cycle Gasoline	-	No	-	10.6	12.8	1.5	.25	25	11	21	24
ST SN50	Gasoline	25	Yes	-	5.5	23.5	1.7	0.37	52	11	13	19
Truck Mounted												
CDF Silver Cloud	Gasoline	88	No	250	0	85	55*	6	68	24	22	106**
CDF Model 1200	Diesel	11	No	760	32	256	15	1.25	79	35	32	620
LF F500E	Gasoline	16	No	-	64	85	55***	1.5	33	30	24	220
ST SN101 M	Gasoline	57	Yes	-	18	68	18	1.5	70	25	13	88

*Attachments are provided to use the drum.
**Empty weight without drum.
***Optional Stainless Steel container.

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8. EQUIPMENT CALIBRATION.

a. Conducting aerosol applications for outdoor flying insect control requires specialized training by operators for the calibration, operation and maintenance of equipment to ensure compliance with label specifications, as well as to ensure proper droplet sizes are produced. Methods of calibrating flow rates for various kinds of ULV equipment are presented below, and Appendix C can be used to record equipment parameters.

b. Equipment with in-line flow meters and temperature gauges. In these cases flow rate calibration is relatively simple, requiring a stop watch and a graduated cylinder marked in tenths of an ounce or in cubic centimeters (milliliters). First, the machine is placed in operation; then, adjust the engine speed to establish proper insecticide tank pressure, and fill the insecticide lines. This may require letting some ''run out'' in order to fill the lines. Set the flow meter to an arbitrary setting (about mid scale). Remove the discharge tube from the dispersal head, and hold it at the same level. Activate the control valve to start discharge. The insecticide is allowed to flow for one minute, and the measurement is made directly in fluid ounces or milliliters per minute. For example, if the machine is operated for one minute, and the total amount of liquid discharged in this time is 128 ml, the flow rate is:

128 ml/min / 29.57 ml in 1 fl oz = 4.3 fl oz / min

If the total flow is higher or lower than the required rate at the existing temperature, flow rate or other parameters must be adjusted to establish proper application rate. Be sure to record the temperature at this setting, since the flow rate must be readjusted for any positive or negative change in temperature of 5 degrees or more.

If a machine is calibrated at a stated insecticide temperature, the machine should be used for operations under the same or nearly identical conditions, or the flow rate may differ greatly from that previously calibrated. Too little flow or output means poor control; too much output means waste, a possibility of paint damage or adverse effects on the environment. Calibration of a machine should be made periodically, usually after 25 hours of operation, or any time when major maintenance is performed. Furthermore, if a change in insecticides is made, recalibration must be performed. A calibrated graph should be plotted for high and low flow rates at temperatures between 60-100° F. For any major change in insecticides or operating conditions, a slide sample of droplets should be obtained.

c. Constant Volume Output Machines. None of the machines produced for commercial or military use have identical systems for control of liquid output. Follow the instructions provided with the equipment to determine flow rate. Although direct readout of flow may be indicated, its accuracy should be checked by the method in (b) above at some ambient temperature within the expected machine operating range.

d. Gravity Flow Machines. Hand-held equipment may employ gravity feed systems using interchangeable orifices for establishing flow rate. For public health ULV operations, usually the smallest orifice provided will produce the desired flow. A large beaker may be used to collect the liquid for transfer to a graduated cylinder for accurate measurement. With the smaller hand-held machines, calibration may offer more technical

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difficulty. Calibration in these cases may require that someone time an application as the operator sprays over a certain area.

e. Sprayers with Fixed Discharge Tubes. Use an accurate liquid level mark and fill the tank to this level before a calibration run. Then measure the amount required to refill to the same level after the run. The boom and lines should be full before and after operation. For accurate measurement the sprayer should be in exactly the same position (preferably level) before and after operation. The volume required to refill and the time of run will determine the flow rate.

9. ULV INSECTICIDES

a. General Considerations.

(1) Although a number of insecticides are labeled for outdoor dispersal, relatively few are needed for military use. Currently available standard stock insecticides will satisfy most requirements. Occasionally non-standard pesticides may be needed to overcome a resistance problem, meet specific legal requirements or satisfy environmental constraints. Also, where the odor of the pesticide is offensive and cannot be tolerated, substitution may be desirable.

(2) Operational application factors must also be considered, and all are equally important. These are: (1) application rate required, (2) swath width, (3) flow rate and vehicle speed and (4) particle size produced by the machine.

b. Standard Stock Insecticides for ULV Ground Dispersal.

(1) *Malathion* (NSN 6840-00-655-9222, 57%, 1gal can), (NSN 6840-00-685-5438, 5 gal can) (*Fyfanon*), (6840-00-926-1481, 96.5%, 54 gal drum) and (6840-01-169-1842, 96.5%, 5 gal can). This is the most commonly used organophosphate in the United States for ULV ground dispersal. Advantages include demonstrated effectiveness against many pest and vector species and its relatively low mammalian toxicity. However, when applied in droplets much larger than those specified in the label, this compound has the ability to damage acrylic-based automobile paints. Machine adjustment must therefore be exact, and application rates followed carefully. Read the Label for specific speeds and rates of application allowed. Links for Malathion: MSDS. Label; Fyfanon: MSDS. Label.

(2) *Permethrin (Kontrol 4-4) with piperonyl butoxide 4.6+4.6%,* 2.5 gal container, (NSN 6840-01-550-5660). This pesticide can be applied as a ULV or as a thermal fog. However, this pesticide is extremely toxic to aquatic organisms. Machine adjustment must therefore be exact, and application rates followed carefully. Read the Label for specific speeds and rates of application allowed. Links: MSDS. Label.

(3) *Pyrethrins, 3% pyrethrins with synergists (ULV fog concentrate)* (NSN 6840-01-104-0780). This insecticide is most commonly used in hand held ULV machines and be applied at full strength or diluted using white mineral oil. Read the Label for specific speeds and rates of application allowed. Links: MSDS. Label.

(4) *Resmethrin (Scourge) 4% with piperonyl butoxide 12%,* 5 gal can (ready to use), (NSN 6840-01-359-8533). This insecticide is a **Restricted Use Pesticide** and must be applied by a certified applicator. The desired application rate may be obtained under different conditions by

changing the flow rate and vehicle speed. Read the Label for specific speeds and rates of application allowed. Links: MSDS. Label.

(5) *Insecticide, Sumithrin with Piperonyl Butoxide, 10%-10%, (Anvil 10+10),* 2.5 gal container, (NSN 6840 01-474-7751). Caution should be used when applying this insecticide it is toxic to fish and bees. The desired application rate may be obtained under different conditions by changing the flow rate and vehicle speed. Read the Label for specific speeds and rates of application allowed. Links: MSDS. Label.

(6) *Insecticide, (Zenivex E20),* 2.5 gal container, (NSN 6840-01-573-4964). This new reduced risk insecticide with Etofenprox, a new active ingredient, was added to standard approved list in Feb 08. Read the Label for specific speeds and rates of application allowed. Links: MSDS. Label.

(7) For Aerial Application, Naled (Dibrom 87.4%™) (NSN 6840-01-270-9765), (*Trumpet EC 78%™*) (NSN 6840-01-532-5414). These two insecticides are extremely corrosive and require application equipment to be corrosion-resistant. These insecticides are standard pesticides used by the AF Aerial Flight Squadron. Links: MSDS. Label.

c. Non-standard Pesticides for ULV Dispersal. Several pyrethroid compounds with synergists are available with approval by major command professionals.

d. Droplet parameters within the aerosol range are specified on the label for each insecticide used for ULV-aerosol dispersal. Table 4 summarizes the label specifications for ULV insecticides used for mosquito control where mandatory droplet sizes are less than 50 μm for 90% of the spray volume.

e. *Label Specifications for Common ULV Insecticides.*

(1) There are certain standards set by federal and state agencies and chemical companies for the performance of a machine and a given pesticide. These standards, as part of the label specifications, must be met so that effective control can be achieved and hazards to personnel and property minimized.

(2) Portions of label specifications for the commonly used standard stock pesticides are quoted below as general guidelines. Before attempting operations or droplet assessment, read the complete label to ensure best results.

(3) *Fyfanon ULV*

(a) 50% of the total spray mass must consist of droplets in the 6 to 18 micron range to achieve adequate dispersal of insecticide over the 300-foot swath. Smaller droplets do not impinge readily on adult mosquitoes.

(b) Droplets should not exceed 32 microns in size. Up to 3% can exceed 32 microns provided the VMD does not exceed 17 microns and no droplets exceed a maximum of 48 microns.

(c) A minimum of two-thirds, preferably four-fifths, of the total spray mass must consist of droplets not exceeding 24 microns in diameter.

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(d) The mass median diameter should not exceed 17 microns.

(e) When Fyfanon droplets are collected on silicone coated slides, the spread factor, if not actually computed for an individual slide, is 0.5.

(f) Sampling distance should be 25 feet from the point of discharge.

(g) Silicone- or Teflon-coated slides may be used for sampling.

(4) *Kontrol 4-4 Formulations.*

(a) Droplets should be produced in the range of 5 - 30 microns.

(b) The volume median diameter produced should be less than 30 microns.

(c) Sampling distance as per ULV machine manufacturer manual.

(d) Silicone coated slides may be used but Teflon® is preferable.

(5) *Anvil 10+10 Formulations.*

(a) Volume median diameter of the droplets produced must be adjusted to produce a droplet size between 8 to 30 microns and 90% of the spray mass must be no larger than 50 microns.

(b) Sampling distance as per ULV machine manufacturer manual.

(c) Silicone-coated slides may be used but Teflon is preferable.

(6) *Resmethrin (Scourge).*

(a) For use in non-thermal backpacks when the material is diluted with oil, the VMD should be 18-50 microns. The label should be consulted for dilution and application rates.

(b) For ground equipment application the volume median diameter of the droplets produced must be adjusted to produce a droplet size between 8 to 30 microns and 90% of the spray mass no larger than 50 microns.

(c) ***Note: these figures are for ground ULV applications, NOT AERIAL applications. Aerial VMD requirements for each chemical are significantly different.***

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Table 4. Summary of ULV Insecticides (Stock and Non-Stock)

Insecticide	Active Ingredient		AI (lbs/gal)	ULV	Thermal Fog	Droplet size Range, µm	VMD µm	Drop Size, Upper limit µm	Diluted or undiluted	Dose Rate		Wind Range, mph
	Name	%								Formulation, Fl Oz/acre	Active Ingredient, lbs/acre	
Fyfanon ULV Mosquito	Malathion	96.5	9.9	Yes	Yes	90 % < 32	<17		Both	0.33 – 4.0	0.0255 – 0.3094	
Dibrom Concentrate	Naled	87.4		Yes	Yes				Both	0.1 – 0.2	0.01 – 0.02	
Trumpet EC	Naled	78		Yes	No	11-20	<15	50	UD	0.25	0.02	
Kontrol 4 4	Permethrin	4.6	0.3344	Yes	Yes	90 % < 48	<30		Both	0.67 – 2.67	0.00175 – 0.007	1 – 10
	Piperonyl Butoxide	4.6	0.3344									
Scourge	Resmethrin	4.14	0.3	Yes	No	90 % < 50	<30		Both	0.5 – 3.0	0.00117 – 0.0070	> 1
	Piperonyl Butoxide	12.42	0.9								0.00351 – 0.0210	
Prentox® Pyronyl™ Oil OR 3610-A	Pyrethrins	3		Yes	No				Both	1.32 – 1.65	0.00210 – 0.0027	≈ 5
	Piperonyl Butoxide	6									0.0043 – 0.0054	
	N-octyl bicycloheptene dicarboximide	10									0.0071 – 0.0089	
ULD BP-300	Pyrethrins	3		Yes	Yes				D	4.0	0.0064	
	Piperonyl Butoxide	6									0.0129	
	N-octyl bicycloheptene dicarboximide	10									0.0216	
Anvil 10 + 10 ULV	Sumithrin	10	0.74	Yes	Yes	90 % < 50	8 – 30		Both	0.21 – 0.62	0.0012 – 0.0036	> 1
	Piperonyl Butoxide	10	0.74								0.0012 – 0.0036	
Zenivex E20	Etofenprox	20	1.48	Yes	Yes	90 % < 50	10 – 30		Both	0.15 – 0.59	0.00175 – 0.007	1 – 10
Anvil 2 + 2 ULV	Sumithrin	2	0.14	Yes	Yes	90 % < 50	8 – 30		UD	1.08 – 3.25	0.0012 – 0.0036	>1
	Piperonyl Butoxide	2	0.14								0.0012 – 0.0036	
Acquahalt	Pyrethrins	5		Yes	No	90 % < 50	< 30		UD	0.28 – 0.76	0.0009 – 0.0025	
	Piperonyl Butoxide	25									0.0045 – 0.0125	
AcquaAnvil	Sumithrin	10	0.847	Yes	No	90 % < 50	8 – 30		Both	0.18 – 0.54	0.0012 – 0.0036	
	Piperonyl Butoxide	10	0.847								0.0012 – 0.0036	
Biomist 4 + 4 ULV	Permithrin	4	0.3	Yes	Yes	90 % < 50	8 – 30		UD	0.75 – 3.0	0.00175 – 0.0070	
	Piperonyl Butoxide	4	0.3								0.00175 – 0.0070	
Duet	Prallethrin	1	0.085	Yes	No	90 % < 50	8 – 30		Both	0.41 – 1.24	0.00030 – 0.0008	
	Sumithrin	5	0.37								0.0012 – 0.0036	
	Piperonyl Butoxide	5	0.37								0.0012 – 0.0036	
Flint 10EC	Permithrin	10		Yes	No				D	0.29 – 1.16	0.0016 – 0.0064	
ULV Mosquito Master 412	Chlorpyrifos	12	0.9	Yes	No	90 % < 50	8 – 30		UD	0.74 – 1.42	0.0050 – 0.0100	> 1
	Permithrin	4	0.3								0.0017 – 0.0030	
Mosquitomist One ULV	Chlorpyrifos	13.6	1	Yes	Yes	90 % < 50	8 – 30		Both	0.64 – 3.70	0.0050 – 0.0289	

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10. APPLICATION PARAMETERS FOR ULV INSECTICIDES

a. **General Considerations**. Any computation of application rate is based upon the area to be treated, insecticide concentration, dispersal unit flow rate and speed at which it moves. Depth of treatment (swath width) and travel speed must be established to enable computation of the flow rate. Dosage is dictated by labels in either fluid ounces or pounds of technical concentrate (actual) per acre. In reality what is being treated is a volume of air above the surface of the earth, called the "space" or "insect zone."

b. **Swath Width**. Perhaps no other single factor in the computation of dosage rate receives so much discussion as the meaning of the term "swath" or "effective swath width." Many arbitrary definitions have been given to this term. The recommendations of the manufacturer or governmental research activities will be acceptable as guidelines. In practice, the operator is governed by the actual area being treated. In addition, applicators may not be able to apply insecticide in the exact manner recommended due to the physical layout or geography of the area. As long as the insecticide is dispersed evenly at recommended dosage rates in more or less equal swaths across the area to be treated, the control procedure will be effective. According to published information, the swath width determined for ULV applications is 300 ft, which is adequate for all but the most densely vegetated areas. Historically, this 300 ft interval came from the center of one road to the center of the next road, demarcating a city block. In early tests, treatment of that area with insecticide resulted in 80% mosquito mortality. Effective control was set at or above 90% in the extensive review by Mount (1998). Effective swath width is the distance at which "effective" control is obtained. Generally, an effective swath width is determined by experimentation and is accomplished by one of two methods: direct evaluation of kill of exposed caged insects, or reduction in landing rate on humans where a natural vector population occurs. Swath width is generally included on the insecticide label. If not, then tests should be conducted to determine the distance at which 90% control is achieved. These tests are highly variable in nature, requiring sufficient replications to achieve an acceptable statistical level, and may require more time than the average mosquito control district or military operations spray team may have..

The Major Command Pest Management Consultant/Entomologist and distributor/formulator should be able to address questions concerning swath width. Once the swath width has been established and a firm recommendation has been made by a company or agency, the operator should make every effort to adhere to the recommendation for swath spacing, vehicle speed, and output to accomplish effective control.

c. **Application Rate**.

(1) General Considerations. Each insecticide has different physical and chemical properties, and biological effectiveness. Therefore, different dosage rates must be established for specific control situations or species involved. The labels generally give a range of application rates or a table of rates with different rates for various scenarios (see Table 4). It is generally given as the weight of active ingredient per unit volume of formulated insecticide. The pest management professional or applicator need to decide what application rate would be appropriate for the specific treatment. The volume of formulated pesticide is also given on the label. If it is not, it can be calculated from the concentration of active ingredient in the formulation (lbs/gal).

(2) **Application Rate Calculations**.

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For most ULV applications the effective swath is 300 ft, and practical vehicle speeds are usually set at either 5 or 10 mph.

Formulation Application Rate (fluid oz/acre), if not given on the label = *Active ingredient rate (lbs/acre) x 128 ÷ Concentration of Active Ingredient in Formulation (lbs/gal).*

Flow Rate (fluid oz/min) = *Vehicle Speed (mph) x Effective Swath (ft) x Application Rate (fluid oz/acre) ÷ 495*

or, if the Application Rate is in gal/acre or L/acre, the flow rate will be in gal/min or L/min, respectively.

Example Using Fyfanon:

The label gives flow rate in fluid oz/min for vehicle speeds of 5, 10, 15, and 20 mph. These flow rates are given for a swath of 300 ft. For non-agricultural lands, it gives a range of application rates from 2-4 fluid oz/acre. The above equation will help to find the flow rate.

Flow rate = 10 mph x 300 ft. swath x 2 fl. oz./acre ÷ 495 conversion factor = 12.12 fl. oz/min.

For an application scenario, it might not be possible to set the required flow rate (due to machine limitations), vehicle speed or swath width. In these cases, pick the two parameters that you can control and calculate the third parameter accordingly:

Vehicle speed and swath width fixed → use above equation for flow rate

Vehicle speed and flow rate fixed:

Swath width (ft) = *[495 x Flow rate (fluid oz/min)] ÷ [Vehicle Speed (mph) x Application rate (fluid oz/acre)]*

Swath width and Flow rate fixed:

Vehicle Speed (mph) = *[495 x Flow rate (fluid oz/min)] ÷ [Swath width (ft) x Application rate (fluid oz/acre)]*

Determination of acres sprayed/hour. It has been established that the effective swath for ULV Fyfanon applications is 300 ft, and practical vehicle speeds are either 5 or 10 mph. Therefore:

Acres covered per hour = *Vehicle speed (mph) x 5280 (ft. in 1 mile) x swath in feet/43,560 (sq. ft in 1 acre)*

For a 5 mph rate: 5 x 5280 x 300/43560 = 181.8 acres/hour.

For a 10 mph rate, this figure would be doubled, or 363.6 acres/hour.

The established flow rate for 5 mph is a maximum of 2.1 fluid ounces per minute or for one hour is 60 x 2.1 = 126 fluid ounces per hour. Therefore 126/181.8 = 0.6931 fluid oz/ac.

The stated weight of technical Fyfanon per gallon is 10.25 pounds per gallon. One fl oz will weigh: 10.25/128 (fl oz in gal) = 0.08 pounds per fluid ounce. Therefore, the deposit rate per acre

is 0.08 x 0.6931 or 0.055 lbs/ac deposit of technical (actual) Fyfanon. For 10 mph, and a maximum flow rate of 4.3 fl oz/min, the application rate is:

(60 x 4.3)/363.6 = 0.71 fluid ounces per acre, or a deposit of 0.08 X 0.71 = 0.0568 pounds per acre deposit of technical Fyfanon.

Simpler procedures can be used to determine the same figures as above. An effective method of determining the acreage covered per minute is:

Speed (mph) x swath (ft.) / 500 = acres covered per minute

For example, a speed of 5 mph with a 300 foot swath gives

5 x 300 / 500 = 3 acres per minute. For a maximum flow rate of 2.1 fl oz/min, the deposit rate per acre is 2.1 / 3 = 0.7 fl oz/ac, or a deposit of 0.08 x 0.7 = 0.056 lbs/ac deposit of technical Fyfanon™. The deposit rate for a 10 mph speed is 10 x 300 / 500 = 6 acres per minute when the flow rate is doubled, and deposition rate is the same 0.056 lb/acre.

11. PRACTICAL CONSIDERATIONS FOR ULV OPERATIONS

a. Although most mosquito populations can be effectively managed by ULV insecticide application, exceptions occur. The target species' biology and flight habits, climatic conditions and the physical environment that affect aerosol cloud dynamics, and other variables combine in complex ways to determine final insecticide efficacy. Some mosquito species have particular activity patterns unique to them. Therefore, using space sprays to control them must occur not only at the right place but at the right time. ***It is crucial to review the biology and activity patterns for each species targeted to determine the best time and place to conduct space spray operations***.

b. Application timing. Schmidt (2003) summarized periods of peak activities for eight common mosquitoes in New Jersey. With this kind of information, we can determine the best time for fogging operations. For example, regardless of time of day in New Jersey, *Oc. trivittatus*, *Oc. canadensis* and *Oc. sollicitans* should be sprayed 30 minutes *before* sunset, *Culex spp.* and *Coquilettidia perturbans* should be sprayed 30 minutes *after* sunset, and sprays to target *Ae. vexans* and *Ochlerotatus cantator* should be made *exactly at sunset* to achieve the best control.

c. Urban physical factors affecting application efficacy. Different species may occur in habitats that negatively affect aerosol dynamics and hinder aerosol clouds from reaching the target. In controlling *Aedes aegypti*, for example, the adult mosquito rests in heavily sheltered sites indoors when not feeding or ovipositing (Reiter 1993). Targeting the mosquito in such indoor locations is largely ineffective using aerial or vehicle-mounted fogging methods because the spray cloud may be physically blocked by windows, vegetation, brick walls and the houses themselves that cause convection currents that interfere with spray cloud dispersion and penetration. Such conditions hamper effective control methods and therefore require additional efforts. Therefore, for controlling urban day-biting vectors like *Ae. aegypti* and *Ae. albopictus*, aerosol applications via vehicle-mounted equipment as well as house-to house indoor applications using hand-held fogging equipment should be made for the best chances of control (Matthews 1996; Mani et al. 2005; Perich et al. 1990, 1992; Reiter, 1993). The use of maximum label rates are also likely necessary to compensate for factors that make control in such urban environments extremely difficult.

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d. Terrain and vegetation type will greatly influence the requirements for droplet size and the ultimate effectiveness of an application. In open terrain with relatively sparse vegetation, greater effective swaths can be obtained. As vegetation density increases, less insecticide penetration occurs. This 'insecticide penetration' factor must be considered for each environment.

e. Wind speed has a profound influence on particle distribution and impingement. For most operations, wind speeds from 1 to 10 miles per hour (mph) will produce the best results. However, data indicate that wind speeds in excess of 10 mph may markedly increase penetration of insecticide into vegetation. Observations of the spray cloud must be made to determine if air flow is carrying the spray cloud into, over, or around the desired target vegetation.

d. Additional Practical Information

The following references/links provide additional information to consider when thermal fog and cold aerosol application methods are to be used to control public health pests.

(1) The World Health Organization's publication entitled "Space spray application of insecticides for vector and public health pest control" (WHO 2003) was co-authored by the Navy Entomology Center in Jacksonville where extensive equipment testing and evaluation is regularly performed. This document and TG 13 complement each other very well to provide excellent references for space spray operations. The link to this document is provided here:

(2) Allman A. E. The Use of Fog Generators in Integrated Vector Control: Thermal Fog & Cold Fog (ULV) Generators. Allman-Sprayers Ltd web site.

(3) William F. Lyon and J. A. Steele. 1998. Mosquito Pest Management, Bulletin 641. The Ohio State University.

(4) The following abstract is reproduced from Mount (1998) and provides very good information on ground ULV application methods and equipment (not otherwise available without internet access).

This review of ultralow-volume (ULV) ground aerosols for adult mosquito control includes discussion on application volume, aerosol generators, droplet size, meteorology, swath, dispersal speed, assay methods, insecticide efficacy, and non-target effects. It summarizes the efficacy of ULV insecticidal aerosols against many important pest and disease-bearing species of mosquitoes in a wide range of locations and habitats in the United States and in some countries of Asia and the Americas. Fourteen conclusions were drawn from the review. 1) ULV ground aerosol applications of insecticide are as efficacious against adult mosquitoes as high- or low-volume aerosols. 2) ULV aerosols with an optimum droplet size spectrum can be produced by several types of nozzles including vortex, pneumatic, and rotary. Droplet size of a particular insecticide formulation is dependent primarily on nozzle air pressure or rotation speed and secondarily on insecticide flow rate. 3) Label flow rates of insecticide for ULV aerosol application can be delivered accurately during routine operations with speed-correlated metering systems within a calibrated speed range, usually not exceeding 20 mph. 4) The most economical and convenient method of droplet size determination for ULV aerosols of insecticide is the waved-slide technique. 5) The efficacy of ULV ground aerosols against adult mosquitoes is related to droplet size because it governs air transport and impingement. The optimum droplet size for mosquito adulticiding

is 8-15 microns volume median diameter (VMD) on the basis of laboratory wind-tunnel tests and field research with caged mosquitoes. 6) In general, ULV aerosols should be applied following sunset when mosquitoes are active and meteorological conditions are favorable for achieving maximum levels of control. Application can be made during daytime hours when conditions permit, but rates may have to be increased. The critical meteorological factors are wind velocity and direction, temperature, and atmospheric stability and turbulence. 7) Maximum effective swaths are obtained with aerosols in the optimum VMD range during favorable meteorological conditions in open to moderately open terrain. The insecticide dosage must be increased in proportion to increased swath to maintain the same level of mosquito control. 8) Dispersal speed within a range of 2.5-20 mph is not a factor affecting efficacy if insecticide rate and optimum droplet size are maintained. 9) The results of caged mosquito assays are comparable with reductions in free-flying natural populations. 10) The field efficacies of mosquito adulticides applied as ULV ground aerosols are predictable from the results of laboratory wind-tunnel tests. 11) Results of field tests in open to moderately open terrain during favorable meteorological conditions indicated that ULV insecticidal aerosol application rates producing 90% or more control of Anopheles, Culex, and Psorophora spp. are below or approximately equal to maximum United States Environmental Protection Agency label rates. Against some Aedes spp., some pyrethroid insecticides must be synergized to produce 90% control at label rates. 12) Results of field tests in residential areas with moderate to dense vegetation and in citrus groves or other densely wooded areas showed that insecticide rates of ULV ground aerosols must be increased 2-3-fold to obtain 90% or more control of adult mosquitoes. However, the maximum rates on some insecticide labels would have to be increased to allow higher application rates. 13) Applications of ULV ground aerosols of insecticide in accordance with label directions following sunset do not pose a serious threat to humans, non-target beneficial animals, or automotive paints. 14) Some aerosol generators operated at high RPM levels exceed the OSHA 8-h hearing hazard criteria of 90 dBA and may require hearing protectors for operators.

The following tables from Mount (1998) provide valuable information on insecticide flow rates, application rates and vehicle speeds for the effective control of mosquitoes under normal conditions.

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Table 6. Rates of ultralow-volume (ULV) ground aerosols of 91–96% malathion (Cythion®, Fyfanon®) for 90% or more control of adult mosquitoes.

Species	Location	Habitat	Aerosol generator	Speed[1] (mph)	Flow rate[2] (fl. oz./min)	Rate[3] (lb. AI/acre)	Reference
Aedes taeniorhynchus (Wied.)	Florida	Open field	Curtis 55,000[4]	5	0.63	0.061 c	Mount et al. (1968)
Ae. taeniorhynchus	Florida	Open field	Leco ULV	10	6.02	0.076 c	Mount et al. (1970b)
Culex quinquefasciatus Say	Texas	Open field	Leco ULV	10	1.58	0.020 c	McNeill and Ludwig (1970)
Ae. taeniorhynchus	Florida	Open field	Leco ULV	10	2.00	0.025 c	Mount and Pierce (1971)
Aedes aegypti (Linn.)	Thailand	Indoors	Leco ULV	3	4.00	0.326 n[5]	Pant et al. (1971)
Ae. taeniorhynchus	Georgia	Residential	Leco ULV	10	3.77	0.048 c	Taylor and Schoof (1971)
Anopheles albimanus (Wied.)	Georgia	Residential	Leco ULV	10	2.37	0.030 c	Taylor and Schoof (1971)
Cx. quinquefasciatus	Georgia	Residential	Leco ULV	10	4.69	0.059 c	Taylor and Schoof (1971)
Ae. taeniorhynchus	Florida	Residential	Leco ULV	5	1.35	0.034 c	Rathburn and Boike (1972a)
Culex nigripalpus Theobald	Florida	Residential	Leco ULV	5	3.01	0.076 c	Rathburn and Boike (1972a)
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	5.63	0.071 c	Mount and Pierce (1972b)
Psorophora columbiae (Dyar and Knab)	Arkansas	Residential	Leco HD-ULV	10	3.00	0.038 c,n	Mount et al. (1973)
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	4.76	0.060 c	Mount et al. (1974c)
Ae. taeniorhynchus, *Aedes sollicitans* (Walker)	N. Carolina	Residential	Leco HD-ULV	10	4.30	0.054 n	Axtell and Dukes (1974)
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	4.93	0.062 c	Mount et al. (1975b)
Anopheles quadrimaculatus Say	Florida	Open field	Leco HD-ULV	10	4.22	0.053 c	Mount et al. (1975b)
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	4.07	0.051 c	Mount et al. (1975c)
Ae. taeniorhynchus	Florida	Open field	Micro-Gen MS	10	4.65	0.059 c	Mount et al. (1975c)
An. quadrimaculatus	Florida	Open field	Leco HD-ULV	10	2.40	0.030 c	Mount et al. (1975c)
An. quadrimaculatus	Florida	Open field	Micro-Gen MS	10	3.80	0.048 c	Mount et al. (1975c)
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	12.85	0.162 c	Mount and Pierce (1976)
Ae. taeniorhynchus	Florida	Open field	Lond. Aire XW	10	15.15	0.191 c	Mount and Pierce (1976)
An. quadrimaculatus	Florida	Open field	Leco HD-ULV	10	9.30	0.117 c	Mount and Pierce (1976)
An. quadrimaculatus	Florida	Open field	Lond. Aire XW	10	9.83	0.124 c	Mount and Pierce (1976)
Ae. taeniorhynchus	Florida	Residential	Various[6]	10	2.13	0.027 c	Rathburn and Boike (1977)
Cx. nigripalpus	Florida	Residential	Various[6]	10	2.13	0.027 c	Rathburn and Boike (1977)
Ae. taeniorhynchus	Florida	Residential	Leco HD-ULV	10	1.40	0.017 c	Rathburn and Boike (1981)
Cx. quinquefasciatus	Florida	Residential	Leco HD-ULV	10	2.10	0.025 c	Rathburn and Boike (1981)
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	2.00	0.024 c	Roberts (1982)
An. quadrimaculatus	Florida	Open field	Leco HD-ULV	10	2.00	0.024 c	Roberts (1982)
An. quadrimaculatus	Arkansas	Open field	Leco HD-ULV	10	3.00	0.036 c	Walker and Meisch (1982)
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	1.24	0.015 c	Roberts (1983)
An. quadrimaculatus	Florida	Open field	Leco HD-ULV	10	0.84	0.010 c	Roberts (1983)
Ae. aegypti	Dom. Rep.	Indoors	Leco HD-ULV	5	9.00	0.218 c[7]	Perich et al. (1990)
Ae. taeniorhynchus	Florida	Residential	Leco HD-ULV	10	2.10	0.025 c	Floore et al. (1991)
Cx. quinquefasciatus	Florida	Residential	Leco HD-ULV	10	2.10	0.025 c	Floore et al. (1991)
Ae. aegypti	Malaysia	Residential	Leco HD-ULV	5	3.01	0.076 c[8]	Vythilingam and Panart (1991)

[1] A speed of 10 mph is indicated when a range of speeds (2.5–20 mph) was used to vary insecticide dose (1 mph = 1.609 km/h).

[2] Flow rate of technical or concentrated insecticide formulation as received from the manufacturer without diluent added by investigator (1 fl. oz./min = 29.7 ml/min).

[3] Rate based on insecticide concentration, flow rate, vehicle speed, and kill of caged (c) or reduction of natural (n) populations of adult mosquitoes, usually within 24 h, over a 300-ft. swath unless otherwise indicated (1 lb. AI/acre = 1.12 kg AI/ha).

[4] Modified for ULV application.

[5] Based on a 150-ft. swath.

[6] Includes Leco® HD-ULV, Micro-Gen® LS2-15, London Aire® XW, Buffalo Turbine® Sonic, Micro Mist®, and Tifa® 100-E-ULV.

[7] Mosquito kills of 100 and 44% outside and inside of houses, respectively.

[8] Mosquito kills of 92 and 39% outside and inside of houses, respectively.

From Mount (1998)

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Table 7. Rates of ultralow-volume (ULV) ground aerosols of chlorpyrifos, fenitrothion, fenthion, and naled for 90% or more control of adult mosquitoes.

Species	Location	Habitat	Aerosol generator	Speed[1] (mph)	Flow rate[2] (fl. oz./min)	Rate[3] (lb. AI/acre)	Reference
			Chlorpyrifos, 6 lb. AI/gal (Dursban®, Mosquitomist®)				
Culex quinquefasciatus	Texas	Open field	Leco ULV	10	1.25	0.005 c	McNeill and Ludwig (1970)
Aedes taeniorhynchus	Florida	Open field	Leco ULV	10	1.81	0.014 c	Mount and Pierce (1971)
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	2.56	0.020 c	Mount and Pierce (1972b)
Psorophora columbiae	Arkansas	Residential	Leco HD-ULV	15	3.20	0.017 n	Mount et al. (1972)
Ae. taeniorhynchus	Florida	Residential	Leco HD-ULV	10	1.05	0.008 c	Rathburn and Boike (1975)
Culex nigripalpus	Florida	Residential	Leco HD-ULV	10	1.66	0.013 c	Rathburn and Boike (1975)
Cx. quinquefasciatus	California	Open field	Military[4]	10	1.33	0.010 c	Husted et al. (1975)
Ae. taeniorhynchus	Florida	Residential	Leco HD-ULV	10	1.34	0.010 c	Rathburn et al. (1981)
Cx. nigripalpus	Florida	Residential	Leco HD-ULV	10	2.13	0.017 c	Rathburn et al. (1981)
Cx. quinquefasciatus	California	Open field	Custom built	6	2.00	0.008 c[5]	Townzen et al. (1987)
			Chlorpyrifos, 12%				
Aedes aegypti	Mexico	Open field	Leco 500	10	6.50	0.008 c	Velazquez-Quintana et al. (1995)
Cx. quinquefasciatus	Mexico	Open field	Leco 500	10	6.50	0.008 c	Velazquez-Quintana et al. (1995)
Ae. taeniorhynchus	Florida	Open field	Leco ULV	10	1.49	0.019 c	Mount and Pierce (1971)
Anopheles quadrimaculatus	Arkansas	Open field	Leco HD-ULV	10	2.22	0.029 c	Walker et al. (1981)
Ps. columbiae	Arkansas	Open field	Leco HD-ULV	10	2.22	0.029 c	Walker et al. (1981)
			Fenitrothion, 95% (Sumithion®, Accothion®)				
Ae. taeniorhynchus	Florida	Residential	Leco HD-ULV	10	2.13	0.028 c	Rathburn et al. (1981)
Cx. nigripalpus	Florida	Residential	Leco HD-ULV	10	2.13	0.028 c	Rathburn et al. (1981)
			Fenthion, 93% (Baytex®)				
Ae. taeniorhynchus	Florida	Open field	Leco ULV	15	0.95	0.008 c	Mount et al. (1970b)
Ae. taeniorhynchus	Florida	Open field	Leco ULV	10	0.97	0.012 c	Mount and Pierce (1971)
Ae. taeniorhynchus	Florida	Residential	Leco HD-ULV	10	1.07	0.013 c	Rathburn and Boike (1975)
Cx. nigripalpus	Florida	Residential	Leco HD-ULV	10	1.07	0.013 c	Rathburn and Boike (1975)
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	1.45	0.018 c	Mount et al. (1978a)
An. quadrimaculatus	Florida	Open field	Leco HD-ULV	10	0.79	0.010 c	Mount et al. (1987a)
			Naled, 85%, 14 lb. AI/gal (Dibrom®)				
Ae. taeniorhynchus	Florida	Open field	Curtis 55,000[6]	5	0.39	0.014 c	Mount et al. (1968)
Ae. taeniorhynchus	Florida	Wooded	Curtis 55,000[6]	5	0.65	0.024 n	Mount et al. (1968)
Ae. taeniorhynchus	Florida	Open field	Leco ULV	15	1.01	0.012 c	Mount et al. (1970b)
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	1.37	0.025 c	Mount and Pierce (1972b)
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	1.37	0.025 c	Mount and Pierce (1972b)
Ps. columbiae	Arkansas	Residential	Leco HD-ULV	10	1.20	0.020 n	Mount et al. (1972)
Ae. taeniorhynchus	Florida	Residential	Leco HD-ULV	10	1.81	0.033 n	Mount and Pierce (1974)

From Mount (1998)

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Table 7. Continued.

Species	Location	Habitat	Aerosol generator	Speed[1] (mph)	Flow rate[2] (fl. oz./min)	Rate[3] (lb. AI/acre)	Reference
			Naled, 85%, 14 lb. AI/gal (Dibrom®)				
Ae. taeniorhynchus	Florida	Residential	Leco HD-ULV	5	0.60	0.020 n[4]	Mount et al. (1978b)
Ae. taeniorhynchus	Florida	Residential	Leco HD-ULV	10	0.75	0.012 c	Rathburn et al. (1981)
Cx. nigripalpus	Florida	Residential	Leco HD-ULV	10	0.75	0.012 c	Rathburn et al. (1981)
Ae. taeniorhynchus	Florida	Residential	Leco HD-ULV	10	1.00	0.018 c	Rathburn et al. (1986)
Cx. quinquefasciatus	Florida	Residential	Leco HD-ULV	10	1.00	0.018 c	Rathburn et al. (1986)
Cx. nigripalpus	Florida	Residential	Leco HD-ULV	10	1.00	0.018 c	Rathburn et al. (1986)

[1] A speed of 10 mph is indicated when a range of speeds (2.5–20 mph) was used to vary insecticide dose (1 mph = 1.609 km/h).
[2] Flow rate of technical or concentrated insecticide formulation as received from the manufacturer without diluent added by investigator (1 fl. oz./min = 29.7 ml/min).
[3] Rate based on insecticide concentration, flow rate, vehicle speed, and kill of caged (c) or reduction of natural (n) populations of adult mosquitoes, usually within 24 h, over a 300-ft. swath unless otherwise indicated (1 lb. AI/acre = 1.12 kg AI/ha).
[4] Modified for ULV application.
[5] Based on 1,000-ft. swath.
[6] Forty-five-minute posttreatment reduction of 85%.

From Mount (1998)

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Table 8. Rates of ultralow-volume (ULV) ground aerosols of bendiocarb and propoxur for 90% or more control of adult mosquitoes.

Species	Location	Habitat	Aerosol generator	Speed[1] (mph)	Flow rate[2] (fl. oz./min)	Rate[3] (lb. AI/acre)	Reference
			Bendiocarb, 25%, 1.67 lb. AI/gal (Ficam®)				
Aedes taeniorhynchus	Louisiana	Open field	Micro-Gen ED	10	2.50	0.006 c	Tapley et al. (1980)
Aedes sollicitans (Walker)	Louisiana	Open field	Micro-Gen ED	10	2.50	0.006 c	Tapley et al. (1980)
Aedes aegypti	Louisiana	Open field	Micro-Gen ED	10	2.50	0.006 c	Tapley et al. (1980)
Anopheles albimanus	Louisiana	Open field	Micro-Gen ED	10	2.50	0.006 c	Tapley et al. (1980)
Culex pipiens	Louisiana	Open field	Micro-Gen ED	10	2.50	0.006 c	Tapley et al. (1980)
Culex quinquefasciatus	Louisiana	Open field	Micro-Gen ED	10	3.60	0.008 c	Tapley et al. (1980)
Aedes nigromaculis (Ludlow)	California	Pasture	Leco HD-ULV	5	2.50	0.003 n[4]	Miller et al. (1982)
Anopheles freeborni Aitken	California	Pasture	Leco HD-ULV	5	2.50	0.003 c[4]	Miller et al. (1982)
Cx. quinquefasciatus	California	Pasture	Leco HD-ULV	5	2.00	0.003 c[4]	Miller et al. (1982)
Culex tarsalis Coquillett	California	Pasture	Leco HD-ULV	5	2.00	0.003 c[4]	Miller et al. (1982)
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	4.62	0.010 c	Roberts (1984)
Anopheles quadrimaculatus	Florida	Open field	Leco HD-ULV	10	3.85	0.008 c	Roberts (1984)
Ae. nigromaculis	California	Pasture	Micro-Gen[5]	5	4.00	0.005 n[4]	Townzen et al. (1987)
Cx. quinquefasciatus	California	Pasture	Micro-Gen[5]	5	4.00	0.010 c[4]	Townzen et al. (1987)
			Propoxur, 1–2 lb. AI/gal (Baygon®)				
Ae. taeniorhynchus	Florida	Open field	Leco ULV	10	12.96	0.034 c	Mount and Pierce (1971)
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	18.88	0.025 c	Mount et al. (1975b)
An. quadrimaculatus	Florida	Open field	Leco HD-ULV	10	12.78	0.017 c	Mount et al. (1975b)
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	4.01	0.009 c	Mount et al. (1978a)
An. quadrimaculatus	Florida	Open field	Leco HD-ULV	10	2.91	0.006 c	Mount et al. (1978a)
Ae. taeniorhynchus	Florida	Residential	Leco HD-ULV	5	9.50	0.024 n[6]	Mount et al. (1978b)
An. quadrimaculatus	Arkansas	Open field	Leco HD-ULV	5	9.12	0.024 c	Walker et al. (1981)
Psorophora columbiae	Arkansas	Open field	Leco HD-ULV	5	5.79	0.018 c	Walker et al. (1981)
An. quadrimaculatus	Arkansas	Wooded	Leco HD-ULV	5	7.00	0.021 c	Walker et al. (1981)
Ae. taeniorhynchus	Florida	Residential	Leco HD-ULV	10	8.52	0.011 c	Rathburn et al. (1981)
Culex nigropalpus	Florida	Residential	Leco HD-ULV	10	8.52	0.011 c	Rathburn et al. (1981)

[1] A speed of 10 mph is indicated when a range of speeds (2.5–20 mph) was used to vary insecticide dose (1 mph = 1.609 km/h).
[2] Flow rate of technical or concentrated insecticide formulation as received from the manufacturer without diluent added by investigator (1 fl. oz./min = 29.7 ml/min).
[3] Rate based on insecticide concentration, flow rate, vehicle speed, and kill of caged (c) or reduction of natural (n) populations of adult mosquitoes, usually within 24 h, over a 300-ft. swath unless otherwise indicated (1 lb. AI/acre = 1.12 kg AI/ha).
[4] Based on 300–2,200-ft. swaths.
[5] Model not specified.
[6] Forty-five-minute reduction of 72%.

From Mount (1998)

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Table 9. Rates of ultralow-volume (ULV) ground aerosols of pyrethrins and resmethrin for 90% or more control of adult mosquitoes.

Species	Location	Habitat	Aerosol generator	Speed[1] (mph)	Flow rate[2] (fl. oz./min)	Rate[3] (lb. AI/acre)	Reference
			Pyrethrins, 4–12% plus piperonyl butoxide, 12–60% (Pyrenone®, Synerol®)				
Aedes taeniorhynchus	Florida	Open field	Leco HD-ULV	10	8.89	0.0086 c	Mount and Pierce (1972b)
Ae. taeniorhynchus, Aedes sollicitans	NC	Residential	Leco HD-ULV	10	4.00	0.0019	Axtell and Dukes (1974)
Anopheles albimanus	El Salvador	Residential	Leco HD-ULV	5	2.50	0.0024 n	Hobbs (1976)
Ae. taeniorhynchus	Florida	Residential	Leco HD-ULV	10	5.60	0.0027 c	Rathburn and Boike (1975)
Culex nigripalpus	Florida	Residential	Leco HD-ULV	10	3.30	0.0016 c	Rathburn and Boike (1975)
Aedes nigromaculis	California	Pasture	Leco HD-ULV	5	10.24	0.0057 a[4,5]	Womeldorf and Mount (1977)
Culex quinquefasciatus	California	Pasture	Leco HD-ULV	5	5.51–10.24	0.0057 c[4]	Womeldorf and Mount (1977)
Anopheles quadrimaculatus	Arkansas	Open field	Leco HD-ULV	10	3.00	0.0016 c	Walker and Meisch (1982)
Culex tarsalis	California	Pasture	Custom built	10	15.70	0.0015 c[4]	Townzen et al. (1988)
			Resmethrin, 2–3.34 lb. AI/gal (SBP-1382®)				
Ae. taeniorhynchus	Florida	Open field	Leco ULV	10	10.30	0.0268 c	Mount and Pierce (1971)
Cx. nigripalpus	Florida	Residential	Leco HD-ULV	5	0.84	0.0073 c	Rathburn and Boike (1972b)
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	16.32	0.0710 c	Mount and Pierce (1972b)
Ae. nigromaculis	California	Pasture	Leco HD-ULV	5	9.83	0.0058 a[4]	Womeldorf and Mount (1977)
Cx. quinquefasciatus	California	Pasture	Leco HD-ULV	5	9.83	0.0058 c[4]	Womeldorf and Mount (1977)
Aedes vexans (Meigen)	Minnesota	Wooded	Custom built	10	1.61	0.0070 n	Sjogren and Frank (1979)
Cx. nigripalpus	Florida	Residential	Leco HD-ULV	10	1.38	0.0036 c	Rathburn et al. (1981)
An. quadrimaculatus	Arkansas	Open field	Leco HD-ULV	5	0.80	0.0070 c	Sandoski et al. (1983)
Psorophora columbiae	Arkansas	Open field	Leco HD-ULV	10	0.80	0.0070 c	Stark et al. (1985)
			Resmethrin, 0.9–1.5 lb. AI/gal plus piperonyl butoxide, 4.5 lb. AI/gal (Scourge®, Oblique®)				
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	8.56	0.0100 c	Mount et al. (1974c)
An. quadrimaculatus	Florida	Open field	Leco HD-ULV	10	2.57	0.0030 c	Mount et al. (1974c)
Cx. quinquefasciatus	California	Pasture	Leco HD-ULV	5	8.89	0.0024 c[4]	Womeldorf and Mount (1977)
An. quadrimaculatus	Arkansas	Open field	Leco HD-ULV	5	0.90	0.0035 c	Sandoski et al. (1983)
Ps. columbiae	Arkansas	Open field	Leco HD-ULV	10	0.92	0.0018 c	Stark et al. (1985)
Aedes melanimon	California	Residential	Custom built	5	2.00	0.078 c	Townzen et al. (1987)
Cx. quinquefasciatus	California	Pasture	Custom built	5	4.00	0.0035 c[4]	Townzen et al. (1987)
Ae. taeniorhynchus	Florida	Residential	Leco HD-ULV	10	3.00	0.0059 c	Floore et al. (1991)
Cx. quinquefasciatus	Florida	Residential	Leco HD-ULV	10	3.00	0.0059 c	Floore et al. (1991)
An. quadrimaculatus	Arkansas	Open field	Leco HD-ULV	15	1.35	0.0020 c	Weathersbee et al. (1991)
An. quadrimaculatus	Arkansas	Open field	Leco HD-ULV	15	0.68	0.0010 c	Groves et al. (1994)

[1] A speed of 10 mph is indicated when a range of speeds (2.5–20 mph) was used to vary insecticide dose (1 mph = 1.609 km/h).
[2] Flow rate of technical or concentrated insecticide formulation as received from the manufacturer without diluent added by investigator (1 fl. oz./min = 29.7 ml/min).
[3] Rate based on insecticide concentration, flow rate, vehicle speed, and kill of caged (c) or reduction of natural (n) populations of adult mosquitoes, usually within 24 h, over a 300-ft. swath unless otherwise indicated (1 lb. AI/acre = 1.12 kg AI/ha).
[4] Dose based on 660–2,640-ft. swaths.
[5] Landing rate reductions were 75–76%.

From Mount (1998)

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Table 10. Rates of ultralow-volume (ULV) ground aerosols of pyrethroid insecticides for 90% or more control of adult mosquitoes.

Species	Location	Habitat	Aerosol generator	Speed[1] (mph)	Flow rate[2] (fl. oz./min)	Rate[3] (lb. AI/acre)	Reference
			Bioresmethrin, 15% plus piperonyl butoxide, 15%				
Aedes taeniorhynchus	G. Cayman	Swamp	Mod. Tifa	10	3.20	0.0027 n[4]	Brooke et al. (1974)
			Cyfluthrin, 3.2 lb. AI/gal				
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	0.02	0.0001 c	Roberts (1985)
Anopheles quadrimaculatus	Florida	Open field	Leco HD-ULV	10	0.04	0.0002 c	Roberts (1985)
			Cypermethrin, 2.48 lb. AI/gal				
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	0.44	0.0014 c	Roberts (1983)
An. quadrimaculatus	Florida	Open field	Leco HD-ULV	10	0.06	0.0002 c	Roberts (1983)
			Deltamethrin, 2%, 0.167 lb. AI/gal				
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	2.26	0.0005 c	Roberts (1982)
An. quadrimaculatus	Florida	Open field	Leco HD-ULV	10	0.09	0.00002 c	Roberts (1982)
			Fenvalerate, 2.4 lb. AI/gal				
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	1.36	0.0042 c	Roberts (1982)
An. quadrimaculatus	Florida	Open field	Leco HD-ULV	10	0.34	0.0010 c	Roberts (1982)
			Fluvalinate, 2 lb. AI/gal				
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	4.50	0.0117 c	Kline et al. (1986)
An. quadrimaculatus	Florida	Open field	Leco HD-ULV	10	0.38	0.0010 c	Kline et al. (1986)
			Lambda cyhalothrin, 2%				
Culex tarsalis	California	Open desert	Micro-Gen G-9HD	5	1.13	0.0003 c[4]	Schaefer et al. (1990)
An. quadrimaculatus	Arkansas	Open field	Leco HD-ULV	15	1.55	0.0003 c	Weathersbee et al. (1991)
			Permethrin, 2–5 lb. AI/gal (Punc®)				
Culex quinquefasciatus	Arkansas	Open field	Leco HD-ULV	10	1.21	0.0030 c	Thompson and Meisch (1977)
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	2.91	0.0076 c	Mount et al. (1978a)
An. quadrimaculatus	Florida	Open field	Leco HD-ULV	10	0.36	0.0009 c	Mount et al. (1978a)
An. quadrimaculatus	Arkansas	Open field	Leco HD-ULV	10	0.50	0.0013 c	Walker and Meisch (1982)
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	0.79	0.0037 c	Kline et al. (1986)
An. quadrimaculatus	Florida	Open field	Leco HD-ULV	10	0.21	0.0010 c	Kline et al. (1986)
Cx. quinquefasciatus	California	Pasture	Custom built	7	4.25	0.0045 c[4]	Townzen et al. (1987)
Cx. tarsalis	California	Pasture	Custom built	7	4.25	0.0045 c[4]	Townzen et al. (1987)
			Permethrin, 12–31% plus piperonyl butoxide, 20–66% (Permanone®, Biomist®, Flak®)				
Aedes sollicitans	New Jersey	Open field	Beecomist HD	5	0.50	0.0033 c	McNelly and Benzon (1995)
An. quadrimaculatus	Arkansas	Open field	Leco 1600	15	0.80	0.0007 c	Groves et al. (1994)
An. quadrimaculatus	Arkansas	Open field	Leco 1600	15	0.80	0.0017 c	Groves et al. (1994)
An. quadrimaculatus	Arkansas	Open field	Leco 1600	10	0.53	0.0035 c	Meisch et al. (1997)

From Mount (1998)

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Table 10. Continued.

Species	Location	Habitat	Aerosol generator	Speed[1] (mph)	Flow rate[2] (fl. oz./min)	Rate[3] (lb. AI/acre)	Reference
		Phenothrin, 40%, 3.04 lb. AI/gal (Sumithrin®, Solo® 40-OS)					
Ae. taeniorhynchus	Florida	Open field	Leco HD-ULV	10	4.74	0.0206 c	Roberts (1981)
An. quadrimaculatus	Florida	Open field	Leco HD-ULV	10	0.36	0.0014 c	Roberts (1981)
		Phenothrin, 20% (Sumithrin®, Solo® 40-OS) plus piperonyl butoxide, 50%					
An. quadrimaculatus	Arkansas	Open field	VecTec Grizzly	15	0.93	0.0012 c	Groves et al. (1994)
Psorophora columbiae	Arkansas	Open field	VecTec Grizzly	15	0.93	0.0012 c	Groves et al. (1994)

[1] Ten miles per hour is indicated when a range of speeds (2.5–20 mph) was used to vary insecticide dose (1 mph = 1.609 km/h).
[2] Flow rate of technical or concentrated insecticide formulation as received from the manufacturer without diluent added by investigator (1 fl. oz./min = 29.7 ml/min).
[3] Rate based on insecticide concentration, flow rate, vehicle speed, and kill of caged (c) or reduction of natural (n) populations of adult mosquitoes, usually within 24 h, over a 300-ft. swath unless otherwise indicated (1 lb. AI/acre = 1.12 kg AI/ha).
[4] Dose based on 600–>2,640-ft. swath.

From Mount (1998)

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Table 11. Summary of rates for ultralow-volume (ULV) ground aerosols of undiluted insecticide for 90% or more control of adult mosquitoes in open to moderately open terrain with favorable meteorological conditions.

Insecticide[1]	Lb. AI/gal	% AI	Lb. AI/acre (fl. oz./min at 10 mph for 300-ft. swath)[2]				
			Aedes spp.	*Anopheles* spp.	*Culex* spp.	*Psorophora* spp.	Mean
Cyfluthrin	3.2	—	0.0001 (0.02)	0.0002 (0.04)	—	—	0.00015 (0.03)
Lambda cyhalothrin	—	2%	—	0.0003 (1.55)	0.0003 (1.55)	—	0.00030 (1.55)
Deltamethrin	—	2%	0.0005 (2.26)	0.00002 (0.09)	—	—	0.00035 (1.38)
Cypermethrin	2.48	—	0.0014 (0.44)	0.0002 (0.06)	—	—	0.0008 (0.25)
Phenothrin + PBO[3]	1.52 + 4.4	20 + 50%	—	0.0012 (0.93)	—	0.0012 (0.93)	0.0012 (0.93)
Fenvalerate	2.4	—	0.0042 (1.36)	0.0010 (0.34)	—	—	0.0026 (0.84)
Bioresmethrin + PBO	—	15 + 15%	0.0027 (0.32)	—	—	—	0.0027 (0.32)
Permethrin + PBO	2.89 + 6.09	31 + 66%	0.0036 (0.95)	0.0020 (0.53)	—	—	0.0028 (0.74)
Pyrethrins + PBO	—	12 + 60%	0.0047 (3.62)	0.0020 (1.54)	0.0029 (2.23)	—	0.0032 (2.46)
Permethrin	3.60	—	0.0057 (1.22)	0.0011 (0.24)	0.004 (0.85)	—	0.0036 (0.77)
Resmethrin + PBO	1.5 + 4.5	18 + 54%	0.0079 (4.05)	0.0024 (1.23)	0.0039 (2.00)	0.0018 (0.92)	0.0040 (2.05)
Bendiocarb	1.67	—	0.006 (2.73)	0.006 (2.73)	0.006 (2.73)	—	0.0060 (2.73)
Fluvalinate	2.0	—	0.0117 (4.50)	0.0010 (0.38)	—	—	0.0064 (2.46)
Phenothrin	3.04	40%	0.021 (5.20)	0.0014 (0.35)	—	—	0.011 (2.75)
Fenthion	9.67	93%	0.013 (1.03)	0.010 (0.79)	0.013 (1.03)	—	0.012 (0.95)
Resmethrin	3.34	40%	0.028 (6.44)	0.0070 (1.61)	0.0056 (1.29)	0.007 (1.61)	0.012 (2.76)
Chlorpyrifos	6.00	—	0.012 (1.54)	—	0.010 (1.28)	0.017 (2.18)	0.013 (1.67)
Propoxur	1.00	—	0.021 (16.16)	0.017 (13.08)	0.011 (8.46)	0.018 (13.85)	0.017 (13.08)
Naled	14.00	85%	0.020 (1.20)	—	0.016 (0.96)	0.020 (1.20)	0.019 (1.14)
Fenitrothion	9.67	93%	0.024 (1.90)	0.029 (2.30)	0.028 (2.22)	0.029 (2.36)	0.028 (2.22)
Malathion	9.70	95%	0.042 (3.32)	0.033 (2.62)	0.039 (3.10)	0.038 (3.00)	0.038 (3.00)

[1] Insecticide formulations listed here are maximum or near maximum concentrations currently registered by US-EPA for ULV ground aerosol application. Other ULV formulations of the same insecticide with similar concentrations may also be registered for use and even more concentrated formulations of some insecticides may be available in the future. However, substantially lower concentrations of some insecticides are also registered, but would represent low-volume (LV) or high-volume (HV) application even though labels indicate ULV application. Dilution of the insecticide formulations listed in this table by the applicator would also represent LV or HV applications rather than ULV application. Common names of insecticides are used in this table. See preceding tables for trade names of insecticides.

[2] 1 lb. AI/acre = 1.12 kg AI/ha; 1 fl. oz./min = 29.7 ml/min; 1 mph = 1.609 km/h; 1 ft. = 0.3048 m.

[3] PBO = piperonyl butoxide.

From Mount (1998)

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12. EQUIPMENT MAINTENANCE AND REPAIR

a. General Considerations. Equipment maintenance and repair are among the most frequently neglected factors in control programs. Many equipment operators understand little beyond the procedures necessary to start and stop the machine or adjust the flow rate as required, and they perform only the essential cleaning functions necessary to ready the machine for use in the next operation. Thus, most of the blame must rest with the operator himself if a machine fails and is unavailable when needed because some routine, but essential, repair or maintenance procedure was not accomplished. Administrative difficulties often occur, such as responsibility for operation being assigned to one department, and repair to another. Much of the equipment is completely foreign even to experienced maintenance or automotive mechanics; therefore, repair is delayed or performed poorly. When a self-reliant operator learns to maintain and repair his equipment properly, he may not have the opportunity to adequately train his replacement, and the process repeats itself. There is no certain solution to this problem, but conditions could be improved if command personnel were to insist upon training specially designated personnel in the procedures necessary to keep equipment operating with a minimum of "down time."

b. Equipment Maintenance Procedures. Because the design and configuration of ULV equipment varies so widely, specific information concerning each unit approved for military use is beyond the scope of this guide. The following are generalized procedures applicable to all ULV dispersal units:

(1) When a new machine is procured, duplicate copies of the operating and maintenance manuals, and keep one with the machine.

(2) Operators should be capable of performing maintenance on all portions of the machine except the engine and compressor systems.

(3) Equipment cleanliness, both internally and externally, is the most vital consideration. Thorough machine cleaning with a solvent is absolutely essential after each operation to maintain equipment in peak operating condition. Pesticides and their solvents can cause irreversible damage to critical parts, especially equipment seals, gaskets and electrical wiring. Consult the manual for specific guidance on solvent to be used. Isopropyl alcohol or other acceptable solvents such as Mineral Oil, Light NF™ (NSN 6505-00-664-0441) or Marvel Mystery Oil (NSN 9150-01-126-4459) should be used to clean insecticide lines each time the machine is secured from operations. If insecticide is spilled on external portions of the machine, cleaning should be accomplished immediately. Some solvents may be harmful to equipment components. Acetone, for example, may damage seals or gaskets used in solenoid valves.

(4) Use a strainer for pesticide filling operations. Foreign matter in pesticides will significantly increase maintenance problems.

(5) Engine and compressor maintenance schedules, whether from the manufacturer or military command, should be carefully followed to ensure long machine life and proper performance.

(6) Spare parts for equipment should be procured when the machine is new to ensure their availability. For contingency operations, spares of the components most subject to failure may be desirable where resupply is difficult.

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(7) The electronic controls and circuitry for aerosol equipment are not complex, and it should not be difficult for a capable technician to determine malfunctions. However, to simplify field repairs, replace the complete circuit board.

c. Routine Maintenance Items.

(1) Grease gun with lubricating grease.
(2) Compressor oil (SAE 20 or 30) as per operator's manual.

d. Essential Operating Spare Parts.

(1) Solenoid valve assemblies and repair kits.
(2) Insecticide line tubing, ferrules and sleeves.
(3) Seals and gaskets.
(4) Flow meters and flow meter repair kits.
(5) Constant delivery pumps and repair kits.
(6) Insecticide and fuel strainer.
(7) Replaceable nozzle assembly components.
(8) Replaceable engine ignition parts.
(9) Air filters for carburetors and compressors.
(10) Replaceable electronic circuit boards and components.

e. Preparation for Storage of Engine/Blower Units. Before storing the aerosol generator the following preparations should be made:

(1) Prepare the insecticide metering system for storage according to instructions in its manual.
(2) Remove the cover and screen on the air intake silencer. Start the engine and pour one pint of lubricating oil (SAE 40) into the blower intake. Shut the engine down immediately. Replace the cover and screen. The oil will coat the entire inner surface of the blower. This will prevent a coat of rust from forming in the blower and will save the cost of a new blower or an expensive repair bill.
(3) Drain the engine carburetor.
(4) Store the aerosol generator under suitable cover so it is protected from the elements.

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APPENDIX A

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APPENDIX B

INSTRUCTIONS FOR DETERMINING FLOW RATE

A. General

1. Two people are needed to perform the calibration.

2. A minimum of two gallons of insecticide in the tank is necessary.

3. Two containers are needed. The large container can be a used plastic bleach or milk bottle with a portion of the top section cut away but leaving the handle. The small container must be a plastic wide mouth conical pharmaceutical graduate. This container must be capable of measuring a minimum of eight fluid ounces or 250 milliliters.

B. Instructions

1. Start the engine. Set the operating air pressure after the machine has warmed up, usually five psi.

2. Turn on the solenoid valve by activating the toggle switch.

3. Open the flow meter valve. Let the ball go to the highest setting, and be sure that there are no bubbles in the lines. Be sure all insecticide lines are filled.

4. Cut off the solenoid valve.

5. Use protective gloves for all subsequent steps. Disconnect the insecticide discharge tube from the fog head. Hold both containers in one hand at the same level as the discharge tube connection, and place the discharge tube over the large container. The discharge line must be held steady during flow rate measurement.

6. Turn on the solenoid valve, and set the flow meter to the point indicated by the flow meter chart for the specific insecticide you are using. Measure from the center of the ball. Allow insecticide to flow into the large container to ensure steady flow and stabilization of temperature. This is approximately a setting of nine for Fyfanon.

7. Record flow meter setting, air pressure, liquid and air temperatures.

8. The person operating the controls will time the flow rate by signaling the person holding the discharge tube to shift it from the large container to the graduate. Timing of flow starts at this instant. Time the flow for 30 seconds. At the end of 30 seconds, signal transfer of the insecticide line back to the large container. Cut off the solenoid valve.

9. Observe the measured flow, and multiply the result by 2 to get the flow rate per minute. Alternatively, 1- or 2-minute readings can be used, but the volume is then divided by 1 or 2 minutes, respectively, to give the rate. Increase or decrease the flow meter setting to obtain the correct flow rate by repeating steps 6, 7 and 8 above.

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10. Reconnect the insecticide line to the fog head. While the machine is still running, turn on the solenoid valve. The flow meter ball setting will rise due to the added venturi action of the nozzle. Readjust the flow meter valve to the setting determined for proper flow rate.

11. Flow rate variations occur from machine to machine. Therefore, a calibration chart based on liquid temperature must be made for your particular machine. Record flow meter settings for each five degrees of temperature between 60 and 100 degrees while maintaining the proper insecticide flow rate. These temperatures can be obtained starting early in the morning and progressing through the day.

C. Cleaning Instructions

1. Remove the filler cap to prevent pressure buildup on the insecticide tank.

2. Remove the insecticide discharge line at the tank. Place this line in a container with approximately one pint of isopropyl alcohol. DO NOT USE ACETONE

D. Cleaning The Machine

1. Remove the air pressure line on the insecticide tank to prevent accidental discharge of contents.

2. Start the machine. Turn on the solenoid valve and adjust the flow meter to the highest setting. Run the machine until all the alcohol is exhausted.

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APPENDIX C

EQUIPMENT CALIBRATION DATA SHEET

Note: Be sure to calibrate the machine before collecting droplet samples.

Please record the following information:

1. Date _____
2. Machine type, manufacturer, serial number_____
3. Machine flow rate in ounces or milliliters per minute_____
4. Machine air pressure _____
5. Machine liquid temperature_____
6. Outside air temperature_____
7. Flow meter setting_____
8. Insecticide used_____
9. Wind speed (MPH)_____

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APPENDIX D

Testing Results of Spray Equipment Used in Vector Control

Introduction

The testing and evaluation of pest management equipment used for vector control in theater operations, humanitarian efforts and on military installation is one of the primary missions of the U.S. Navy's Naval Entomology Center of Excellence at NAS JAX in Jacksonville, FL. Besides testing the operation, safety, and durability of spray equipment, the performance characteristics of equipment is also tested. These performance characteristics include precision of flow rate control and the droplet spectra generated by the equipment. The assessment of droplet spectra has been traditionally done by either a hot-wire droplet measurement system (DC-III (AIMS), KDL labs, Inc) or by waving a glass slide through the spray and capturing droplets on either Teflon, magnesium oxide, or silicon-coated slides. Another method for measuring droplet size is with laser diffraction equipment such as that used by the USDA-ARS's Aerial Application Technology group in College Station.

In a joint effort between NECE and USDA, a series of equipment evaluations were started in 2005 with the goal of evaluating the droplet spectra generated by spray application equipment used by military personnel to control arthropods. Most of the equipment was evaluated with water- and oil-based insecticides under a variety of flow rates and/or pressures. The objectives of this work were to present not only information on spray system droplet size generated by different sprayers, but also to present a methodology by which other similar systems can be evaluated to provide applicators with sprayer system performance data. There was a range in droplet sizes generated by the different sprayers with the different spray solutions tested. These data allow users to select the spray solution and sprayer that generates the droplet size spectra that meet their specific spray application scenarios. For example, if an applicator is making a barrier control application where the desired result is that droplets impact and are retained on the surface, a sprayer that generates larger droplets (i.e. > 40 μm) would be selected. If the application called for a spray that moves laterally downwind to impact flying insects, a sprayer that generates a spray cloud with a volume median diameter (VMD) between 3 to 25 μm would be selected. This work provides a database detailing the droplet size characteristics resulting from equipment, operational settings, and spray formulation combinations which aid and support decisions made by field users.

Equipment Tested

To date, over 61 pieces of equipment have been tested with future tests planned to evaluate all equipment with National Stock Numbers (NSN). The equipment was divided into hand-held, truck-mounted, thermal fogging, and ultra-low-volume (ULV) categories. In each of the sections below, a short summary of the test conducted within each category is provided. The publication that was produced from each of the tests is shown at the end of the summary with a link to the article by clicking on the manuscript citation. The article contains all of the testing procedures and data for all of the equipment and spray formulations tested.

Hand-Held Equipment

The atomization characteristics from four hand-held sprayers (LECO P-1, Colt ULV Aerosol Generator, ULVAFAN MK2, Turbair ElectraFan 12) and a Stihl SR400 backpack sprayer were evaluated using water- and oil-based solutions. The effects on droplet size spectrum for three insecticides (Tempo SC Ultra, Anvil 10+10, and Aqua-Reslin) were also evaluated. Generic

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solutions were used to simulate the physical properties of the active ingredient solutions. There were significant differences in the droplet spectrum generated by the different sprayers. The VMD of the equipment tested ranged from 14.9-90.5 μm for the water-based solutions and 11.7-92.4 μm for the oil-based solutions.

Hoffmann, WC, TW Walker, VL Smith, DE Martin, and BK Fritz. Droplet-size Characterization of Handheld Atomization Equipment Typically Used in Vector Control. J. of American Mosquito Control Association 23(3):315-320. 2007.

Truck-Mounted Equipment

The droplet size spectra produced by eleven truck-mounted sprayers and three spray formulations were evaluated. The droplet size spectra were measured by a laser diffraction instrument, a hot wire system (DC-III probe), and rotating slides. The information presented can be used by applicators to select the sprayer that produces the droplet size spectra needed for their particular application situation. There were considerable differences in the droplet size spectra produced by the different sprayers tested. The VMD for the water-based sprays ranged from 4.7 to 211 microns depending on the sprayer, and the percent of spray volume contained in droplets less than 20 microns ranged between 0.5-98.9 percent. The VMD for the oil-based sprays ranged from 9.4 to 125.3 microns and the percent of spray volume contained in droplets less than 20 microns ranged between 2.4 - 97.9 percent. The correlations between the VMD measured by the laser system and the mass median diameter, Sauter diameter, and VMD measured by the DC-III probe were all significant. Generally, the slide VMD's were numerically similar to the VMD's from the laser system and the Sauter diameter from the DC-III probe.

Hoffmann, WC, TW Walker, DE Martin, JAB Barber, T. Gwinn, VL Smith, D. Szumlas, Y. Lan, and BK Fritz. Characterization of Truck-Mounted Atomization Equipment Used in Vector Control. J. of American Mosquito Control Association 23(3):321-329. 2007.

Thermal Fogging Equipment

A total of 56 replicated spray tests, comprised of 11 sprayers and five pesticides were completed for this study. There was a wide range in the droplet size spectra produced by the different sprayers tested. The volume median diameter ($D_{V0.5}$) measurements for the sprays diluted in diesel ranged from 2.6 μm to 75.5 μm depending on the sprayer and the %Vol<20μm ranged between 12.0-100%. The $D_{V0.5}$ measurements for the sprays diluted in water ranged from 27.9 μm to 59.9 μm and the %Vol<20μm ranged between 8.5-30.7%. The cold fogging equipment tested generated spray clouds with $D_{V0.5}$ around 100 μm and would not be considered desirable for vector control spatial sprays. The droplet sizes measured by the swinging slide method did not consistently match the droplet sizes measured by the laser diffraction instrument. Based on these trials, the use of the swinging slide method for sampling thermal fog sprays is not recommended without additional study examining the relationship between laser diffraction based droplet size measurements and swinging slide.

Hoffmann, W. C., Walker, T. W., Fritz, B. K., Gwinn, T., Smith, V. L., Szumlas, D., Quinn, B., Lan, Y., Huang, Y. and Sykes, D. Spray characterization of thermal fogging equipment typically used in vector control. JAMCA Vol. 24(4):550-559. 2008.

ULV Equipment

The droplet spectra produced by eight different ULV sprayers with oil- and water-based spray solutions were evaluated along with two thermal foggers using diesel-based spray solutions.

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The volume median diameter from the sprayers ranged from 14.8 to 61.9 μm for the oil-based spray solutions and 15.5 to 87.5 μm for the water-based spray solutions. The two thermal foggers generated sprays with a volume median diameter of 3.5 μm.

W.C. Hoffmann, T.W. Walker, B.K. Fritz, M. Farooq, V.L. Smith, C.A. Robinson, and Y. Lan. Spray Characterization of ULV Sprayers Typically Used in Vector Control. Unpublished but submitted to JAMCA for publication.

Note: The following tables (Figures 3-9) from the publications above provide excellent atomization parameters for a large variety of truck-mounted and hand-held cold aerosol and thermal fog equipment using ULV insecticides that are either oil-, water- or diesel-based. This information can be valuable when choosing which sprayer and insecticide combination to use for your control operations.

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Table 4. Spray droplet spectra data from the laser diffraction system for sprayers applying BVA 13 ULV Oil.

Sprayer	Flow rate (oz/min)	$D_{V0.1}$ (µm ± SD)	$D_{V0.5}$ (µm ± SD)	$D_{V0.9}$ (µm ± SD)	%vol <20 µm
Pro-Mist ULV Fogger	4	2.20 ± 0.1	20.4 ± 0.3	34.7 ± 0.2	48.8
15 MP	18	4.1 ± 0.1	25.0 ± 0.2	42.2 ± 0.4	35.0
	1.5	2.7 ± 0.1	22.3 ± 0.2	34.6 ± 0.1	40.0
Clarke Grizzly®	4	3.5 ± 0.2	17.5 ± 0.5	38.6 ± 1.7	56.4
B&G Phoenix Fogger					
680	4	3.4 ± 0.2	20.1 ± 1.1	44.0 ± 3.2	49.8
London Fog XKD	4	2.3 ± 0.1	9.4 ± 0.1	20.7 ± 0.5	88.6
@100 psi	18	5.1 ± 0.1	30.5 ± 0.8	76.7 ± 3.7	33.1
@120 psi	18	4.7 ± 0.4	26.1 ± 1.4	99.6 ± 5.0	40.1
	1	1.3 ± 0.1	4.8 ± 0.6	12.5 ± 2.9	97.9
London Fog 18–20	4	5.3 ± 0.8	13.8 ± 0.8	25.0 ± 1.6	76.8
	18	6.1 ± 0.1	14.6 ± 0.2	26.6 ± 0.6	73.1
	0.8	3.4 ± 0.4	19.9 ± 1.2	40.5 ± 2.3	50.3
London Fog MAG	4	3.5 ± 0.1	14.7 ± 0.7	32.3 ± 1.8	66.5
AST Terminator					
No. 24 Orifice	4	3.6 ± 0.3	17.2 ± 0.2	38.8 ± 0.1	54.6
No. 35 Orifice	4	4.6 ± 0.3	21.7 ± 0.8	46.4 ± 1.9	45.2
No. 41 Orifice	4	4.2 ± 0.3	21.6 ± 1.0	46.7 ± 2.3	45.6
Whitmire Micro-Gen					
Model G4	4	3.5 ± 0.2	17.5 ± 0.5	36.5 ± 0.6	57.2
Buffalo Turbine	128	43.9 ± 3.0	125.3 ± 12.0	212.4 ± 28.4	2.5
Model CSM2	103	43.2 ± 2.1	122.5 ± 7.3	204.5 ± 15.6	2.4

Figure 1. Droplet spectra of **Truck**-mounted **cold aerosol** sprayers using **oil-based sprays** (from Hoffman et al. 2007a).

Table 3. Spray droplet measurements from the laser diffraction system for water-based sprays.

Sprayer	Spray solution[1]	Flow rate (oz/min)	$D_{V0.1}$ (µm ± SD)	$D_{V0.5}$ (µm ± SD)	$D_{V0.9}$ (µm ± SD)	%vol <20 µm
Pro-Mist ULV Fogger 15 MP	Water + 90% NIS	4	14.9 ± 0.6	34.7 ± 1.4	40.9 ± 6.2	31.9
		18	14.8 ± 0.3	32.2 ± 1.2	57.2 ± 0.7	19.6
Clarke Grizzly®	Water + 90% NIS	4	8.8 ± 0.3	25.9 ± 0.6	46.4 ± 1.0	33.5
B&G Phoenix Fogger 680	Water + 90% NIS	4	6.9 ± 0.5	25.1 ± 0.3	46.8 ± 1.3	36.0
		9	7.2 ± 1.0	27.8 ± 0.2	51.9 ± 0.4	31.1
London Fog XKD	Water + 90% NIS	4	1.3 ± 0.0	4.7 ± 0.2	11.9 ± 0.4	98.9
		17	7.6 ± 0.1	29.7 ± 1.1	112.5 ± 6.7	35.9
London Fog 18–20	Water + 90% NIS	4	11.1 ± 0.2	19.2 ± 0.1	29.8 ± 0.4	54.1
		18	8.1 ± 0.1	15.3 ± 0.1	25.7 ± 0.0	72.7
London Fog MAG	Water + 90% NIS	4	7.6 ± 0.1	25.6 ± 0.7	60.6 ± 1.8	37.6
LECO ULV Fog Generator Model MD	Water + 90% NIS	4	6.0 ± 0.0	12.6 ± 1.3	29.2 ± 4.3	79.7
Whitmire Micro-Gen Model G4	Water + 90% NIS	4	8.6 ± 0.5	23.3 ± 0.3	42.2 ± 0.7	39.5
AST Terminator	Water + 90% NIS	4	7.6 ± 1.2	20.4 ± 2.7	48.5 ± 6.0	40.6
Buffalo Turbine Model CSM2	Water + 90% NIS	128	93.1 ± 15.3	211.0 ± 31.1	315.1 ± 37.1	0.5
		120	73.0 ± 2.7	174.4 ± 26.0	269.8 ± 54.1	0.9
Clark Pro-Mist ULV Fogger	Aqua-Reslin® (1:4)	4	15.6 ± 8.0	26.5 ± 0.4	36.1 ± 0.4	13.3
B&G Phoenix Fogger 680	Aqua-Reslin (1:4)	4	8 ± 0.1	20.7 ± 0.7	38.4 ± 1.7	47.8
London Fog XKD	Aqua-Reslin (1:4)	4	2.1 ± 0.1	8.5 ± 0.6	19.9 ± 1.5	90.1
London Fog 18–20	Aqua-Reslin (1:4)	4	8.2 ± 0.0	15.3 ± 0.0	24.8 ± 0.0	74.1
AST Terminator	Aqua-Reslin (1:4)	4	5.5 ± 0.1	19.0 ± 0.7	41.9 ± 1.5	35.4

[1] NIS, nonionic surfactant.

Figure 2. Droplet spectra of **Truck**-mounted **cold aerosol** sprayers using **water-based sprays** (from Hoffman et al. 2007a).

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Table 1. Spray droplet spectra data from the laser diffraction system and Teflon-coated slides for London Fog™ (LF) and Curtis Dyna-Fog® (CDF) thermal fogging sprayers with pesticides diluted in diesel (pest:diesel), with maximum temperature measured at the spray nozzle.

Sprayer	Flow rate (oz/min)[1]	Pesticide	Dilution (pest:diesel)	Laser diffraction data[2]				Slide data		Max. temperature (°C) (°F)[3]
				$D_{V0.1}$ (µm ± SD)	$D_{V0.5}$ (µm ± SD)	$D_{V0.9}$ (µm ± SD)	%Vol<20 µm	$D_{V0.5}$ (µm)	%Vol<20 µm	
LF Eliminator	(10.7)	Fyfanon	1:18.3	1.2 ± 0.2	4.2 ± 0.9	9.7 ± 0.9	99.5	12.9	74.3	187 (368)
		Anvil 10+10	1:7.7	1.3 ± 0.1	4.4 ± 0.7	9.9 ± 1.8	99.9	24.7	36.5	138 (280)
		Kontrol 30-30	1:39	1.2 ± 0.0	3.8 ± 0.0	8.6 ± 0.1	100.0	29.5	20.0	196 (384)
LF F500	(53.3)	Fyfanon	1:91.5	1.6 ± 0.4	5.4 ± 1.4	11.4 ± 2.2	99.8	5.7	100.0	171 (340)
		Anvil 10+10	1:38.5	1.2 ± 0.1	4.0 ± 0.5	8.7 ± 1.4	100.0	6.2	100.0	209 (409)
		Kontrol 30-30	1:195	19.6 ± 4.6	45.4 ± 4.7	68.6 ± 5.0	10.8	9.9	91.8	210 (410)
CDF Golden Eagle	(10.7)	Fyfanon	1:18.3	1.2 ± 0.0	4.0 ± 0.2	9.5 ± 0.6	100.0	N/A[4]	N/A	193 (380)
		Anvil 10+10	1:7.7	1.3 ± 0.1	4.5 ± 0.4	9.9 ± 0.9	100.0	7.1	91.4	226 (440)
		Kontrol 30-30	1:39	1.5 ± 0.1	5.6 ± 0.4	14.0 ±0.8	97.7	16.1	56.7	224 (436)
		Kontrol 30-30	Undiluted	0.9 ± 0.0	2.6 ± 0.1	4.2 ± 0.1	99.9	N/A	N/A	242 (468)
	(22.0)	Kontrol 30-30	Undiluted	1.6 ± 0.3	5.6 ± 1.4	11.3 ± 1.7	99.9	N/A	N/A	238 (460)
CDF Silver Cloud	(53.3)	Fyfanon	1:91.5	1.0 ± 0.1	3.1 ± 0.4	6.0 ± 1.5	100.0	14.2	63.0	299 (570)
		Anvil 10+10	1:38.5	1.1 ± 0.0	3.6 ± 0.1	7.5 ± 0.4	100.0	11.3	93.0	264 (508)
		Kontrol 30-30	1:195	19.2 ± 0.6	75.5 ± 2.3	137.1 ± 1.9	12.0	N/A	N/A	211 (412)
		Kontrol 30-30	1:39	4.7 ± 2.9	17.9 ± 5.0	66.9 ± 3.5	52.9	N/A	N/A	N/A
CDF 1200	(53.3)	Fyfanon	1:91.5	1.2 ± 0.2	4.0 ± 0.9	9.9 ± 2.1	99.8	6.2	100.0	207 (405)
		Kontrol 30-30	1:195	1.3 ± 0.2	4.5 ± 1.2	13.4 ±2.4	97.8	42.2	20.8	338 (640)
CDF Trailblazer	(10.7)	Fyfanon	1:18.3	1.4 ± 0.1	5.1 ± 0.6	12.8 ± 1.0	98.9	7.8	81.5	302 (576)
		Anvil 10+10	1:7.7	0.9 ± 0.0	2.6 ± 0.1	4.3 ± 0.3	99.8	8.8	88.1	254 (489)
		Kontrol 30-30	1:39	2.6 ± 0.8	8.6 ± 1.8	16.4 ± 1.9	96.3	15.0	71.9	286 (546)
		Aqualuer	1:26.8	1.1 ± 0.1	3.6 ± 0.6	8.0 ± 1.9	99.9	14.2	74.5	317 (602)

[1] Flow rate represented the application rate if the sprayer was moving at 8 km/h (5 mph); however, sprayer was not moving during these studies.
[2] $D_{V0.1}$, $D_{V0.5}$, $D_{V0.9}$, droplet diameter (µm) at which 10%, 50%, and 90%, respectively, of the spray volume is contained in droplets smaller than this value; %Vol<20 µm, percentage of spray volume contained in droplets <20 µm.
[3] Maximum temperature measured at the release point (e.g., nozzle or outlet) of the spray.
[4] N/A, data were not collected.

Figure 5. Droplet spectra of **truck**-mounted and hand-held **thermal fog sprayers** using **diesel-based sprays** (from Hoffman et al. 2008).

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Table 4. Atomization parameters for oil-based sprays.

Sprayer	Spray solution	$D_{V0.1}$ (µm ± SD)	$D_{V0.5}$ (µm ± SD)	$D_{V0.9}$ (µm ± SD)	%vol < 52 µm
Colt ULV	BVA 13 ULV Oil	3.2 ± 1.1	11.7 ± 1.7	28.6 ± 6.8	99.3
Leco P-1	BVA 13 ULV Oil	11.9 ± 0.4	49.3 ± 1.0	101.9 ± 9.4	53.6
Stihl SR400					
Setting 1	BVA 13 ULV Oil	9.4 ± 1.8	36.9 ± 2.2	90.4 ± 4.9	66.4
Setting 2	BVA 13 ULV Oil	15.6 ± 0.1	55.8 ± 0.4	109.9 ± 2.7	46.9
Setting 3	BVA 13 ULV Oil	21.9 ± 0.8	73.2 ± 11.2	265.9 ± 18.5	35.1
Setting 4	BVA 13 ULV Oil	21.5 ± 0.6	71.3 ± 2.9	142.4 ± 11.9	34.9
Setting 5	BVA 13 ULV Oil	22.5 ± 0.8	76.2 ± 4.5	156.0 ± 16.2	32.8
Setting 6	BVA 13 ULV Oil	23.1 ± 0.4	76.8 ± 2.1	165.7 ± 11.4	31.9
Turbair Electrafan	BVA 13 ULV Oil	37.5 ± 7	84.3 ± 1	116.8 ± 4.4	18.4
ULVAFAN	BVA 13 ULV Oil	36.6 ± 17.6	92.4 ± 44.5	225.2 ± 47.1	26.4
Colt ULV	Anvil 10+10®	7.8 ± 0.2	14.1 ± 0.1	27.2 ± 2.0	99.4
Leco P-1	Anvil 10+10	14.4 ± 0.1	51.5 ± 2.2	95.0 ± 5.1	51.3
Stihl SR400					
Setting 1	Anvil 10+10	6.0 ± 2.6	33.4 ± 4.1	83.5 ± 3.1	70.2
Setting 4	Anvil 10+10	22.1 ± 0.1	70.0 ± 0.3	138.1 ± 0.3	35.2
Setting 6	Anvil 10+10	23.6 ± 0.6	74.0 ± 1.3	147.7 ± 2.94	32.7
ULVAFAN	Anvil 10+10	38.5 ± 5.2	81.3 ± 3.2	111.7 ± 3.6	20.2

Figure 6. Droplet spectra of **hand**-held **cold aerosol sprayers** using **oil-based sprays** (from Hoffman et al. 2007b).

Table 1. Atomization parameters for oil-based spray solutions.

Sprayer	Rate (oz/min)	Pressure (psi)	$D_{V0.1}$ (µm ± SD)	$D_{V0.5}$ (µm ± SD)	$D_{V0.9}$ (µm ± SD)	Vol < 20 µm (%)
Birchmeier B245: ULV Nozzle	4	Fixed	16.2 ± 0.1	45.0 ± 0.9	79.2 ± 1.6	15.7
Birchmeier B245: LV Nozzle	4	Fixed	11.5 ± 0.1	38.8 ± 1.2	70.0 ± 1.6	23.2
ADAPCO Guardian 190ES	4	5	7.1 ± 0.9	14.8 ± 1.3	30.3 ± 4.9	70.8
ADAPCO Guardian 190ES	10	5	8.5 ± 0.5	16.8 ± 0.6	30.8 ± 1.1	63.3
B&G Porta-Pak	2.4	Fixed	18.3 ± 0.7	61.9 ± 2.1	112.0 ± 1.8	11.5
CDF MiniLite	4	Fixed	6.5 ± 1.2	17.9 ± 0.4	36.4 ± 0.5	56.8
CDF L30	4	Fixed	2.6 ± 0.4	25.7 ± 0.7	41.2 ± 0.5	32.4
CDF Anileator	4	Fixed	10.1 ± 1.5	39.1 ± 1.5	79.8 ± 1.8	25.1
Clarke P1	1	Fixed	10.0 ± 0.7	35.0 ± 1.6	66.5 ± 5.8	29.0
CDF Maxi-Pro 4	4	4	7.5 ± 0.3	20.3 ± 0.0	42.3 ± 0.3	49.0
CDF Maxi-Pro 4	4	6	7.8 ± 0.1	15.3 ± 0.1	29.3 ± 0.2	69.5

Figure 7. Droplet spectra of **hand**-held **cold aerosol sprayers** using **oil-based sprays** (from Hoffman et al. 2007b).

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Table 3. Atomization parameters for water-based sprays.

Sprayer	Spray solution	$D_{V0.1}$ (µm ± SD)	$D_{V0.5}$ (µm ± SD)	$D_{V0.9}$ (µm ± SD)	%vol < 52 µm
Colt ULV	Aqua-Reslin® (1:1)	9.7 ± 1.8	16.0 ± 1.6	26.7 ± 2.2	99.2
Colt ULV	Aqua-Reslin (1:3)	9.6 ± 1.6	14.9 ± 0.6	23.3 ± 3.0	99.9
Leco P-1	Aqua-Reslin (1:1)	33.6 ± 1.9	75.3 ± 6.3	137.3 ± 5.8	25.3
Leco P-1	Aqua-Reslin (1:3)	32.6 ± 3.5	71.8 ± 4.6	113.1 ± 6.0	26.2
Stihl SR400					
Setting 1	0.1% Triton X	18.0 ± 0.3	52.1 ± 0.8	102.6 ± 2.5	50.5
Setting 2	0.1% Triton X	21.0 ± 0.2	60.0 ± 0.3	118.4 ± 1.4	42.0
Setting 3	0.1% Triton X	23.6 ± 0.4	67.6 ± 1.2	138.1 ± 3.6	35.9
Setting 5	0.1% Triton X	25.5 ± 1.1	74.1 ± 3.3	160.9 ± 1.5	32.0
Setting 6	0.1% Triton X	27.2 ± 1.2	78.5 ± 2.9	166.3 ± 8.8	29.4
Stihl SR400					
Setting 1	Tempo SC Ultra®	22.4 ± 0.6	62.1 ± 1.0	122.4 ± 2.5	40.3
Setting 4	Tempo SC Ultra	30.0 ± 0.3	83.5 ± 0.3	159.0 ± 0.5	26.1
Setting 6	Tempo SC Ultra	31.6 ± 0.2	89.3 ± 0.5	184.8 ± 3.5	23.4
Stihl SR400					
Setting 1	Aqua-Reslin (1:1)	20.9 ± 1.2	49.9 ± 0.2	98.3 ± 2.0	53.4
Setting 1	Aqua-Reslin (1:44)	16.5 ± 0.9	45.6 ± 1.3	81.8 ± 3.7	59.9
Setting 4	Aqua-Reslin (1:44)	28.2 ± 0.6	76.2 ± 1.6	137.3 ± 1.4	29.1
Setting 6	Aqua-Reslin (1:44)	30.0 ± 0.15	81.2 ± 0.4	152.5 ± 3.1	26.6
Stihl SR400 with electrostatic nozzle	Aqua-Reslin (1:44)	26.2 ± 0.1	71.1 ± 0.4	126.9 ± 1.4	31.6
Turbair Electrafan	0.1% Triton X	33.3 ± 1.1	47.2 ± 0.9	74.4 ± 2.2	64.6
ULVAFAN	0.1% Triton X	36.7 ± 4.1	63.3 ± 5.3	117.4 ± 4.3	32.0
ULVAFAN	Tempo SC Ultra	59.0 ± 5.5	90.5 ± 5.6	123.7 ± 4.6	6.2

Figure 8. Droplet spectra of **hand**-held **cold aerosol sprayers** using **water-based sprays** (from Hoffman et al. 2007b).

Table 2. Atomization parameters for water + nonionic surfactant (0.25% v/v) spray solutions.

Sprayer	Rate (oz/min)	Pressure (psi)	$D_{V0.1}$ (µm ± SD)	$D_{V0.5}$ (µm ± SD)	$D_{V0.9}$ (µm ± SD)	Vol < 20 µm (%)
Birchmeier B245: ULV Nozzle	4	Fixed	42.9 ± 1.8	77.4 ± 3.8	125.0 ± 9.3	1.63
Birchmeier B245: LV Nozzle	4	Fixed	25.0 ± 1.2	60.3 ± 2.1	98.9 ± 3.4	7.2
Arro-Gun Mozzie	4	Fixed	12.0 ± 0.5	42.6 ± 0.7	79.8 ± 1.6	20.5
ADAPCO Guardian 190ES	4	5	10.3 ± 0.3	20.9 ± 0.3	36.2 ± 0.4	46.2
ADAPCO Guardian 190ES	10	5	10.2 ± 0.2	21.5 ± 0.1	37.2 ± 0.1	45.6
B&G Porta-Pak	4	Fixed	34.2 ± 1.3	87.5 ± 3.3	149.4 ± 5.6	3.2
CDF MiniLite	4	Fixed	12.4 ± 1.2	26.4 ± 0.6	43.5 ± 0.7	28.8
CDF L30	4	Fixed	16.4 ±0.2	27.9 ± 0.0	40.0 ± 0.2	17.8
CDF Maxi-Pro 4	4	4	9.9 ± 0.2	15.5 ± 0.1	23.1 ± 0.3	78.6
CDF Maxi-Pro 4	4	5.5	12.0 ± 0.2	19.1 ± 0.1	29.7 ± 0.2	55.1
CDF Maxi-Pro 4	4	6	11.9 ± 0.3	20.0 ± 0.5	34.2 ± 0.9	50.0

Figure 9. Droplet spectra of **hand**-held **cold aerosol sprayers** using **water-based sprays** (from Hoffman et al. 2007b).

Table 3. Atomization parameters for diesel-based spray solutions.

Sprayer	Rate (oz/min)	Solution	$D_{V0.1}$ (µm ± SD)	$D_{V0.5}$ (µm ± SD)	$D_{V0.9}$ (µm ± SD)	Vol < 20 µm (%)
CDF Golden Eagle	4	Diesel	1.1 ± 0.0	3.4 ± 0.1	6.8 ± 0.3	100.0
CDF Golden Eagle	4	Diesel:Kontrol 1:39	1.1 ± 0.0	3.5 ± 0.1	7.2 ± 0.1	100.0
CDF Golden Eagle	4	Diesel:Fyfanon 1:91.5	1.1 ± 0.0	3.5 ± 0.1	7.2 ± 0.3	100.0
CDF Golden Eagle	4	Diesel:Kontrol 1:195	1.1 ± 0.0	3.4 ± 0.2	7.0 ± 0.6	100.0
LF Eliminator	4	Diesel	1.1 ± 0.0	3.3 ± 0.1	6.5 ± 0.0	99.7
LF Eliminator	4	Diesel:Kontrol 1:39	1.1 ± 0.1	3.6 ± 0.3	7.7 ± 1.0	99.9
LF Eliminator	4	Diesel:Fyfanon 1:91.5	1.1 ± 0.0	3.3 ± 0.1	6.7 ± 0.2	100.0
LF Eliminator	4	Diesel:Kontrol 1:195	1.1 ± 0.1	3.3 ± 0.3	6.5 ± 1.0	100.0

Figure 9. Droplet spectra of **hand**-held **cold aerosol sprayers** using **water-based sprays** (from Hoffman et al. 2007a).

Table 2. Atomization parameters for water + nonionic surfactant (0.25% v/v) spray solutions.

Sprayer	Rate (oz/min)	Pressure (psi)	$D_{V0.1}$ (µm ± SD)	$D_{V0.5}$ (µm ± SD)	$D_{V0.9}$ (µm ± SD)	Vol < 20 µm (%)
Birchmeier B245: ULV Nozzle	4	Fixed	42.9 ± 1.8	77.4 ± 3.8	125.0 ± 9.3	1.63
Birchmeier B245: LV Nozzle	4	Fixed	25.0 ± 1.2	60.3 ± 2.1	98.9 ± 3.4	7.2
Arro-Gun Mozzie	4	Fixed	12.0 ± 0.5	42.6 ± 0.7	79.8 ± 1.6	20.5
ADAPCO Guardian 190ES	4	5	10.3 ± 0.3	20.9 ± 0.3	36.2 ± 0.4	46.2
ADAPCO Guardian 190ES	10	5	10.2 ± 0.2	21.5 ± 0.1	37.2 ± 0.1	45.6
B&G Porta-Pak	4	Fixed	34.2 ± 1.3	87.5 ± 3.3	149.4 ± 5.6	3.2
CDF MiniLite	4	Fixed	12.4 ± 1.2	26.4 ± 0.6	43.5 ± 0.7	28.8
CDF L30	4	Fixed	16.4 ±0.2	27.9 ± 0.0	40.0 ± 0.2	17.8
CDF Maxi-Pro 4	4	4	9.9 ± 0.2	15.5 ± 0.1	23.1 ± 0.3	78.6
CDF Maxi-Pro 4	4	5.5	12.0 ± 0.2	19.1 ± 0.1	29.7 ± 0.2	55.1
CDF Maxi-Pro 4	4	6	11.9 ± 0.3	20.0 ± 0.5	34.2 ± 0.9	50.0

Figure 10. Droplet spectra of **hand**-held **thermal fog sprayers** using **diesel-based sprays** (from Hoffman et al. 2007b).

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APPENDIX E

DIRECTIONS FOR DETERMINING PARTICLE SIZE OF AEROSOLS AND FINE SPRAYS

I. Excellent reviews of particle measurement have been published by A.H. Yeomans (USDA Publication ET-267: 1949), Matthews (1992) and Rathburn (1970) and are included below. Additional information may be obtained from references in their bibliographies. Specific guidelines for droplet measurement can also be obtained from the Navy Entomology Center of Excellence (below). If assistance is required in collecting droplets or analyzing prepared slides, please contact one of the following for assistance:

a. Navy:

Officer in Charge, Navy Entomology Center of Excellence, ATTN: Testing and Evaluation, Box 43, Bldg 931 Child St, Naval Air Station, Jacksonville, FL 32212

b. Army:

(1) Commander, USAPHC-North, Entomological Sciences, ATTN: MCHB-AN-ES, Bldg 4411, Ft George G. Meade, MD 20755-5225

(2) Commander, USAPHC-South, Entomological Sciences, ATTN: MCHB-AS-ES, 2472 Schofield Road, 2nd Floor, Fort Sam Houston, TX 78234-6233

(3) Commander, USAPHC-West, Entomological Sciences, ATTN: MCHB-AS-ES, Box 339500-MS 115, Ft Lewis, WA 98433-9500

II. A "Hot Wire" calibration method, using equipment such as the KLD Labs DC-III Droplet Analyzer, provides a quick means of calibrating ULV sprayers. The hot wire method takes 15 minutes and provides an instantaneous readout on the spectrum of droplet sizes. Newer hot wire systems have electronic data collection systems, which can be downloaded onto a computer for record keeping and digital display of data. The hot wire is actually a thin metal filament that is very delicate and therefore easily broken, so extreme care is needed when using such a system.

III. Impinged slide analysis. The best method that has been found for determining the particle size of insecticidal aerosols and fine sprays is to deposit a sample on a glass slide and measure the particles under a high-power microscope. The following directions for determining droplet sizes and other spray cloud characteristics is taken from a report from the United States Department of Agriculture, Agricultural Research Administration, Bureau of Entomology and Plant Quarantine (ET-267, May 1949) written by A.H. Yeomans. This paper describes in detail the technique as it is now used, and should contain most of the information needed to determine important droplet size and spray cloud parameters.

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Aerosol Droplet Analysis Test Kit Components*

(*Mention of a product or supply source does not constitute an endorsement of the products named or criticism of similar ones not mentioned. Mention of trade names does not constitute a guarantee or warranty of the products by the AFPMB, the Military Services, or the Department of Defense.)

An inexpensive droplet analysis kit can be produced using the following components:

1. Four foot long ¾ inch diameter or 1 inch diameter wooden dowel
2. One each ¼ inch diameter X 1 ½ inch long bolt (can use 10/32 inch up to ¼ inch diameter bolt)
3. Two flat washers, ¼ inch (or size to match other diameter bolts, if used).
4. One wing nut, ¼ inch

Note. Use the above materiel to construct a wooden dowel stick with slotted end, bolt, washers, and wing nut to clamp slides into the slotted stick. The stick can be made using a 3/4 or 1 inch diameter dowel, approximately 4 feet long. Cut a slit, using a saw, approximately 4 inches long in one end of the dowel so that it can hold a Teflon®-coated slide. Next drill a hole, using a drill bit which matches the diameter of the 1 ½ inch bolt, through the dowel approximately 3 inches down from the dowel end with the slit. Microscope slides placed one inch into the slit can then be tightened into the slit using a ¼ inch x 1 ½ inch bolt inserted into the drilled hole and fastened with washers and wing nut. Wood dowel, bolts, flat washers, and wing nuts can be purchased at a local hardware store.

5. Graduated Cylinder, polypropylene economy graduated, size 250 ml. The graduated cylinder is used for measuring flow rates. A source is:

Fisher Scientific
Phone: 1-800-926-1166
Nalgene polypropylene economy graduated cylinder
Catalogue No. 08-572-6E
Unit Price - $10.67 each, Case of 12 for $85.40

6. A set of Teflon-coated, numbered slides, cover slides, and spacers should be obtained for droplet collection. Careful attention to the instructions will allow you to effectively evaluate your equipment. Distributors of aerosol droplet samplers and coated microscope slides (Teflon- and magnesium oxide).

BioQuip Products
7803 LaSalle Avenue
Gardenia, CA 90248-3602
Phone: (310) 324-0620
Fax: (310) 324-7931
Slide Cost: $110.40 for a box of 25 or $23.35 for a box of 5.

John W. Hock Company
7409 NW 23rd Avenue
Gainesville, Florida 32606
Phone: 352-378-3209

ED_002896_00040479-00053

7. Cover slides (NSN 6640-00-494-3808)

8. Mailer and/or shipper tubes:

Fisher Scientific
Phone: 1-800-926-1166
Catalogue No. 03-526: $154.41 for 300 foam mailers that hold 10 slides each
Catalogue No 03-526A: :$87.10 for 300 mailing sleeves for 03-526 above

9. Paper gasket. Make paper gaskets, using manila folders or 3/5 cards. First cut the gasket materiel to the same dimensions as the slide (1"x3"). Then carefully cut out the center so that you have a "window frame" effect. The edges of the "window frame" should be about ¼ inches thick. This gasket is used as a spacer between Teflon-coated slide and the 1"x 3" slide used as a cover.

10. Tape. Cellophane tape is commonly found in most convenience stores. Use cellophane tape at both ends of the slide and wrap it securely.

11. Cleaning Brush, size 2-inch diameter x 6-inch long brush, with overall length of 20 inches. Brush is used to clean graduated cylinder after use.

Fisher Scientific
Nessler tube brush
Catalogue No 03-621-B
Cost: $20.28 each; Pack of 12 EA for $207.99

12. Other ULV sampling equipment

a. A magnification of 100X is recommended when performing droplet measurements, although many workers use a magnification of 400X. This practice is satisfactory for uncovered slides, but difficult to achieve with covered slides.

b. For field or contingency operations, a 100X magnifier may be used as an alternative to a more expensive, heavy and often difficult to obtain compound microscope. Using this device and a fixed spread factor, field measurement and assessment of droplet spectra can be satisfactorily performed with minimal measurement error.

a. Eyepiece (ocular) micrometer with etched scale of 100 numbered divisions that fit into the eyepiece's base. Note: Prior to purchase, ensure that the micrometer will fit into the model of the microscope with which it will be used. Cost is approximately $50.00.

b. Stage micrometer. Used to calibrate the ocular scale in the eyepieces. Glass slide, with a millimeter divided into 100 parts of 10 microns each. Approximate cost is $75.00 to $150.00. Note: Prior to purchase, ensure that the micrometer will fit the model of the microscope that it will be used with. Cost is approximately $50.00.

Preparation of Slides if ready-to-use slides are not purchased

Particles of relatively nonvolatile materials can be measured before they evaporate. To prevent excessive spreading, filming, or coalescence, the slide must be coated with an oleophobic

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substance that will cause the individual droplets to maintain their convexity to some degree. Two of the most satisfactory materials for this purpose proved to be a 1-percent alcoholic solution of mannitan monolaurate, and a silicon product marketed under the trade name Dri-film 9987. The slides are first immersed in a cleaning solution, dried, then immersed in the oleophobic coating solution, and redried. When dry the slides should be lightly polished with a soft cloth. They may be stored in ordinary slide boxes for several days before they are used.

Particles of volatile materials, which evaporate rapidly, cannot be measured directly, but their size can be estimated by measuring the craters they leave at the points of contact on slides coated with magnesium oxide or carbon soot. It is important to apply the right thickness of coating for the range of particle sizes' anticipated.

Deposition of Particles on Slides

A sample of an aerosol or spray cloud can be deposited on a slide by impingement or by settling.

Deposition by impingement may be accomplished by moving the slide through an aerosol or spray cloud, or by moving the aerosol or spray cloud past a slide in fixed position. The velocity of movement in either case must be adjusted to the particle size range expected. The velocity must be increased as the average particle size decreases. Since the deposition is in proportion to the particle size, compensation must be made for this factor. The Cascade and Micro Impacters developed in England, wherein an aerosol cloud is drawn through a series of orifices to vary the velocity, and impinged on a different slide at each orifice, is useful only with very small particles, mostly out of the range of insecticidal aerosols and sprays.

Deposition by settling should be limited to particle size ranges below 20 microns in diameter. It may be accomplished by two means. An aerosol of spray cloud is released in an enclosure and allowed to settle onto slides placed on the floor or bottom. The cloud must be mixed to be uniform, and the aerosol or spray released in such a way as to prevent impingement on the sides or ceiling of the enclosure. The amount released should be small enough to prevent coalescing in the air or too heavy a deposit on the slide. Adequate time must be allowed for all the smaller particles to settle a distance equal to the height of the enclosure. Convection currents should be prevented as much as possible. A second and more rapid method is to draw the aerosol or spray cloud through an electrical precipitator in which slides have been placed. When the machine is turned on, all particles in the field are precipitated in a matter of seconds. Deposition by settling results in a slide representative of the entire range of particle sizes in the sample, with each size present in true proportion so that no adjustment or weighting is necessary.

Procedure for Collecting Droplets from Aerosol Equipment

1. Proceed as follows:

   a. Set the machine to the proper flow rate according to the instructions that come with the machine.

   b. Move the fogging head to a horizontal position so the insecticide flow is parallel to the ground. This position is for testing only; move back to the original position for operating purposes (45°).

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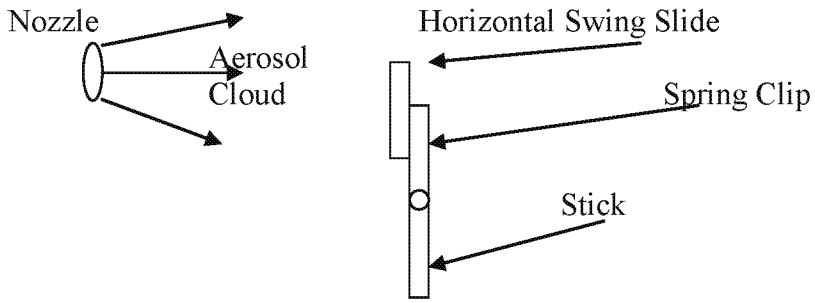
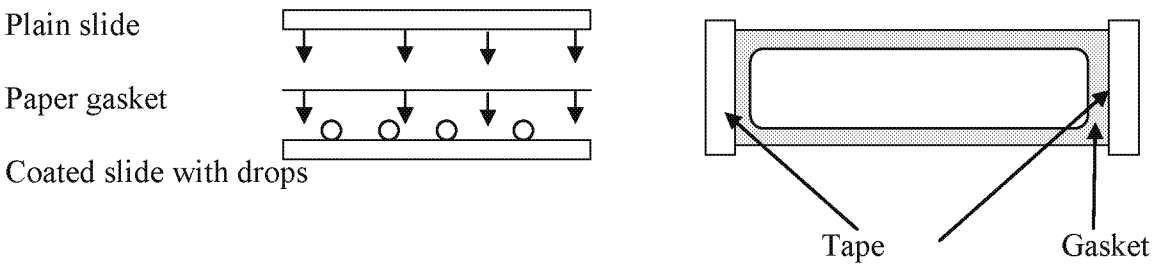
c. With the machine operating properly, take three slide samples at eight (8) feet. The wind speed should not exceed ten mph for the test. The slide is attached to a six foot stick (broom handle satisfactory) with a large spring clip at the end. Tape the clip to the stick firmly. Wave the slide through the insecticide cloud vertically to the flow. The exposure time for the swing should be approximately one (1) second. The droplets must be collected on the numbered side of the slide, so face it towards the cloud.



d. After the sample is obtained, put one of the spacing papers provided on the slide (slide number side up), and cover the paper and the exposed slide with a plain microscope slide, making a "sandwich" of the two slides and the paper.



e. Use cellophane tape at both ends of the slide and wrap it securely.

f. Wrap the slides in tissue paper to prevent breaking and return them in the mailing box provided.

g. If there are any questions, contact NECE, DSN 942-4624, COM 904-542-4624.

h. After the sample is obtained, put one of the spacing papers provided on the slide (slide number side up), and cover the paper and the exposed slide with a plain microscope slide, making a "sandwich" of the two slides and the paper.

2. Record pertinent equipment and weather information on the data sheet provided in Appendix C.

3. If unable to read and evaluate these slides yourself, submit slides to commands listed at the beginning of this section.

Microscope Eyepiece Micrometer Calibration

1. Measurement of objects with the microscope requires calibration of the instrument. Two scales are required: an ocular or eyepiece micrometer (a glass disc bearing a scale of 50 or 100 divisions

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numbered at intervals to facilitate reading), and a stage micrometer (a glass slide with a scale divided into units of 0.1 mm and 0.01 mm).

2. Remove eyepiece, unscrew the top lens, and note the circular shelf inside. Clean the eyepiece micrometer disc with lens paper and place it carefully in the eyepiece so that it rests on the shelf. Replace the top lens and insert the eyepiece in the microscope. With moderate illumination, the scale should be in sharp focus. If its numbers are backwards, the disc is upside down and must be turned over. This scale, regardless of the objective used, will appear the same size when viewed within the eyepiece, and must be calibrated for each lens combination (i.e. eyepiece and objective) provided by the microscope. The stage micrometer is used for that purpose. With the lowest power objective in position, place the stage micrometer on the stage and focus on its scale. The ruling may be in 0.01 mm units throughout its length or only at one end, with the remainder divided into 0.1 mm units. Rotate the eyepiece until its scale is either superimposed on, or very close and parallel to, the stage scale. Move the stage micrometer so that its zero line is exactly even with, or superimposed on, that of the eyepiece scale. Then, as far as possible from that point, find another at which the two scales coincide. Count the spaces on each scale between these points and determine the actual distance on the stage micrometer. That distance divided by the number of eyepiece spaces gives the actual length measured by one space on the eyepiece scale. Following is an actual calibration using a 10x eyepiece and a 16 mm (10x) objective:

100 eyepiece spaces = 65 stage spaces, each 0.01 mm long

100 eyepiece spaces = 0.65 mm

1 eyepiece space = 0.0065 mm or 6.5 microns

In the same manner, calibrate all other objectives of the microscope and record the values obtained. Because scale lines on the stage micrometer have appreciable thickness at high magnification, readings are more accurate if taken at their edges in finding points at which the two scales coincide. The calibration value is inversely proportional to the magnification. Thus, the distance measured by one eyepiece space with a 43x (4mm) objective is 10/43 that when the 10x (16 mm) objective is used. Actually, lenses vary slightly from their stated magnifications, making it necessary to calibrate each lens combination. Also, calibrations are accurate for a given instrument only if the length of the body tube remains unchanged.

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Aerosol Droplet Test (Sample)

EYEPIECE DIVISIONS	TALLY	N	DN	%(DN/EDN)	% ACCUM
1	///// ///// ///// ///// //	22	22	03	03
2	///// ///// ///// ///// ///// ///// //	32	64	07	10
3	///// ///// ///// ///// ///// ///// /	31	93	11	21
4	///// ///// ///// ///// ///// ///	28	112	13	34
5	///// ///// ///// ///// ///// ////	29	145	17	51
6	///// ///// ///// ///// /	21	126	15	66
7	///// ///// /////	15	105	12	78
8	///// /////	10	80	09	87
9	///// ///	8	72	08	95
10	////	4	40	05	100
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					
25					
26					
27					
28					
29					
30					
TOTALS					

AVERAGE DROPLET DIAMETER = _____MICRONS
(TOTAL D x N / TOTAL NO. DROPLETS)
VMD calculation : 5 X 5 X 0.5 = 12.5 microns.

See the end of this section for additional measurement instructions.

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Aerosol Droplet Test (Worksheet)

EYEPIECE DIVISIONS	TALLY	N	DN	%(DN/EDN)	% ACCUM
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					
25					
26					
27					
28					
29					
30					
TOTALS					

AVERAGE DROPLET DIAMETER = _____MICRONS
(TOTAL D x N / TOTAL NO. DROPLETS)

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Determination of Particle Size

After the sample of aerosol or spray has been deposited on a slide, it is placed under a microscope and the individual particles are measured with an eyepiece micrometer. A mechanical stage on the microscope is necessary. The diameter as measured on the slide is then corrected for the amount of spread that has taken place, and the diameter of the original sphere is determined.

At least 200 particles should be measured, according to DalaValle (5). The more homogeneous the aerosol or spray, the fewer particles need be counted. All particles should be counted as they are seen in the field. An accurate method is to measure all particles from one edge of a slide to the other and that pass through the micrometer scale as the slide is moved by the mechanical stage. Under some conditions of impingement, particles of the smaller size groups are congregated along the margin of the slide. Measurements in such areas should be avoided.

It is sometimes useful to photograph the particles or to project them on a screen through a microscope. Better results have been obtained, however, by measuring the particles directly as seen in the microscope. It is often more convenient to measure in terms of the divisions of an eyepiece micrometer, and convert these divisions into microns after the median has been determined.

Impinged Slides. Samples may be collected by impingement on a coated slide by waving the slide through an aerosol or spray cloud, or by drawing the aerosol or spray past a slide in fixed position, such as in a wind tunnel. The slide should be nearly perpendicular to the movement of the aerosol or spray. In either case the rate of deposition has been demonstrated to be in ratio to the square of the diameter. This rate of deposition was suggested by the Central Aerosol Laboratory of Columbia University and was based on Sell's law. To compensate for the decrease in the rate of deposition as the particle decreases in size, each diameter is multiplied by the number of particles of that size, f and expressed as the percent of the total of such products. Representative data illustrating this method are given in Table 1.

Table 1. Representative count of aerosol particles impinged on microscope slides.

Diameter (scale Divisions)	**Number of Particles**	**Diameter Times Number of Particles**	**Percent of Total of Column 3**	**Accumulative Percentage**
0.5	2	1	0.2	0.2
1.0	26	26	6.3	6.5
1.5	33	49.5	11.9	18.4
2.0	82	164	39.5	57.9
2.5	34	85	20.5	78.4
3.0	17	51	12.3	90.7
3.5	4	14	3.4	94.1
4.0	5	20	4.8	98.9
4.5	1	4.5	1.1	99.9
Total	**204**	**415.0**		

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The diameter is used in the first power only, since the particles impinge in the ratio to D^2, but the mass diameter is computed on the basis of their volume, which is a ratio to D^3. Therefore, the number of particles is multiplied by D^3/D^2, or by D.

The accumulative percentages from the last column are plotted on the arithmetic probability scales in Figure 1. The 50-percent point of the line so plotted is taken as the median of the particles as they appear on the slide. In this example the 50-percent point has a value of 2 scale divisions, or 30 microns, as each division we predetermined to equal 15 microns.

A correction factor must be determined for each slide. The original spherical droplet as it is impinged on the slide becomes a convex lens, and the extent of its spread from its original shape can be calculated by determining the focal length of the lens so formed. This method is described in Porton Report 2463 (6), a digest of which is appended. In the example cited the correction factor is 0.40; therefore 30 microns x 0.40 gives a median particle diameter of 12.0 microns.

Settled Slides. The median diameter of all the particles collected on a slide by gravitational settling or by electric precipitation is determined by calculating the volume of each particle. The diameter of the particles is measured in microns. The volume is determined by multiplying the cube of the corrected diameter by $\pi/6$, or 0.5236. The volume of the particles of each diameter is expressed as a percentage of the total volume. Representative data illustrating this method are given in Table 2. The accumulative percentages from Table 2 are also plotted on the arithmetic probability scale in Figure 1 and the median particle diameter is determined to be 4.05 microns.

Table 2. A representative count of aerosol particles settled on a microscope slide. (Volume = $1/6\ \pi D^3 = 0.5236D^3$)

Diameter (microns)	Number of Particles	Volume (micron^3)	Percent of Total Volume	Accumulative Percentage
1.4	1	1	0.01	0.01
2.1	55	267	3.3	3.31
2.8	101	1161	14.5	17.81
3.5	50	1119	14.0	31.81
4.2	57	2211	27.7	59.51
4.9	11	677	8.5	68.01
5.6	20	1838	23.0	91.01
6.3	4	524	60.6	97.61
7.0	1	180	2.3	99.91
Totals	300	7978		

Figure 1. Percentage or total volume of aerosol samples below each stated particle diameter impinged and settled on microscope slides. The mass median diameter is determined from the 50-percent point. The correction factor for spread has been applied to the data for the settled slide (from Table 2) but not the data for the impinged slide (Table 1).

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Spread-Factor Ratios to Determine Correction Factor

f'/2A	Correction Factor	f'/2A	Correction Factor	f'/2A	Correction Factor
1.48	0.60	2.0	0.48	4.0	0.375
1.50	0.58	2.1	0.47	4.8	0.35
1.55	0.55	2.2	0.46	5.0	0.34
1.60	0.54	2.3	0.45	5.5	0.33
1.65	0.53	2.6	0.44	6.0	0.32
1.70	0.52	2.65	0.43	6.8	0.31
1.75	0.51	2.8	0.42	7.0	0.30
1.80	0.50	3.1	0.41	8.0	0.29
1.95	0.49	3.3	0.40	9.0	0.28
				10.0	0.27

Dukes et al. (1993) published correction factors for mosquito adulticides and the following table is provided as a more current and accurate reference.

Table 1. Table of correction factors(2r/2A)[1] for various spread factors (f'/2A)[2] for liquids having a refractive index of 1.5.

f'/2A	.00	.01	.02	.03	.04	.05	.06	.07	.08	.09
1.0	1.00	.98	.96	.95	.94	.93	.91	.89	.88	.86
1.1	.85	.84	.83	.82	.81	.80	.79	.78	.77	.76
1.2	.75	.74	.73	.72	.71	.70	.69	.69	.68	.67
1.3	.66	.66	.65	.64	.63	.63	.62	.62	.61	.61
1.4	.60	.60	.59	.59	.59	.58	.58	.58	.57	.57
1.5	.57	.56	.56	.56	.55	.55	.55	.54	.54	.54
1.6	.54	.53	.53	.53	.53	.53	.52	.52	.52	.52
1.7	.52	.51	.51	.51	.51	.51	.51	.51	.51	.50
1.8	.50	.50	.50	.50	.50	.50	.50	.49	.49	.48
1.9	.49	.49	.49	.49	.49	.49	.48	.48	.48	.48

[1]Correction Factor = $\frac{\text{2 X radius (r) of a spherical droplet}}{\text{diameter of droplet lens (2A)}}$

[2]Spread Factor = $\frac{\text{focal length change (f') from the base to the top of the droplet lens}}{\text{diameter of the droplet lens (2A)}}$

The following tables from Dukes et al. (1993) provide correction factors for common insecticide formulations.

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Table 3. Correction factors for aerosol droplets collected on Teflon® coated microscope slides from six different batch samples[1] of Cythion® determined within a two day period of May, 1993.

DATE[2]	MANUFACTURER BATCH NUMBER	PERCENT MALATHION	Lbs. AI per gallon	CORRECTION FACTOR
08-1479	*	91.0	9.33	0.69
3-18-83	*	91.0	9.33	0.68
2-22-87	*	91.0	9.33	0.69
06-10-88	W805258261	91.0	9.33	0.68
03-27-89	W901248105	91.0	9.33	0.68
05-07-92[3]	300 20122-03	95.0	9.79	0.68

[1]Batch samples of Cythion maintained at JAMSRL for reference.
[2]Date shipment received at JAMSRL, Panama City, Fl.
[3]Distributed by Cyanamid and manufactured by Cheminova of Lemvig, Denmark.
*Batch number not available.

Table 4. Correction factors for aerosol droplets of insecticides, formulations and diluents deposited on Teflon® coated slides.

INSECTICIDE (FORMULATION)	DILUTION RATIO (percent volume)	CORR. FACTOR	INSECTICIDE (FORMULATION)	DILUTION RATIO (percent volume)	CORR. FACTOR
CARBAMATES			PYRETHROIDS		
Baygon 1 MOS	Undiluted	0.66	Biomist 4 + 20	Undiluted	0.56
Ficam ULV	Undiluted	0.66	Omar 10	Undiluted	0.61
			Permanone 10 EC	Undiluted	0.56
ORGANOPHOSPHATES			Permanone 10 EC/Orchex Oil	50.0/50.0	0.55
Baytex	Undiluted	0.75	Permanone 10 EC/Orchex Oil	66.7/33.3	0.57
Baytex/Soybean Oil/Diesel Oil	1.5/33.5/65.5	0.58	Punt 10 R200-ND	Undiluted	0.59
Cythion 91 %	Undiluted	0.68	Pyrocide Fogging Formula	Undiluted	0.61
Cythion 95 %	Undiluted	0.68	SBP 1382 40MF	Undiluted	0.66
Cythion/Diesel Oil	6.0/94	0.58	SBP 1382 49MF/Klearol	9.0/90.0	0.61
Cythion/Soybean Oil	25.0/75.0	0.64	SBP 1382 40MF/Soibean Oil	40.0/60.0	0.59
Cythion/SBP-1382-40MF/Soy Oil	27.7/0.3/75.0	0.61	Scourge/BVA-13 Oil	20.0/80.0	0.65
Cythion/SBP-1382-40MF/HAN	34.0/1.0/65.0	0.70	Scourge/Klearol	59.5/40.5	0.60
Cythion/SBP-1382-40MF/HAN	58.0/1.0/41.0	0.70	Scourge/Klearol	2.9/97.1	0.60
Dibrom 14	Undiluted	0.72	Scourge/Soybean Oil	59.5/40.5	0.63
Dibrom 14/HAN	10.0/90.0	0.63	Scourge/Soibean Oil	2.9/97.1	0.63
Dibrom 14/Soybean Oil	10.0/90.0	0.63			
Dibrom 14/Soibean Oil	25.0/75.0	0.63	OILS and DILUENTS		
Dibrom 8 EC/Water/Nalco-Trol	28.0/71.9/0.1	0.70	BVA-13 Oil	Undiluted	0.54
Dow Mosquito Fogging Conc.	Undiluted	*	Heavy Aromatic Naphtol	Undiluted	*
Dursban One ULV Mosquitocide	Undiluted	0.59	Soybean Oil	Undiluted	0.63
Dursban 1.5 ULV Mosquitocide	Undiluted	0.60			

*Droplets evaporate too rapidly to determine spread factor.

Literature Cited

1. Gibbs, WE. 1924. Clouds and smokes. Philadelphia.
2. Goodhue, LD and WN Sullivan. 1943. Making and testing aerosols. American Association for the Advancement of Science. Publication 20: 157-162.
3. Goodhue, LD, PT Diamond and RL Riley. 1945. Determination of particle size of liquefied gas as aerosols. U.S. Department of Commerce. O.T.S., PB 76015.
4. Goodhue, LD and RL Riley. 1946. Particle-size distribution in liquefied-gas aerosols. Journal of Economic Entomology. 39: 223-226.
5. DalaValle, JH. 1943. Micrometrics. New York.
6. May, KR. 1942. The Cascade Impactor--an apparatus for sampling solid and liquid particulate clouds. British Commonwelth Scientific Office. Porton Report. 2463, 7 pp.
7. Dukes, J C, CB Rathburn and CF Hallmon. 1993. Aerosol droplet correction factors for mosquito adulticides: a review and modifications to the focal length change method utilizing Teflon® coated microscope slides. J. Florida Mosq. Cont. Assoc. 64 (2): 67-72.

ED_002896_00040479-00063

(A digest of Porton Report No. 2463)

1. Use a compound high-power microscope.
2. Use a flat mirror.
3. Remove condenser.
4. Use outside light.
5. Focus on particle, and measure and record exact diameter.
6. Set reading on fine focus adjustment at zero.
7. Manipulate coarse focus adjustment and mirror until some distant object (window frame) is in as sharp a focus as possible, using the drop as a lens.
8. Then focus downward with fine focus adjustment until the drop is in clear focus. The difference between the No. 6 reading of the fine focus adjustment (zero) and the no. 8 reading is the focal-length change.
9. Compute f' (focal-length change) and 2A (diameter of particle)

Example: The diameter of a particle covering 4 divisions in an eyepiece micrometer (1 division = 15.4 microns) would be 4 x 15.4 microns, or 61.6 microns. With a focal-length change of 206 microns, the spread factor of the particle would be 206/61.6, or 3.3.

With this factor of 3.3, the correction factor (f'/2A) for this drop would be 0.40 (see below for spread-factor ratios).

Additional tips for measuring and analyzing droplets

(1) Measure 100-200 droplets on an exposed slide. Several horizontal sweeps or observation of isolated portions, selected at random, near the center of the slide will be satisfactory. Mark the counts in groups of five to simplify the procedure. A laboratory type hand tally counter or similar device will aid in maintaining a running total of droplets. Record the individual size totals and the grand total at the bottom of the (N) column.

(2) Using ten droplets counted as part of the sample, determine the spread factor by the method described below. Droplets measured with a diameter of five or more divisions on the ocular scale will yield the most accurate results.

(3) Multiply the number of droplets in the (N) column by the eyepiece divisions for each size recorded. For example, 32 drops x 2 eyepiece divisions = 64 (Encl. 4). Record this number in the column marked "DN".

(4) Total the numbers in the (DN) column and record the number at the bottom of the page, for example 859.

(5) Divide each number in the % DN/EDN column by the (DN) total, and round the answers to the nearest whole number. For example 22 divided by 859 = 0.0256 = 0.03, or 3%. Record this number in the (% DN/EDN) column.

(6) Accumulate the total by adding each number in the (% DN/EDN) column to the (% ACCUM) number in the extreme right hand column. For example: 03+07 = 10; 10+11=21. The total of this column must equal 100 percent.

ED_002896_00040479-00064

(7) Using semi-log graph paper, plot the accumulated percent for eyepiece division opposite the eyepiece number (vertical axis). Plot each number until 100% is reached.

(8) Using a straight edge, draw a line through the dots for the "best fit."

(9) To determine the VMD, draw a vertical line from the 50% mark on the cumulative percent (horizontal) axis to the point where it intersects with the line drawn in (h) above. In this case, the intersection point is exactly at the five (5) eyepiece division mark.

(10) Multiply this number by the calibrated size of the eyepiece division in microns. For the microscope used in this example, each eyepiece division equals five (5) microns, therefore 5 x 5 = 25 microns.

(11) Using the computed spread factor, correct for droplet spread. In this example 25 (microns) x 0.5 (computed spread factor) = 12.5 microns VMD. Computation of the spread factor for this slide is not shown, but selected for simplified calculations.

(12) Percentage of droplets above or below a given size may be computed by referring to the chart. For example, if the percent less than 18 microns is to be determined, divide 18 by 5 (microns in each eyepiece division), and then divide it again by the spread factor. 18 divided by 5 = 3.6, divided by 0.5 = 7.2. Locate 7.2 on the eyepiece division axis using a straight edge, and move across until this line intersects with the line drawn in (8) above. Draw a vertical line down from this point to locate the percentage, in this case 82%.

(13) Statistical Evaluation of Recovered Droplet Data. For routine evaluation of droplet data, collecting particles on impinged slides is satisfactory. Data recovered from settling chamber studies require the third power, or cube of the diameter, to be employed for particle assessment. This procedure is more involved mathematically. Additionally, there are a variety of statistical analyses that can be used to interpret the data.

ED_002896_00040479-00065

APPENDIX F

SOURCES OF SUPPLY FOR ULV AEROSOL INSECTICIDE SPRAY EQUIPMENT AND NOZZLES*

A. Primary ULV Dispersal Equipment Manufacturers

1. Beecomist Systems Incorporated, 31 Meeting House Rd, Telford, PA 18969 (215) 542-8909, FAX: (215) 721-0751. AGULVE, PROMIST 15 and 25.

2. Clarke, 159 North Garden Ave., Roselle, IL 60172 (630) 894-2000, FAX: (800) 832-9344. Truck and hand-held sprayers.

3. Curtis-Dyna Products, P.O. Box 297, Westfield, IN 46074 (317) 896-2561. Truck and hand-held sprayers.

4. London Fog Company, 505 Brimhall Ave., Long Lake, MN 55356 (612) 473-5366, FAX: (612) 473-5302. Truck and hand-held sprayers.

*Mention of a product or supply source does not constitute an endorsement of the products named or criticism of similar ones not mentioned. Mention of trade names does not constitute a guarantee or warranty of the products by the AFPMB, the Military Services, or the Department of Defense.

ED_002896_00040479-00066

B. Additional Spray Equipment Manufacturers**

Aakar Exports
401, Shantam Complex, Near Ageta Tennis Court,
Ahmedabad, Gujarat- 380 052, INDIA
Tel: 91-79-27461944
Telifax: 91-79-40054009
Email: shethmalay@hotmail.com, aakarexports@hotmail.com
http://www.indiamart.com/agriculturesprayer

ADAPCO Inc. (NEW info)
550 Aero Lane
Sanford, Florida 32771-6342
Contact: Bill Reynolds
Office: (800) 367 0659
Fax: (866) 330 9888
breynolds@e-adapco.com
http://www.adapcoinc.com
(OLD Adapco Inc. Info:
2800 South Financial Court
Sanford, Florida 32773-8118
Tel: (407)330-4800)

Ag Spray Equipment, Inc.
1100 New Industry Lane
Hopkinsville, KY 42240
Tel: 270-886-0296, 800-637-7172
fax: 270-885-7392
agspray@agspray.com
http://www.agspray.com/

AgSouth
P.O. Box 627
Union City, Tennessee 38281
Tel: 1-731-885-3806
Fax: 1-731-885-3813
info@agsouthco.com
http://www.agsouthco.com/

AgTec
2811 U.S. 31
Plymouth, IN 46563
Tel: (574) 784-2905
Fax: (574) 784-3284
http://www.agtecsprayers.com/

Air-O-Fan
507 East Dinuba Avenue
Reedley, California, 93654
Tel: (559) 638-6546
Fax: (559) 638-9262
info@airofan.com
http://www.air-o-fan.com/

Allman-Sprayers Ltd
Birdham Road
Chichester
West Sussex
PO20 7BT
Tel: +44 (0) 1243 512511
Fax: +44 (0) 1243 511171
sales@allman-sprayers.co.uk
http://www.allman-sprayers.co.uk/

American Spring & Pressing Works Pvt Ltd
Post Box. No.7602, Aspee House, B.J. Patel Road, Malad West,
Mumbai - 400 064., Maharashtra, India
Phone : +91-22-28822331, 32, 33, 35
Fax : +91-22-28822336
aspee@aspee.net
http://www.aspee.com/

AMR Industries, Inc.
12734 Branford St., No. 20
Arleta, CA 91331
Tel: 818-834-4480
Fax: 818-834-4490
http://www.amrindustriesinc.com

AMS Inc.
105 Harrison Street
American Falls, ID 83211
Tel: 800-635-7330, 208-226-2017
Fax: 208-226-7280
ams@ams-samplers.com
http://www.ams-samplers.com/main.shtm

ARRO-GUN Spray Systems
7575 Tamra Drive
Reno, Nevada 89506
Tel: 775-972-4782
Fax: 775-972-8254
Contact: Dan Ariaz
dariaz@aol.com
www.arro-gun.com

B & G Chemical & Equipment Company, Inc.
10539 Maybank
Dallas, TX 75220
Contact: Emmitt Bewley
Tel: 214-357-5741, 800-345-9387
phoenixfogger@aol.com
http://www.phoenixfogger.com/
http://www.stingersprayssystems.com/

BEI, Incorporated
1375 Kalamazoo Street
South Haven, MI 49090
Tel: 269-637-8541
Fax: 269-637-4233
info@bei-inc.com

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http://www.bei-inc.com/

Berthoud Sprayers
EXEL Gsa – BP 424 - 69653
VILLEFRANCHE s/s
Cedex, FRANCE
Tél : +33 (0)4 74 62 48 30
Fax : +33 (0)4 74 62 37 51
Contact : Mr Henry Ormancey
http://www.berthoud.fr/default_gb.htm

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Fax: +41 (0)56 485 81 82
info@birchmeier.com
http://www.birchmeier.com/

Braglia Srl
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Masone - Reggio Emilia, Italy (UE) - P.I.
00443530357
Tel: +39 0522 340648
Fax: +39 0522 345025
braglia@braglia.it
http://www.braglia.it/database/braglia/braglia.nsf?OpenDatabase

Bubco Incorporated
816 "B" Black Diamond Way
Lodi, CA 95240
Tel: 209-367-3885, 800-474-2532
Fax 209-367-3895
info@bubco.com
http://www.bubco.com/

Buffalo Turbine
180 Zoar Valley Road
Springville, NY 14141
Tel: 716-592-2700
Fax: 716-592-2460
info@buffaloturbine.com
http://www.buffaloturbine.com/

C & S Turf Care Equipment
6207 Dressler Road N.W.
North Canton, Ohio 44720
Tel: 330-966-4511, 1-800-872-7050
Fax: 330-966-0956
http://www.csturfequip.com/Default.aspx

Chema Industries
37 Victor Emmanuel Square – Smouha
Alexandria 21615
EGYPT
Tel: +203 424 1313
Fax: +203 429 2120
Contact: Mrs. Karar
Email: chema@elisra.net
http://www.chema.com.eg

C.I.M.A. S.p.A. - Loc. Molino Quaroni
27040 - Montu' Beccaria (Pv) - Italia
tel. +39-0385-246636
fax +39-0385-246637
export@cima.it
http://www.cimaitalia.it/

Clarke Mosquito Control
110 E. Irving Park Rd.
4th Floor
Roselle, Illinois 60172
Tel: (800) 323-5727
Fax: (800) 832-9344
Email: info@clarkemosquito.com
http://www.clarkemosquito.com/

Contree Sprayer Products
W9898 Jackson Rd
Beaver Dam, WI 53916
Tel: 800-433-3579
Fax: 920-356-0228
contree@contree.com
http://www.contree.com/

Croplands Equipment
PO Box 2441
Dry Creek SA 5441 Australia
Tel: 1800 999 162
Fax: 1800 623 778
jorg.kitt@au.nufarm.com
http://www.croplands.com.au/

Curtis Dyna-fog Ltd
P.O. Box 297
17335 US 31 North
Westfield, IN 46074-0297
Tel: 317/896-2561
Fax: 317/896-3788
info@dynafog.com
http://www.dynafog.com/home.html

Deere & Company
One John Deere Place
Moline IL 61265-8098
Tel: 309-765-8000
http://www.deere.com/en_US/ProductCatalog/FR/category/FR_SPRAYERS.html

DEMCO • Dethmers Mfg Company
4010 320th Street, P.O. Box 189
Boyden, IA 51234-0189
Tel: (712) 725-2311, 800-543-3626
Fax: (712) 725-2380
sales@demco-products.com
http://www.demco-products.com/

Durand-Wayland, Inc.
P.O. Box 1404

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LaGrange GA 30241
Tel: 800 241-2308 (USA & Canada), 706 882-8161
Fax: 706 882-0052
http://www.durand-wayland.com/

Enviromist
PO Box 1246 Berri 5343 South Australia
Tel: 1800624044, +61 (08) 85824077
Fax: +61 (08) 85823857
sales@enviromist.com.au
http://www.enviromist.com.au/

Epoca S.p.a
Via S. Andrea, 24 Z.I. Loc.
San Lazzaro 36061 Bassano del Grappa (VI) - Italy - P.IVA 00597620244
Tel: +39 0424 884911
mailto:info@sprayers.it
http://www.sprayers.it/

FIMCO
800 Stevens Point Drive
Suite DD836
Dakota Dunes, SD 57049
Tel: 800-831- 0027
Fax: 800- 494-0440
http://www.fimcoindustries.com/

Flexicoil
flexicoil@minacs.com
http://www.flexicoil.com/field_sprayers.asp

Fogmaster Corporation
1051 SW 30th Avenue
Deerfield Beach, FL 33442
Tel: 954/ 481-9975
Fax: 954/ 480-8563
info@fogmaster.com
http://www.fogmaster.com/

F/S Manufacturing Inc.
1102 Center St.
West Fargo, ND 58078
Tel: 701-281-1729, 1-800-333-2314
Fax: 701-281-1412
fsmfg@fsmfg.com
http://www.fsmfg.com/
Garrards Pty Ltd.
P.O. Box 5477
Brendale MDC
Queensland 4500
Tel: (07) 3881 1693
Fax: (07) 3881 1781
http://www.garrards.com.au/Rega_Pumps_and_Sprayers.cfm

Gempler's
P.O. Box 44993
Madison, WI 53744-4993
Tel: 1-800-382-8473
Fax: 1-608-662-3301
customerservice@gemplers.com
http://www.gemplers.com/

GNC Industries, Inc.
1808 Airport Rd.
Pocahontas, AR 72455
Tel: 1-800-462-2005
Fax: 1-870-248-9905
sales@gnci.org
http://www.gncindustries.com/

Goldacres
1 Northwestern Rd, ST Arnaud VIC 3478
Telephone: 03 5477 3999
Fax: 03 5477 3908
http://www.goldacres.com.au/company

GPS Sprayers Ltd
3C Station Yard
Thame, Oxfordshire, OX9 3UH
Tel: UK 01844 215533
Fax: UK 01844 215577
enquiries@gps-sprayers.co.uk
http://www.gps-sprayers.com/

GreenTech
108-112 Wing Street,
WINGFIELD,
South Australia 5013
Tel: (08) 8359 5362
Fax: (08) 8359 5364
sales@greentechspraysystem.com
http://www.greentechspraysystem.com/

Gremar, Inc.
4800 Corporate Drive
P.O. Box 65003
West Des Moines, IA 50265
Tel: 800-597-3867
Fax: 515-221-9243
info@gremarinc.com
http://www.gremarinc.com/

Hagie Manufacturing Company
P.O. Box 273
Clarion, IA 50525-0273
Tel: 515-532-2861, 800-247-4885
Fax: 515-532-3553
http://www.hagie.com/english/

Hardi® West Coast
8550 W. Roosevelt Avenue
Visalia, California 93291-9458
Contact: Tom Kinzenbaw
Phone: 563-386-1730, 559 651-4016
Fax 559 651-4160
tlk@hardi-us.com

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http://www.hardi-us.com/html/hardir_north_america.html

Horvick Incorporated
4350 48th Ave. North
Fargo, ND 58102-7404
Tel: (800) 373-4084, (701) 280-2862
catalog@horvick.com
http://www.horvick.com/

Howe Enterprises, LCC
36880 Washington Loop Road
Punta Gorda, FL 33982
Tel: 239-707-8585
killmosquito@yahoo.com
http://www.killmosquito.net/

H. D. Hudson Manufacturing Company
500 N. Michigan Avenue
Chicago, IL 60611-3669
Tel: 800-977-7293
ladybug@hdhudson.com
http://www.hdhudson.com/

Hypro
375 5th Ave NW
New Brighton, MN 55112
Tel: 800-424-9776
Fax: 800-323-6496
sales@hypropumps.com
http://www.hypropumps.com/spray/en-us

Idromeccanica Bertolini Spa
Via F.lli cervi, 35/1 - 42100
Reggio Emilia (RE) - ITALY
Tel. +39 0522-306641
Fax +39 0522-306648
email@bertolinipumps.com
http://www.bertolinipumps.com/nuovosito/

J.F. Oakes
Tel: 662-746-7276
Fax: 662-746-4568
sales@jfoakes.com
http://www.jfoakes.com/

Jacto Inc.
9650 SW Herman Rd
Tualatin, Oregon 97062
Tel: (503) 885-8723, (800) 522-8610
Fax: (800) 511-3671
info@jacto.com
http://www.jacto.com/

John Bean Sprayers
P.O. Box 1404
LaGrange GA 30241
Tel: 800 241-2308 (USA & Canada), 706 882-8161
Fax: 706 882-0052
http://johnbeansprayers.durand-wayland.com/

Joseph H. Chalfoun
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Telfax: 961- 9- 444390/444116/440257
chalfoun@dm.net.lb
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KB Manufacturing, LLC
35545 290th St. SW
Fisher, MN 56723
Tel: 218-281-7435
kbmfg@hughes.net
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Kellands Agricultural Limited,
Brimpsfield Rd, Birdlip,
Gloucestershire, GL4 8JH
Tel: 01452 863900
Fax: 01452 863400
http://www.kellands.co.uk/

Kisan76
info@kisan76.com
http://www.kisan76.com/

Knight Farm Machinery Ltd
Tel: +44 (0)1780 722200
Fax: +44 (0)1780 722201
mail@knight-ltd.co.uk
http://www.knight-ltd.com/

Leinbachs, Inc
P.O. Box 11786 Winston-Salem N.C. 27116
100 Northstar Drive Rural Hall N.C. 27045
Tel: (336) 969-0359
leinbachs@leinbachs.com
http://www.leinbachs.com/inc.html

London Foggers
505 Brimhall Avenue
P.O. Box 406
Long Lake (Minneapolis), MN 55356
Tel: 952-473-5366
Fax: 952-473-5302
bbonnett@usinternet.com
http://www.londonfoggers.com/

MAROLEX Sp.z o.o.
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05-092 Dziekanów Leśny k. Warszawy
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Tel: (+48 22) 732 23 53
info@marolex.pl
http://www.garden-sprayers.com/htm_en/gardening_agriculture_household_sprayers/

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Maruyama U.S., Inc.
Post Office Box 2107
Denton, TX 76202
Tel: 940 383-7400
Fax: 940 393-7466
maruyama@maruyama-us.com
http://www.maruyama-us.com/

The McGregor Company
Equipment Manufacturing Division
28232 Endicott RD, P.O. Box 740
Colfax, WA 99111
Tel: (509)397-4360, (800)727-9160
Fax: (509)397-6306
mcgregoreq@mcgregor.com
http://www.mcgregoreq.com/

Metallurgy Prapopoulos Bros S.A.
Aristotelous 123
263 35, Patras, Greece
Tel: +30 - 2610 - 641642
Facsimile: +30 - 2610 – 641641
info@prapopoulos.gr
http://www.prapopoulos.gr/

Michigan Metalcraft, LLC
151 W. Main St. P.O. Box 176
Farwell, Michigan 48622
Tel : 989-588-4383
Fax: 989-588-3193
info@farmspraying.com
http://www.farmspraying.com/

Micron Sprayers Ltd
Three Mills, Bromyard
Herefordshire, HR74HU
ENGLAND
Tel: +44 (0) 1885 482397
Fax: +44 (0) 1885 483043
Contact: John Clayton, Tim Sander
Email: micron@micron.co.uk
http://www.micron.co.uk

New Mountain Innovations
22 Pennsylvania Avenue
Niantic, CT 06357
Tel: 860-691-1876
Contact: Herb Nyberg
info@larvasonic.com
http://www.larvasonic.com/

Norris-Wiener, Inc., Bay State Spray Equipment
90 Chelmsford Rd.
North Billerica, MA 01862-1345
Tel: 800-553-2501
Fax: 978-670-5633
http://www.norriswiener.com

On-Target Spray
31421 SW Olympic
Drive
Wilsonville, Oregon 97070
Tel: (503) 329-8120
Fax: (503) 682-9496
info@ontargetspray.com
http://www.ontargetspray.com/

PBM Supply & Mfg., Inc.
Manufacturing, Distribution, Sales and Repair Facility
324 Meyers Street
Chico, CA 95928
Toll Free: 800-688-1334
Fax: 530-345-9903
pbm@pbmsprayers.com
http://www.pbmsprayers.com/

Public Health Equipment and Supply Co.
P.O. Box 10458
San Antonio, TX 78210
Phone: 210-532-3846, 800-284-0106
Fax: 210-532-9703
phesco@phesco.com
http://www.phesco.com/

Pioneer West Inc
64088 McDonald Lane
La Grande, Oregon 97850
Tel: (541) 663-WEST
http://www.pioneerwestinc.com/

Progressive Ag, Inc
1336 McWilliams Way
Modesto, CA 95352
Tel: 800-351-8101, 209-567-3232
Fax: 209-567-3233
http://www.proaginc.com/

Proptec
11429 Upton Road
Bath, MI 48808
Tel: 866-641-4671, 517-641-4671
Fax: 517-641-4671
sales@proptec.com
http://www.proptec.com/

R & D Sprayers
419 Hwy 104
Opelousas, LA USA 70570
Tel: 337-942-1001
Fax: 337-942-7841
rdspray@co2sprayers.com
http://www.co2sprayers.com/

Raz Sprayers
10 Ben David st. Ind. Zone
Rishon-Le-Zion Israel 75130
Tel: 972-3-9643252

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Fax:972-3-9643239
http://www.razspray.com/

Really Innovations
605 Townsend Rd
Cocoa, FL 32926
TeL 800-940-8024
Fax 321-639-6927
really.nolen@reallyinnovations.com
http://www.reallyinnovations.com/

Redball, LLC
140 30th Ave SE
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Benson, MN 56215
Tel: 877.332.2551, 320.843.4932
Fax: 320.843.2503
info@redballproducts.com
http://www.redballproducts.com/

RHS, Inc.
2005 West Oregon Street
Hiawatha, KS 66434
Tel: (800) 247-3808
Fax: (785) 742-7174
mhanzlicek@rhs-inc.com
http://www.rhs-inc.com/

Sands Agricultural Machinery Ltd
Main Road, Brunstead, Stalham, Norwich,
Norfolk
England NR12 9ER
Tel: +44 (0)1692 580522
Fax: +44 (0)1692 583324
sales@samltd.co.uk
http://www.samltd.co.uk/

Silver Wheels
511 E Main St.
St. Nazianz, WI. 54232-0127
Tel: 800.247.5557
http://www.silverwheels.com/

Slimline Manufacturing
559 Okanagan Ave., East
Penticton, British Columbia
Canada, V2A 3K4
Tel: 1-800-495-6145, 1-250-493-6145
Fax: 1-250-492-7756
http://www.turbomist.com/

Spectrum Electrostatic Sprayers Inc.
7500 San Felipe, Suite 860
Houston, Texas, USA 77063
Tel: (713) 783-5771
Fax: (713) 783-5772
sesinc@spectrumsprayer.com
http://www.spectrumsprayer.com/

Spray Innovations Inc.
1107 17th Street
Columbus, NE 68601
Tel: 402-564-6002
Fax: 402-564-7005
spray@sprayinnovations.com
http://www.sprayinnovations.com/

Sprayer Specialties, Inc.
5149 NW 111th Drive
Grimes, IA 50111
Tel: 800-351-1587
http://www.sprayers.com/

Swingtec GmbH
Achener Weg 59,
P.O Box 1322,
Isny – Germany 88307
Tel: +49 (0) 7562 708-0
Fax: +49 (0) 7562 708 111
info@swingtec.de
www.swingtec.de

Taizhou Luqiao Ziqiang Sprayer Factory
Hardware & Plastic Industrial Park, Pengjie
Town, Luqiao Zone, Taizhou, Zhejiang, China
Tel: 86-576-2723679
Fax: 86-576-2723678
ziquiang@zqsprayer.com
http://www.zqsprayer.com

Wylie Sprayers
702 East 40th Street
Lubbock, TX 79404
Tel: 888-249-5162, 806-763-1335
Fax: 806-763-1092
jimt@nts-online.net
http://www.wyliesprayers.com/

Yuma Farm & Home Supply
2222 East 16TH Street
Yuma, AZ
Tel: (928) 329-9113
Fax: (928) 329-0281
yumafarmhome@aol.com
http://sunsprayers.com/

Yung Chi Y. C. Industries
No.23-1, Huei Lai St.
Yuan Lin Town Changhua Hsien, 510 Taiwan
Tel: 886-4-8724620
Fax: 886-4-8726501
webmaster@yungchi.com.tw
http://www.yungchi.com.tw

C. Nozzle Manufacturers**

ABB C-E Services, Inc.
200 Great Pond Dr.

ED_002896_00040479-00072

Windsor, CT 06095
Phone: 860-285-5400, 800-243-1946
Fax: 860-285-9611

Air Bubble Jet
361 York Road West, Yorkton, SK
Phone/Fax: 306-782-8362
kelly@becquet.com
http://www.becquet.com/kelly/gvm00.htm

AllSpray
P.O. Box 87707
Carol Stream, IL 60188-7707
Tel: 630-933-0123
Fax: 630-681-9396
info@AllSpray.com
http://www.allspray.com

BETE Fog Nozzle, Inc.
50 Greenfield Street
Greenfield, MA 01301
Tel: 413-772-0846, 800-235-0049
Fax: 413-772-6729
techservice@bete.com
http://www.bete.com/

BEX Incorporated
37709 Schoolcraft Road
Livonia, Michigan 48150-1009
Tel: 734-464-8282
Fax: 734- 464-1988
Sales@bex.com
http://www.bex.com/

Bowles Fluidics Corp.
6625 Dobbin Rd.
Columbia, MD 21045
Phone: 410-381-0400
Fax: 410-381-2718
sales@bowlesfluidics.com
http://www.bowlesfluidics.com

Corona Integrated Technologies Inc
6215 Overstone Drive
West Vancouver, BC
Canada V7W 1X7
Tel: 888-878-9433
Fax: 604-738-9918
Contact: Mike Baxter
http://www.smokemachines.com/

CP Products Co., Inc.
418 S. Price Road
Tempe, AZ 85281
Tel: 480/303-0600
Fax: 480/303-0909
cjbaecker@cpproductsinc.com
http://www.cpproductsinc.com/

Delavan
P.O. Box 969-T
Bamberg, SC 29003
Phone: 800-982-6943 (toll free)
Fax: 803-245-4146
http://www.delvaninc.com

Greenleaf Technologies
P.O. Box 1767, Covington, LA, 70434
Tel: 1-800-881-4832, 985-892-3870
Fax: (985) 898-0336
sales@turbodrop.com
http://www.turbodrop.com/

Iontech, Ion Technologies Corp.
4815 Para Dr.
Cincinnati, OH 45237
Phone: 513-641-3100
Fax: 513-641-3560
info@ion-tech.com
http://www.ion-tech.com

Jasmic
PO Box 369
Hazlet, New Jersey 07730
Tel: 888-452-7642
Fax: 888-732-5552
info@jasmic.net
http://www.jasmic.net/

Jireh Enterprise
2317 South Street
Toms River, NJ 08753
http://www.jirehenterprise.com

Lechler Inc.
445 Kautz Rd.
St. Charles, IL 60174
Tel: 630-377-6611
Fax: 630-377-6657, 800-444-7069
info@lechlerag.com
http://www.lechler.com

Micronair (contact Micron Sprayers Ltd)
Three Mills, Bromyard
Herefordshire, HR74HU
ENGLAND
Tel: +44 (0) 1885 482397
Fax: +44 (0) 1885 483043
Email: micron@micron.co.uk
http://www.micron.co.uk

National Environmental Service Company
7 Hampshire Drive
Mendham, NJ 07945
Tel: 973 543 4586
http://www.drdust.com

Orthos Liquid Systems, Inc.

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POB 1970
Bluffton, SC 29910
Tel: 843-987-7200
Fax: 843-987-7203
http://www.duesen-schlick.com

Quality Spray Products Co.
478 W. Wrightwood Ave.
Elgin, IL 60120
Tel: 630-530-0170
Fax: 630-530-4571

Pem Fountain Co.
P.O. Box 32565, RPO Village Gate
Richmond Hill, ON L4C0A2
Phone: 905-737-7264
Fax: 905-884-8941
sales@pemfountain.ca
http://www.pemfountain.com

PNR America LLC
One Civic Center Plaza, Suite 508
Poughkeepsie, NY 12601
Tel: 866-625-8458
Fax: 845-483-7755
tsnyder@pnramerica.com
http://www.pnramerica.com

Pro Dyne Corp.
136 Green Tree Rd.
Phoenixville, PA 19460
Phone: 888-776-3963 (toll free)
Fax: 610-789-7007
prodyne@prodynecorp.com
http://www.prodynecorp.com

Rexnord Industries
P.O. Box 2022
Milwaukee, WI 53201
Tel: 414-643-2320, 866-739-6673
Fax: 414-643-3078
http://www.rexnord.com

Root-Lowell Manufacturing Co.
1000 Foreman Road · P.O. Box 289
Lowell, MI 49331-0289
Tel: 616-897-9211
Fax: 616-897-8223
dharvey@rlflomaster.com
http://rlflomaster.com/

Spraying Systems Co.
Mobile Systems Division
PO Box 7900
Wheaton, IL 60189-7900
Tel: 630-665-5000
http://www.teejet.com/ms/teejet/

Steinen, William Manufacturing Co.
29 E. Halsey Rd.
Parsippany, NJ 07054
Phone: 973-887-6400, 800-724-3343 (toll free)
Fax: 973-887-4632
info@steinen.com
http://www.steinen.com

Waterbury Companies, Inc.
P.O. Box 1812
Waterbury, CT 06722
Tel: 800-845-3495
customerservice@watco.com
http://www.timemist.com/

Wilger Inc.
255 Seahorse Drive
Lexington, TN 38351-6538
Tel: (877)968-7695, (731)968-7695
Fax: (877)968-7613, (731)968-7613
wilgeresc@bellsouth.net
http://www.wilger.net/home.html

**Mention of a product or supply source does not constitute an endorsement of the products named or criticism of similar ones not mentioned. Mention of trade names does not constitute a guarantee or warranty of the products by the AFPMB, the Military Services, or the Department of Defense. Thanks to CDR (U. S. Navy Ret.) Joseph Conlon for supplying these last two lists.

ED_002896_00040479-00074

Message

From: Sims, Diann [Sims.Diann@epa.gov]
Sent: 4/26/2018 2:23:42 PM
To: Perry, Tracy [Perry.Tracy@epa.gov]; Garber, Kristina [Garber.Kristina@epa.gov]
Subject: FW: CADPR Contact

Flag: Flag for follow up

See below the link that CADPR shared with NFMS previously. Just FYI. We don't need to do anything with it at this point.

From: Wilhoit, Larry@CDPR [mailto:Larry.Wilhoit@cdpr.ca.gov]
Sent: Wednesday, April 18, 2018 11:44 AM
To: 'Tony Hawkes - NOAA Federal' <tony.hawkes@noaa.gov>
Cc: Steinmann, Kimberly@CDPR <Kimberly.Steinmann@cdpr.ca.gov>; cathy.tortorici@noaa.gov; David Baldwin <David.Baldwin@noaa.gov>; Nancy Golden <nancy_golden@fws.gov>; George Noguchi <george_noguchi@fws.gov>; Sims, Diann <Sims.Diann@epa.gov>
Subject: RE: CADPR Contact

Tony,

Yes, I did retire, a little over a year ago. However, I am working part time as a retired annuitant. Kim Steinmann has taken my position at DPR and she can answer most of your questions.

I am not sure what file you are referring to, but I have uploaded 3 files with malathion data that I sent you in the past and another file with data such as percent acres treated for all AIs in 2015; these are in one zip file at https://www.dropbox.com/s/tcn6f1fj31xc5za/malathion_all.zip?dl=0. I believe Kim as created a file with PUR statistics by AI for 2016, and if you want that, you can ask her.

You have my permission to use the data in any of these files. Let me know if this is what you need or if you have questions about the data.

Hope all is good with you,
Larry

From: Tony Hawkes - NOAA Federal <tony.hawkes@noaa.gov>
Sent: Tuesday, April 17, 2018 2:53 PM
To: Wilhoit, Larry@CDPR <Larry.Wilhoit@cdpr.ca.gov>; Sims.Diann@epa.gov
Cc: Cathy Tortorici - NOAA Federal <cathy.tortorici@noaa.gov>; David Baldwin <David.Baldwin@noaa.gov>; Nancy_Golden <Nancy_Golden@fws.gov>; George Noguchi <George_Noguchi@fws.gov>
Subject: Fwd: CADPR Contact

Hi Larry, Diann Sims at EPA suggested I contact you. Didn't you retire a few years ago?

The agencies have been working together evaluating the available pesticide usage data. You generated a spreadsheet for EPA with California usage data that included calculations of percent crop treated with malathion and possibly other chemicals. Diann did not want to share the spreadsheet with us without your approval first. She suggested we contact you and request it directly. Can you please provide this spreadsheet to us? We'd like to understand how the values where calculated as well as any qualifiers you have listed concerning the values.

Thank you,
Tony

---------- Forwarded message ----------
From: **Sims, Diann** <Sims.Diann@epa.gov>
Date: Tue, Apr 17, 2018 at 11:54 AM
Subject: CADPR Contact
To: "tony.hawkes@noaa.gov" <tony.hawkes@noaa.gov>
Cc: "Becker, Jonathan" <Becker.Jonathan@epa.gov>, "Perry, Tracy" <Perry.Tracy@epa.gov>, "Garber, Kristina" <Garber.Kristina@epa.gov>, "Atwood, Donald" <Atwood.Donald@epa.gov>

Tony,

Here is the contact. Please share as appropriate. Larry.Wilhoit@cdpr.ca.gov

--
Tony Hawkes, PhD
National Marine Fisheries Service
Office of Protected Resources
510 Desmond Drive SE Suite 103
Lacey, WA 98503

Telephone: (360) 753-4374
tony.hawkes@noaa.gov

Message

From: Lennartz, Steven [Lennartz.Steven@epa.gov]
Sent: 6/28/2018 6:21:19 PM
To: Connolly, Jennifer [Connolly.Jennifer@epa.gov]; Garber, Kristina [Garber.Kristina@epa.gov]
Subject: RE: [EXTERNAL] follow up on ag data relevant to HI
Attachments: 2015AgBaseline_Protocols.pdf

We were previously using the 1980 HI land cover data, which had several agriculture land cover classes. We had opted for the NOAA cultivated class because it was newer. However, there is a 2015 update to the HI land cover which would could consider for future versions. Here's how the classes stack up. I've also attached the metadata.

1980 ALUM Category	2015 Crop Category
Aquaculture	Aquaculture
Avocado	Tropical Fruits
Banana	Banana
Banana/Guava	Diversified Crop
Banana/Other	Diversified Crop
Banana/Papaya	Diversified Crop
Banana/Wetlands	Diversified Crop
Coffee	Coffee
Dairy	Dairy
FIELD CROPS	Diversified Crop
Flowers	Flowers / Foliage / Landscape
Foliage and Nursery	Flowers / Foliage / Landscape
Foliage and Nursery/Forage and Grain	Flowers / Foliage / Landscape
Guava	Tropical Fruits
Macadamia Nuts	Macadamia Nuts
Macadamia Nuts/Other	Macadamia Nuts
ORCHARDS	Tropical Fruits
Other	Tropical Fruits
Papaya	Papaya
Papaya/Guava	Papaya
Pineapple	Pineapple
Sugarcane	Sugar
Vegetables / Melons	Diversified Crop
Vegetables / Melons - Flowers	Diversified Crop
Vegetables / Melons - Foliage and Nursery	Diversified Crop
Vegetables / Melons - Papaya/Macadamia Nuts	Diversified Crop
Wetlands	Taro

From: Connolly, Jennifer
Sent: Thursday, June 28, 2018 1:03 PM
To: Garber, Kristina <Garber.Kristina@epa.gov>
Cc: Lennartz, Steven <Lennartz.Steven@epa.gov>
Subject: RE: [EXTERNAL] follow up on ag data relevant to HI

Deliberative Process / Ex. 5

Does that answer what you are looking for? Deliberative Process / Ex. 5 I think it would be helpful.

Steve, anything to add?

Jen

From: Garber, Kristina
Sent: Thursday, June 28, 2018 10:11 AM
To: Connolly, Jennifer <Connolly.Jennifer@epa.gov>
Subject: FW: [EXTERNAL] follow up on ag data relevant to HI

For HI, Deliberative Process / Ex. 5 ? There's no CDL.

From: Golden, Nancy [mailto:nancy_golden@fws.gov]
Sent: Thursday, June 28, 2018 9:45 AM
To: Garber, Kristina <Garber.Kristina@epa.gov>; Leona Laniawe <leona_laniawe@fws.gov>
Cc: Sims, Diann <Sims.Diann@epa.gov>
Subject: Re: [EXTERNAL] follow up on ag data relevant to HI

Hi Kris,

Deliberative Process / Ex. 5

Leona, does that capture it? Anything else?

Thanks, Nancy

On Wed, Jun 27, 2018 at 10:41 AM, Garber, Kristina <Garber.Kristina@epa.gov> wrote:

> Hi Nancy,

Diann Sims told me that you have some questions about agricultural data for Hawaii. Deliberative Process / Ex. 5

Deliberative Process / Ex. 5 ?

Thanks,

Kris

Kris Garber, Senior Science Advisor

Environmental Fate and Effects Division

Office of Pesticide Programs

US Environmental Protection Agency

Mail Code: 7507P

1200 Pennsylvania Ave NW

Washington DC 20460-0001

Phone: 703-347-8940

Hawaii Statewide Agricultural Land Use Baseline 2015

Metadata and Crop Mapping Protocols

The 2015 Hawaii Statewide Agricultural Land Use Baseline layer was created to provide a snapshot of contemporary commercial agricultural land use activity in Hawaii. It is based upon an assemblage of geospatial datasets, primarily high-resolution WorldView-2 satellite imagery (2011 – 2013) used as a base layer for digitization. Additional datasets used in this work include GIS layers ('Agriculture and Farming', 'Inland Water Resources', and 'Cadastral and Land Descriptions') provided by the state of Hawaii, Office of Planning Statewide GIS Program and other data provided by major land owners and managers. County Real Property Tax and Agricultural Water Use data were also used to identify commercial farm operations. Data for both real property tax assessment and agricultural water use were collected from each county that provided their most recent records, generally from 2014-2015. Not all properties that receive County agricultural tax assessment rates or reduced water cost for agricultural uses were mapped due to the small scale of some of their operations. These data sources were used to verify mapped commercial farms and identify operations that might have been missed using the imagery alone.

Digitized crop locations and boundaries were verified through a combination of on-the-ground site visits, meetings and presentations of draft layers with agricultural stakeholders and landowners, solicitations through a publicly accessible online web mapping portal, and spot-checking using Google Earth™ and other high resolution imagery sources.

The 2015 Hawaii Statewide Agricultural Land Use Baseline layer represents our best efforts to capture the scale and diversity of commercial agricultural activity in Hawaii in 2015 and should be used for informational purposes only.

Sponsor of the 2015 Hawai'i Crop Layer. Funding for the 2015 Hawaii Statewide Agricultural Land Use Baseline was provided by the Hawai'i State Department of Agriculture.

Authors of the 2015 Hawai'i Crop Layer. The University of Hawai'i at Hilo Spatial Data Analysis and Visualization (SDAV) Laboratory carried out the work under the direction of Dr. R.L. Perroy, Project Manager Jeffrey Melrose, and Cartographer and Geospatial Analyst Sylvana Cares. UH Hilo Undergraduate student interns Leilani Yamasaki and Ian Seely also contributed many hours to this work.

No Warranty. We have made every effort to make all images, maps, graphs, data, and other information provided as a result of this project as accurate and error-free as possible. However, we do not guarantee the accuracy of any images, maps, graphs, data, or other information. All content is provided without warranty of any kind and is not intended for any regulatory use whatsoever.

The following protocols were used to generate the 2015 Hawaii Statewide Agricultural Land Use Baseline data layers.

- This project focused on commercial agricultural operations with a three acre minimum crop mapping area. Understanding the importance of smaller scale producers to Hawai'i's agricultural community, commercial operations less than three acres were included where they could be reasonably identified.
- Mapped units follow actual cropped areas as identified in satellite imagery, not TMK parcel boundaries.
- Agricultural lands which did not display actual vegetation growth but appeared to be part of an active agricultural rotation (freshly tilled fields, etc.) were included in acreage summaries.
- For papaya and other crops that rotate every 3-4 years, mapping efforts were focused on active production areas and did not include fallowed fields.
- Packing or processing facilities and in-field access roads were generally included in acreage summaries.
- Homes, reservoirs, un-farmable gulches and major roadways separating field areas were not mapped.
- "Agri-scaping" of private residences was not mapped.
- Small backyard orchards and what appear to be home use vegetable gardens were not mapped
- Equestrian uses, including arenas, boarding and riding facilities, were not mapped.
- Rooster cultivation was not mapped.
- Piggeries were not mapped. Available data on pork production is summarized in the text and figures of the accompanying report.
- Poultry farms were not mapped. Available data on egg and poultry production is summarized in the text of the accompanying report.

Crops categories and considerations

- **Aquaculture** includes active shrimp farms, working fishponds, algae raceways, and research/production facilities.
- **Banana** includes fields in contiguous plantings >2-3 acres. Throughout the state, bananas are also cultivated in gulches, along farm boundaries and in small patches within smaller, diversified farm operations. These smaller mixed plantings are generally incorporated into the surrounding farm and labeled as Diversified Crop.
- **Coffee** includes both larger plantation-type scale operations on Moloka'i, Maui and Kaua'i; and smaller farm planting >2.0 acres in coffee growing regions like Kona, Ka'u and elsewhere. The North and South Kona region on Hawai'i Island were difficult to map accurately based on a practice some farmers have adopted to plant both macadamia nuts and coffee on the same ground. The mature nature of these orchard-dominated agricultural practices in Kona made it challenging to confidently discern these coffee-macadamia crop boundaries from satellite imagery.
- **Commercial Forestry** plantings include both short and long rotation tree crops. Plantings include eucalyptus and other species intended for timber, fiber or energy production and higher value hardwoods like koa and mahogany that are planted for eventual commercial harvest and not part of native forest restoration efforts

- **Dairy** includes core milking and on-farm processing facilities and the surrounding pastures and crop lands under active dairy use.
- **Diversified Crops** includes a wide range of products that are grown either outdoors or in greenhouses. The category includes many of Hawai'i's small farms and much of its local, fresh vegetablesincluding tomato, cucumbers leaf crops, beans, asparagus, etc. This category also includes smaller plantings of crops such as banana, tropical fruit, papaya, coffee etc. that were too small to map out individually. Also included in the category are export crops such as Okinawan sweet potato, basil and Asian spices that make up an estimated 500-1,000 acres of farm land on 'Oahu and South Hilo.
- **Flowers, Foliage and Landscaping** includes a wide variety of products including hot house/shade cloth grown orchids, antheriums, tropical flowers, potted nursery plants, field stock, sod farms, and landscape trees.
- **Macadamia Nut** includes orchards that appear to be actively maintained and harvested. Orchards range from hundreds of acres in South Kona, Ka'ū and Puna to small and mid-size plantings throughout Hawai'i Island. Portions of abandoned planting near Wailuku, Maui, were not counted.
- **Papaya** is a 3-4 year crop and was mapped in areas where significant commercial papaya production occurs. Mapping efforts focused on just those lands that appeared to be planted and in active production. Fallow lands that might be available for future papaya planting were not mapped but are an important part of what the papaya industry needs to produce in a sustainable fashion. Smaller plantings of papaya on mixed-crop farms are mapped as Diversified Crop.
- **Pasture** includes areas in active commercial cattle operation where fencing is apparent and water troughs and/or cattle trails can be identified. Small pasture plots in diverse rural homestead areas were not the focus of this mapping effort but were included where larger parcels and contiguous pasture areas were present. Lands that were formerly in pasture use but have now been purchased by the military (Ke'āmūku, S. Kohala), the National Park Service (Kahuku Ranch, Kau) or withdrawn from pasture in favor of native forest restoration (for example; Keauhou Ranch, Kau or Hakalau, Preserve, Hāmākua) were not included in the pasture layer. Range lands that are fenced and grazed only seasonally are mapped as pasture lands.
- **Pineapples** includes areas planted in large to mid-size operations (primarilyon 'Oahu and Maui). Smaller pineapple plantings that are mixed within smaller diversified farm operations are mapped and labeled as Diversified Crop.
- **Seed Production** includes all arable lands in use by the seed companies at the time of this survey. Net acres actually planted in corn, or other seed crops, will be substantially less than the gross acres depicted. The industry estimates that they use approximately 25% of their farmable land at any time for growing crops. Areas depicted include in-field roads, pollen drift buffers and areas managed for future crop rotation.
- **Sugar** includes areas planted in sugarcane. The majority of this crop is grown by Hawai'i Commercial and Sugar Company (HC&S) in the central plain of Maui. HC&S provided the GIS data they use to manage their agricultural field operations. Several smaller sugar plantings were identified on other islands where the crop is used to produce rum or cane is carved into swizzle sticks for the visitor market.
- **Taro** includes crop grown only in wetland settings. Dry land taro is included in the Diversified Crop category.

- **Tropical Fruit** includes a range of products such asrambutan, avocado, longan, lychee, citrus, cacao and other orchard fruit trees, that are planted for commercial harvest. Small backyard orchards were not mapped and are not counted in summary tropical fruit acreage.

1980 ALUM Layer -> 2015 Crop Category Conversion

The previous statewide agricultural footprint layer prepared for the state of Hawaii, the ALUM (Agricultural Land Use Maps) layer, was released in 1980. ALUM crop categories were used as the basis for the 2015 Agricultural Land Use Baseline, with minor modifications shown below.

1980 ALUM Category	2015 Crop Category
Aquaculture	Aquaculture
Avocado	Tropical Fruits
Banana	Banana
Banana/Guava	Diversified Crop
Banana/Other	Diversified Crop
Banana/Papaya	Diversified Crop
Banana/Wetlands	Diversified Crop
Coffee	Coffee
Dairy	Dairy
FIELD CROPS	Diversified Crop
Flowers	Flowers / Foliage / Landscape
Foliage and Nursery	Flowers / Foliage / Landscape
Foliage and Nursery/Forage and Grain	Flowers / Foliage / Landscape
Guava	Tropical Fruits
Macadamia Nuts	Macadamia Nuts
Macadamia Nuts/Other	Macadamia Nuts
ORCHARDS	Tropical Fruits
Other	Tropical Fruits
Papaya	Papaya
Papaya/Guava	Papaya
Pineapple	Pineapple
Sugarcane	Sugar
Vegetables / Melons	Diversified Crop
Vegetables / Melons - Flowers	Diversified Crop
Vegetables / Melons - Foliage and Nursery	Diversified Crop
Vegetables / Melons - Papaya/Macadamia Nuts	Diversified Crop
Wetlands	Taro

Message

From: Anderson, Brian [Anderson.Brian@epa.gov]
Sent: 11/30/2018 6:23:52 PM
To: Rossmeisl, Colleen [Rossmeisl.Colleen@epa.gov]; Panger, Melissa [Panger.Melissa@epa.gov]; Garber, Kristina [Garber.Kristina@epa.gov]
Subject: agenda

Just sent this to FWS as a proposal.

Hi Patrice,

We've been thinking about how to structure the meeting on the 10th.

We're thinking something like the following? What do you think?

- Introduction: 5 min (maybe you and me set it up?
  - Purpose: We are going to present progress on the action area development related to agricultural uses of malathion.
  - Deliberative Process / Ex. 5
  - Each agency will give their perspective on the pros and cons of the methods at the end of the presentation.

- Presentation – (EPA and FWS). 30 min
- Perspectives on pros and cons of the methodology and recommendations
  - EPA: 10 minutes
  - FWS: 10 minutes
  - NMFS: 10 minutes
  - USDA: 10 minutes
- Discussion: 30 minutes
- Wrap up and next steps: 15 minutes

Brian Anderson
Office of Pesticide Programs
Environmental Fate and Effects Division
703.305.0067

Message

From: Miller, Wynne [Miller.Wynne@epa.gov]
Sent: 6/13/2018 11:51:00 AM
To: Sims, Diann [Sims.Diann@epa.gov]; Garber, Kristina [Garber.Kristina@epa.gov]
Subject: FW: OPP General Follow-up: ESA usage workgroup final workplan
Attachments: milestones_timeline_interagency_usage_ 5-30-18_final .docx

Just so you can easily access the timeline … here's what was sent forward.

From: Keigwin, Richard
Sent: Wednesday, June 06, 2018 7:31 AM
To: Bertrand, Charlotte <Bertrand.Charlotte@epa.gov>; Beck, Nancy <Beck.Nancy@epa.gov>; Baptist, Erik <Baptist.Erik@epa.gov>; Wise, Louise <Wise.Louise@epa.gov>
Cc: Keller, Kaitlin <keller.kaitlin@epa.gov>; Dinkins, Darlene <Dinkins.Darlene@epa.gov>; Messina, Edward <Messina.Edward@epa.gov>; Echeverria, Marietta <Echeverria.Marietta@epa.gov>; Miller, Wynne <Miller.Wynne@epa.gov>
Subject: OPP General Follow-up: ESA usage workgroup final workplan

As we discussed during yesterday's OPP General, attached is the milestones and timeline that has been developed to work through the incorporate of usage data into ESA assessments. This will likely be a discussion topic at an upcoming MOA Workgroup meeting.

We will also get you a timeline for completing our work on the revised biological evaluations for carbaryl and methomyl, which incorporate the ideas put forward in the "strawman".

From: Echeverria, Marietta
Sent: Thursday, May 31, 2018 8:58 AM
To: Keigwin, Richard <Keigwin.Richard@epa.gov>; Messina, Edward <Messina.Edward@epa.gov>
Cc: Miller, Wynne <Miller.Wynne@epa.gov>
Subject: ESA usage workgroup final workplan

Rick and Ed,

As discussed in our general yesterday, attached is the "final" workplan for the EPA/FWS/NMFS/USDA workgroup charged with incorporating usage information into the ESA consultation process. Please let Wynne or me know if you have any questions.

Regards,
Marietta

Message

From: Anderson, Brian [Anderson.Brian@epa.gov]
Sent: 3/11/2019 8:00:08 PM
To: Rossmeisl, Colleen [Rossmeisl.Colleen@epa.gov]; Garber, Kristina [Garber.Kristina@epa.gov]; Panger, Melissa [Panger.Melissa@epa.gov]; Connolly, Jennifer [Connolly.Jennifer@epa.gov]; Donovan, Elizabeth [Donovan.Elizabeth@epa.gov]
Subject: FW: response to comments
Attachments: Response to comments ESA Revised methods_03.7.19.md.docx

Got input from Dyner - I have not looked yet.

b

From: Dyner, Mark
Sent: Monday, March 11, 2019 3:45 PM
To: Anderson, Brian <Anderson.Brian@epa.gov>
Cc: Knorr, Michele <knorr.michele@epa.gov>
Subject: RE: response to comments

Brian,

Thanks for the chance to review. A lot of hard work went into this. For the most part Attorney Client / Ex. 5

Attorney Client / Ex. 5

Happy to talk through any questions you have.

Mark

Mark Dyner
Office of General Counsel
(202) 564-1754

From: Anderson, Brian
Sent: Friday, March 08, 2019 8:32 AM
To: Dyner, Mark <dyner.mark@epa.gov>
Subject: FW: response to comments

Hi Mark,

Attached is our response to comment draft on the Services comments on our proposed revised methods. We'd really appreciate your review of this document to see if you think we addressed any concerns that you had with the

comments. We tried to incorporate your thoughts from the NMFS review and our disussions about the USDA comments.

Thanks again Mark!!

Brian

From: Anderson, Brian
Sent: Thursday, March 07, 2019 11:04 PM
To: Echeverria, Marietta (Echeverria.Marietta@epa.gov) <Echeverria.Marietta@epa.gov>
Cc: Garber, Kristina (Garber.Kristina@epa.gov) <Garber.Kristina@epa.gov>; Rossmeisl, Colleen (Rossmeisl.Colleen@epa.gov) <Rossmeisl.Colleen@epa.gov>; Panger, Melissa (Panger.Melissa@epa.gov) <Panger.Melissa@epa.gov>; Peck, Charles (Peck.Charles@epa.gov) <Peck.Charles@epa.gov>; Connolly, Jennifer (Connolly.Jennifer@epa.gov) <Connolly.Jennifer@epa.gov>; Donovan, Elizabeth (Donovan.Elizabeth@epa.gov) <Donovan.Elizabeth@epa.gov>
Subject: response to comments

Marietta,

Attached is the draft response to comments document for your reviewing pleasure.

I want to thank the team for their work on this. They really put together a very thoughtful and thorough response in a short amount of time. I can't say enough about the dedication of this team.

A few things we want to flag for you are below – these are just issues in the comments that we thought are notable. They are not really surprises, but particular comments that are worth noting.

Thanks for your comments,

ESA Team

Comments on Usage Data:

Deliberative Process / Ex. 5

Use of Apical vs Sublethal Endpoints:

Deliberative Process / Ex. 5

Collaboration:

Deliberative Process / Ex. 5

1% and 5% cutoff values for overlap:

Deliberative Process / Ex. 5

Message

From: Lennartz, Steven [Lennartz.Steven@epa.gov]
Sent: 6/27/2018 12:37:03 PM
To: Garber, Kristina [Garber.Kristina@epa.gov]
Subject: RE: description of public comments submitted on diazinon, chlorpyrifos and malathion usage data

Thanks Kris

From: Garber, Kristina
Sent: Tuesday, June 26, 2018 2:40 PM
To: Panger, Melissa <Panger.Melissa@epa.gov>; Lennartz, Steven <Lennartz.Steven@epa.gov>; Atwood, Donald <Atwood.Donald@epa.gov>
Cc: Perry, Tracy <Perry.Tracy@epa.gov>; Sims, Diann <Sims.Diann@epa.gov>
Subject: description of public comments submitted on diazinon, chlorpyrifos and malathion usage data

Mosquito adulticide team.

Tracy Perry put together the attached description of comments received on the first 3 op BEs, with a focus on those containing usage data. I thought that the first one would be of interest to your efforts.

Kris

Kris Garber, Senior Science Advisor
Environmental Fate and Effects Division
Office of Pesticide Programs
US Environmental Protection Agency
Mail Code: 7507P
1200 Pennsylvania Ave NW
Washington DC 20460-0001

Phone: 703-347-8940

Message

From: Anderson, Brian [Anderson.Brian@epa.gov]
Sent: 5/8/2018 3:56:19 PM
To: Panger, Melissa [Panger.Melissa@epa.gov]; Lennartz, Steven [Lennartz.Steven@epa.gov]; Garber, Kristina [Garber.Kristina@epa.gov]; Connolly, Jennifer [Connolly.Jennifer@epa.gov]
Subject: NASS questions

At my general with Marietta yesterday, we were talking about the NASS dataset. Questions came up that I'm trying to address in clear terms. A couple of questions are in yellow.

Is the below information accurate and clear?

Thanks

b

Hi Marietta,

Deliberative Process / Ex. 5

Please let me know if you have questions.

There are three data sets:

1. The SUUM
    a. Chemical and crop specific (state level) - estimates how many acres are treated with a chemical based on survey data.
2. The CDL
    a. Use category specific spatial layer (sub-county) - tells us where potential use sites are located – many crops in the CDL are grouped to reduce false negatives. Therefore, a CDL layer will include labeled and non-labeled crops.
    b. BEAD is crosswalking the vocabularies in the SUUM and CDL – EFED can do it if BEAD is unable to.
3. The NASS
    a. Crop specific (not chemical specific -spatial scale is state or county)- tells us how many acres are planted by state (maybe county) per crop.
    b. Used for two purposes:
        i.
        ii.

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5 Is this right?

Deliberative Process / Ex. 5

4. Deliberative Process / Ex. 5

Deliberative Process / Ex. 5/?

a.

b. Deliberative Process / Ex. 5

Table 2. Malathion Agricultural Usage by Crop and State (Data Averaged Over Reporte

Crop Group	Crop	Data Source
Root and Tuber Vegetables (including leaves)	Beets (Garden)	**
Root and Tuber Vegetables (including leaves)	Beets (Garden)	CDPR - CCACR (2011-2015)
Root and Tuber Vegetables (including leaves)	Carrots	CDPR - CASR (2011-2015)
Root and Tuber Vegetables (including leaves)	Carrots	AMRD (2011-2015)
Root and Tuber Vegetables (including leaves)	Carrots	AMRD (2011-2015)
Root and Tuber Vegetables (including leaves)	Carrots	AMRD (2011-2015)
Root and Tuber Vegetables (including leaves)	Carrots	AMRD (2011-2015)
Root and Tuber Vegetables (including leaves)	Chayote fruit/roots	**
Root and Tuber Vegetables (including leaves)	Chayote fruit/roots	CDPR - CCACR (2011-2015)
Root and Tuber Vegetables (including leaves)	Horseradish	**
Root and Tuber Vegetables (including leaves)	Horseradish	CDPR - CCACR (2011-2015)
Root and Tuber Vegetables (including leaves)	Parsnip	CDPR - CCACR (2011-2015)
Root and Tuber Vegetables (including leaves)	Potatoes	CDPR - CASR (2011-2015)
Root and Tuber Vegetables (including leaves)	Potatoes	AMRD (2011-2015)
Root and Tuber Vegetables (including leaves)	Potatoes	AMRD (2011-2015)
Root and Tuber Vegetables (including leaves)	Potatoes	AMRD (2011-2015)
Root and Tuber Vegetables (including leaves)	Potatoes	AMRD (2011-2015)
Root and Tuber Vegetables (including leaves)	Potatoes	AMRD (2011-2015)
Root and Tuber Vegetables (including leaves)	Potatoes	AMRD (2011-2015)
Root and Tuber Vegetables (including leaves)	Potatoes	AMRD (2011-2015)
Root and Tuber Vegetables (including leaves)	Potatoes	AMRD (2011-2015)
Root and Tuber Vegetables (including leaves)	Potatoes	AMRD (2011-2015)
Root and Tuber Vegetables (including leaves)	Potatoes	AMRD (2011-2015)

Root and Tuber Vegetables (including leaves)	Potatoes	AMRD (2011-2015)
Root and Tuber Vegetables (including leaves)	Potatoes	AMRD (2011-2015)
Root and Tuber Vegetables (including leaves)	Potatoes	AMRD (2011-2015)
Root and Tuber Vegetables (including leaves)	Potatoes	AMRD (2011-2015)
Root and Tuber Vegetables (including leaves)	Potatoes	AMRD (2011-2015)
Root and Tuber Vegetables (including leaves)	Radish	**
Root and Tuber Vegetables (including leaves)	Radish	CDPR - CCACR (2011-2015)
Root and Tuber Vegetables (including leaves)	Rutabagas	**
Root and Tuber Vegetables (including leaves)	Rutabagas	CDPR - CCACR (2011-2015)
Root and Tuber Vegetables (including leaves)	Salsify	**
Root and Tuber Vegetables (including leaves)	Sweet Potato	**
Root and Tuber Vegetables (including leaves)	Sweet Potato	CDPR - CASR (2011-2015)
Root and Tuber Vegetables (including leaves)	Turnips	**
Root and Tuber Vegetables (including leaves)	Turnips	CDPR - CCACR (2011-2015)
Root and Tuber Vegetables (including leaves)	Yams	**
Bulb Vegetable	Garlic	CDPR - CASR (2011-2015)
Bulb Vegetable	Leeks	**
Bulb Vegetable	Leeks	CDPR - CCACR (2011-2015)
Bulb Vegetable	Onions	CDPR - CCACR (2011-2015)
Bulb Vegetable	Onions	AMRD (2011-2015)
Bulb Vegetable	Onions	AMRD (2011-2015)
Bulb Vegetable	Onions	AMRD (2011-2015)
Bulb Vegetable	Onions	AMRD (2011-2015)
Bulb Vegetable	Onions	AMRD (2011-2015)
Bulb Vegetable	Onions	AMRD (2011-2015)
Bulb Vegetable	Onions	AMRD (2011-2015)
Bulb Vegetable	Green Onion and Shallots	**
Bulb Vegetable	Green Onion and Shallots	CDPR - CCACR (2011-2015)
Leafy Vegetables (except Brassica)	Amaranth (Chinese spinach)	**

Leafy Vegetables (except Brassica)	Amaranth (Chinese spinach)	CDPR (2011-2015)
Leafy Vegetables (except Brassica)	Arugula	**
Leafy Vegetables (except Brassica)	Celery	CDPR - CASR (2011-2015)
Leafy Vegetables (except Brassica)	Celtuce	**
Leafy Vegetables (except Brassica)	Chervil	**
Leafy Vegetables (except Brassica)	Chrysanthemum (edible)	**
Leafy Vegetables (except Brassica)	Chrysanthemum (edible)	CDPR - CCACR (2011-2015)
Leafy Vegetables (except Brassica)	Corn Salad	**
Leafy Vegetables (except Brassica)	Corn Salad	CDPR (2011-2015)
Leafy Vegetables (except Brassica)	Dandelion	**
Leafy Vegetables (except Brassica)	Dandelion	CDPR (2011-2015)
Leafy Vegetables (except Brassica)	Dock (Sorrel)	**
Leafy Vegetables (except Brassica)	Endive	**
Leafy Vegetables (except Brassica)	Endive	CDPR - CCACR (2011-2015)
Leafy Vegetables (except Brassica)	Florence fennel	**
Leafy Vegetables (except Brassica)	Florence fennel	CDPR - CCACR (2011-2015)
Leafy Vegetables (except Brassica)	Kale	**
Leafy Vegetables (except Brassica)	Kale	CDPR - CCACR (2011-2015)
Leafy Vegetables (except Brassica)	Kohlrabi	**
Leafy Vegetables (except Brassica)	Kohlrabi	CDPR - CCACR (2011-2015)
Leafy Vegetables (except Brassica)	Lettuce (leaf and head)	CDPR - CASR (2011-2015)
Leafy Vegetables (except Brassica)	Lettuce (leaf and head)	AMRD (2011-2015)
Leafy Vegetables (except Brassica)	Mustard Greens	**
Leafy Vegetables (except Brassica)	Mustard Greens	CDPR - CCACR (2011-2015)
Leafy Vegetables (except Brassica)	Mustard Spinach	**
Leafy Vegetables (except Brassica)	Orach	**
Leafy Vegetables (except Brassica)	Parsley	**
Leafy Vegetables (except Brassica)	Parsley	CDPR - CCACR (2011-2015)
Leafy Vegetables (except Brassica)	Purslane	**
Leafy Vegetables (except Brassica)	Radicchio	**
Leafy Vegetables (except Brassica)	Radicchio	CDPR - CCACR (2011-2015)
Leafy Vegetables (except Brassica)	Swiss Chard	**

Leafy Vegetables (except Brassica)	Swiss Chard	CDPR - CCACR (2011-2015)
Leafy Vegetables (except Brassica)	Watercress	**
Leafy Vegetables (except Brassica)	Watercress	CDPR (2011-2015)
Brassica (Cole) Leafy Vegetables	Broccoli	CDPR - CASR (2011-2015)
Brassica (Cole) Leafy Vegetables	Broccoli Raab/Rappini	**
Brassica (Cole) Leafy Vegetables	Broccoli Raab/Rappini	CDPR - CCACR (2011-2015)
Brassica (Cole) Leafy Vegetables	Brussels sprout	CDPR - CCACR (2011-2015)
Brassica (Cole) Leafy Vegetables	Cabbage	CDPR - CASR (2011-2015)
Brassica (Cole) Leafy Vegetables	Cabbage	AMRD (2011-2015)
Brassica (Cole) Leafy Vegetables	Cabbage	AMRD (2011-2015)
Brassica (Cole) Leafy Vegetables	Cabbage	AMRD (2011-2015)
Brassica (Cole) Leafy Vegetables	Cabbage	AMRD (2011-2015)
Brassica (Cole) Leafy Vegetables	Cabbage	AMRD (2011-2015)
Brassica (Cole) Leafy Vegetables	Cabbage	AMRD (2011-2015)
Brassica (Cole) Leafy Vegetables	Cabbage	AMRD (2011-2015)
Brassica (Cole) Leafy Vegetables	Cabbage	AMRD (2011-2015)
Brassica (Cole) Leafy Vegetables	Cauliflower	CDPR - CASR (2011-2015)
Brassica (Cole) Leafy Vegetables	Cavalo Broccolo	**
Brassica (Cole) Leafy Vegetables	Chinese broccoli (Gai lon)	**
Brassica (Cole) Leafy Vegetables	Chinese broccoli (Gai lon)	CDPR (2011-2015)
Brassica (Cole) Leafy Vegetables	Chinese cabbage (bok choy and Nappa)	**
Brassica (Cole) Leafy Vegetables	Chinese cabbage (bok choy and Nappa)	CDPR - CCACR (2011-2015)
Brassica (Cole) Leafy Vegetables	Chinese mustard (Gai choy)	**
Brassica (Cole) Leafy Vegetables	Chinese mustard (Gai choy)	CDPR (2011-2015)
Brassica (Cole) Leafy Vegetables	Collards	**
Brassica (Cole) Leafy Vegetables	Collards	CDPR - CCACR (2011-2015)
Brassica (Cole) Leafy Vegetables	Mizuna	**
Brassica (Cole) Leafy Vegetables	Mustard Spinach	**

Brassica (Cole) Leafy Vegetables	Rape greens	**
Brassica (Cole) Leafy Vegetables	Rape greens	CDPR (2011-2015)
Legume Vegetables	Beans (Succulent)	CDPR - CASR (2011-2015)
Legume Vegetables	Beans (Succulent)	AMRD (2011-2015)
Legume Vegetables	Beans (Succulent)	AMRD (2011-2015)
Legume Vegetables	Beans (Succulent)	AMRD (2011-2015)
Legume Vegetables	Beans (Succulent)	AMRD (2011-2015)
Legume Vegetables	Beans (Succulent)	AMRD (2011-2015)
Legume Vegetables	Beans (Succulent)	AMRD (2011-2015)
Legume Vegetables	Beans (Succulent)	AMRD (2011-2015)
Legume Vegetables	Beans (Succulent)	AMRD (2011-2015)
Legume Vegetables	Beans (Succulent)	AMRD (2011-2015)
Legume Vegetables	Beans (Succulent)	AMRD (2011-2015)
Legume Vegetables	Beans (Succulent)	AMRD (2011-2015)
Legume Vegetables	Beans (Succulent)	AMRD (2011-2015)
Legume Vegetables	Dry Beans/Peas	CDPR - CASR (2011-2015)
Legume Vegetables	Dry Beans/Peas	AMRD (2011-2015)
Legume Vegetables	Dry Beans/Peas	AMRD (2011-2015)
Legume Vegetables	Dry Beans/Peas	AMRD (2011-2015)
Legume Vegetables	Dry Beans/Peas	AMRD (2011-2015)
Legume Vegetables	Dry Beans/Peas	AMRD (2011-2015)
Legume Vegetables	Dry Beans/Peas	AMRD (2011-2015)
Legume Vegetables	Dry Beans/Peas	AMRD (2011-2015)
Legume Vegetables	Dry Beans/Peas	AMRD (2011-2015)
Legume Vegetables	Dry Beans/Peas	AMRD (2011-2015)
Legume Vegetables	Dry Beans/Peas	AMRD (2011-2015)

Legume Vegetables	Lima Beans	AMRD (2011-2015)
Legume Vegetables	Lima Beans	AMRD (2011-2015)
Legume Vegetables	Lima Beans	AMRD (2011-2015)
Legume Vegetables	Lima Beans	AMRD (2011-2015)
Legume Vegetables	Lima Beans	AMRD (2011-2015)
Legume Vegetables	Lima Beans	AMRD (2011-2015)
Legume Vegetables	Lima Beans	AMRD (2011-2015)
Legume Vegetables	Lima Beans	AMRD (2011-2015)
Legume Vegetables	Peas (Fresh/Green/ Sweet)	CDPR - CCACR (2011-2015)
Legume Vegetables	Peas (Fresh/Green/ Sweet)	AMRD (2011-2015)
Legume Vegetables	Peas (Fresh/Green/ Sweet)	AMRD (2011-2015)
Legume Vegetables	Peas (Fresh/Green/ Sweet)	AMRD (2011-2015)
Legume Vegetables	Peas (Fresh/Green/ Sweet)	AMRD (2011-2015)
Fruiting Vegetable	Eggplant	**
Fruiting Vegetable	Eggplant	CDPR - CCACR (2011-2015)
Fruiting Vegetable	Okra	**
Fruiting Vegetable	Okra	CDPR - CCACR (2011-2015)
Fruiting Vegetable	Peppers	CDPR - CASR (2011-2015)
Fruiting Vegetable	Peppers	AMRD (2011-2015)
Fruiting Vegetable	Peppers	AMRD (2011-2015)
Fruiting Vegetable	Peppers	AMRD (2011-2015)
Fruiting Vegetable	Peppers	AMRD (2011-2015)
Fruiting Vegetable	Peppers	AMRD (2011-2015)
Fruiting Vegetable	Peppers	AMRD (2011-2015)
Fruiting Vegetable	Peppers	AMRD (2011-2015)
Fruiting Vegetable	Peppers	AMRD (2011-2015)
Fruiting Vegetable	Tomatillos	**

Fruiting Vegetable	Tomatillos	CDPR - CCACR (2011-2015)
Fruiting Vegetable	Tomato	CDPR - CASR (2011-2015)
Fruiting Vegetable	Tomato	AMRD (2011-2015)
Cucurbit Vegetables	Cantaloupe	CDPR - CASR (2011-2015)
Cucurbit Vegetables	Cantaloupe	AMRD (2011-2015)
Cucurbit Vegetables	Cantaloupe	AMRD (2011-2015)
Cucurbit Vegetables	Cantaloupe	AMRD (2011-2015)
Cucurbit Vegetables	Cantaloupe	AMRD (2011-2015)
Cucurbit Vegetables	Cantaloupe	AMRD (2011-2015)
Cucurbit Vegetables	Cantaloupe	AMRD (2011-2015)
Cucurbit Vegetables	Cantaloupe	AMRD (2011-2015)
Cucurbit Vegetables	Cucumbers	CDPR - CASR (2011-2015)
Cucurbit Vegetables	Cucumbers	AMRD (2011-2015)
Cucurbit Vegetables	Cucumbers	AMRD (2011-2015)
Cucurbit Vegetables	Cucumbers	AMRD (2011-2015)
Cucurbit Vegetables	Cucumbers	AMRD (2011-2015)
Cucurbit Vegetables	Cucumbers	AMRD (2011-2015)
Cucurbit Vegetables	Cucumbers	AMRD (2011-2015)
Cucurbit Vegetables	Cucumbers	AMRD (2011-2015)
Cucurbit Vegetables	Cucumbers	AMRD (2011-2015)
Cucurbit Vegetables	Cucumbers	AMRD (2011-2015)
Cucurbit Vegetables	Cucumbers	AMRD (2011-2015)
Cucurbit Vegetables	Casaba	**
Cucurbit Vegetables	Crenshaw	**
Cucurbit Vegetables	Honey balls	**
Cucurbit Vegetables	Honeydew	CDPR - CCACR (2011-2015)
Cucurbit Vegetables	Muskmelon	**

Cucurbit Vegetables	Persian	**
Cucurbit Vegetables	Pumpkins	CDPR - CCACR (2011-2015)
Cucurbit Vegetables	Pumpkins	AMRD (2011-2015)
Cucurbit Vegetables	Pumpkins	AMRD (2011-2015)
Cucurbit Vegetables	Pumpkins	AMRD (2011-2015)
Cucurbit Vegetables	Pumpkins	AMRD (2011-2015)
Cucurbit Vegetables	Pumpkins	AMRD (2011-2015)
Cucurbit Vegetables	Pumpkins	AMRD (2011-2015)
Cucurbit Vegetables	Pumpkins	AMRD (2011-2015)
Cucurbit Vegetables	Pumpkins	AMRD (2011-2015)
Cucurbit Vegetables	Pumpkins	AMRD (2011-2015)
Cucurbit Vegetables	Pumpkins	AMRD (2011-2015)
Cucurbit Vegetables	Pumpkins	AMRD (2011-2015)
Cucurbit Vegetables	Pumpkins	AMRD (2011-2015)
Cucurbit Vegetables	Pumpkins	AMRD (2011-2015)
Cucurbit Vegetables	Pumpkins	AMRD (2011-2015)
Cucurbit Vegetables	Pumpkins	AMRD (2011-2015)
Cucurbit Vegetables	Pumpkins	AMRD (2011-2015)
Cucurbit Vegetables	Pumpkins	AMRD (2011-2015)
Cucurbit Vegetables	Pumpkins	AMRD (2011-2015)
Cucurbit Vegetables	Pumpkins	AMRD (2011-2015)
Cucurbit Vegetables	Pumpkins	AMRD (2011-2015)
Cucurbit Vegetables	Pumpkins	AMRD (2011-2015)
Cucurbit Vegetables	Squash (summer and winter)	CDPR - CASR (2011-2015)
Cucurbit Vegetables	Squash (summer and winter)	AMRD (2011-2015)
Cucurbit Vegetables	Squash (summer and winter)	AMRD (2011-2015)
Cucurbit Vegetables	Squash (summer and winter)	AMRD (2011-2015)
Cucurbit Vegetables	Squash (summer and winter)	AMRD (2011-2015)

Cucurbit Vegetables	Squash (summer and winter)	AMRD (2011-2015)
Cucurbit Vegetables	Squash (summer and winter)	AMRD (2011-2015)
Cucurbit Vegetables	Squash (summer and winter)	AMRD (2011-2015)
Cucurbit Vegetables	Squash (summer and winter)	AMRD (2011-2015)
Cucurbit Vegetables	Squash (summer and winter)	AMRD (2011-2015)
Cucurbit Vegetables	Squash (summer and winter)	AMRD (2011-2015)
Cucurbit Vegetables	Squash (summer and winter)	AMRD (2011-2015)
Cucurbit Vegetables	Squash (summer and winter)	AMRD (2011-2015)
Cucurbit Vegetables	Squash (summer and winter)	AMRD (2011-2015)
Cucurbit Vegetables	Squash (summer and winter)	AMRD (2011-2015)
Cucurbit Vegetables	Squash (summer and winter)	AMRD (2011-2015)
Cucurbit Vegetables	Watermelons	CDPR - CASR (2011-2015)
Cucurbit Vegetables	Watermelons	AMRD (2011-2015)
Cucurbit Vegetables	Watermelons	AMRD (2011-2015)
Cucurbit Vegetables	Watermelons	AMRD (2011-2015)
Cucurbit Vegetables	Watermelons	AMRD (2011-2015)
Cucurbit Vegetables	Watermelons	AMRD (2011-2015)
Cucurbit Vegetables	Watermelons	AMRD (2011-2015)
Cucurbit Vegetables	Watermelons	AMRD (2011-2015)
Cucurbit Vegetables	Watermelons	AMRD (2011-2015)
Cucurbit Vegetables	Watermelons	AMRD (2011-2015)
Cucurbit Vegetables	Watermelons	AMRD (2011-2015)
Cucurbit Vegetables	Watermelons	AMRD (2011-2015)
Citrus	Grapefruit	CDPR - CASR (2011-2015)
Citrus	Grapefruit	AMRD (2011-2015)
Citrus	Grapefruit	AMRD (2011-2015)

Citrus	Kumquat	CDPR - CCACR (2011-2015)
Citrus	Lemons	CDPR - CASR (2011-2015)
Citrus	Limes	**
Citrus	Limes	CDPR - CCACR (2011-2015)
Citrus	Oranges	CDPR - CASR (2011-2015)
Citrus	Oranges	AMRD (2011-2015)
Citrus	Tangelos	CDPR - CCACR (2011-2015)
Citrus	Tangelos	NASS (2011, 2015)
Citrus	Tangerines	CDPR - CCACR (2011-2015)
Citrus	Tangerines	NASS (2011, 2015)
Citrus	Other Citrus Hybrids	**
Pome Fruit	Pears	CDPR - CASR (2011-2015)
Pome Fruit	Pears	AMRD (2011-2015)
Pome Fruit	Pears	AMRD (2011-2015)
Stone Fruits	Apricots	CDPR - CASR (2011-2015)
Stone Fruits	Cherries (sweet and tart)	CDPR - CASR (2011-2015)
Stone Fruits	Cherries (sweet and tart)	AMRD (2011-2015)
Stone Fruits	Cherries (sweet and tart)	AMRD (2011-2015)
Stone Fruits	Cherries (sweet and tart)	AMRD (2011-2015)
Stone Fruits	Nectarines	CDPR - CASR (2011-2015)
Stone Fruits	Peaches	CDPR - CASR (2011-2015)
Stone Fruits	Peaches	AMRD (2011-2015)
Stone Fruits	Peaches	AMRD (2011-2015)
Stone Fruits	Peaches	AMRD (2011-2015)
Stone Fruits	Peaches	AMRD (2011-2015)
Stone Fruits	Peaches	AMRD (2011-2015)

Stone Fruits	Peaches	AMRD (2011-2015)
Stone Fruits	Peaches	AMRD (2011-2015)
Stone Fruits	Peaches	AMRD (2011-2015)
Stone Fruits	Peaches	AMRD (2011-2015)
Stone Fruits	Peaches	AMRD (2011-2015)
Stone Fruits	Peaches	AMRD (2011-2015)
Berry and Small Fruit	Blueberries	CDPR - CASR (2011-2015)
Berry and Small Fruit	Blueberries	NASS (2011, 2015)
Berry and Small Fruit	Blueberries	NASS (2011, 2015)
Berry and Small Fruit	Blueberries	NASS (2011, 2015)
Berry and Small Fruit	Blueberries	NASS (2011, 2015)
Berry and Small Fruit	Blueberries	NASS (2011, 2015)
Berry and Small Fruit	Blueberries	NASS (2011, 2015)
Berry and Small Fruit	Currants	**
Berry and Small Fruit	Currants	CDPR (2011-2015)
Berry and Small Fruit	Gooseberries	**
Berry and Small Fruit	Caneberries	CDPR - CCACR (2011-2015)
Berry and Small Fruit	Caneberries	AMRD (2011-2015)
Berry and Small Fruit	Caneberries	AMRD (2011-2015)
Berry and Small Fruit	Blackberry	CDPR - CCACR (2011-2015)
Berry and Small Fruit	Boysenberry	CDPR - CCACR (2011-2015)
Berry and Small Fruit	Loganberry	CDPR (2011-2015)
Berry and Small Fruit	Raspberry	CDPR - CCACR (2011-2015)
Berry and Small Fruit	Grapes	CDPR - CASR (2011-2015)
Berry and Small Fruit	Grapes (table and raisin)	CDPR - CASR (2011-2015)
Berry and Small Fruit	Grapes (wine)	CDPR - CASR (2011-2015)

Berry and Small Fruit	Grape vines (overwinter nursery stock)	**
Berry and Small Fruit	Strawberries	CDPR - CASR (2011-2015)
Berry and Small Fruit	Strawberries	AMRD (2011-2015)
Berry and Small Fruit	Strawberries	AMRD (2011-2015)
Berry and Small Fruit	Strawberries	AMRD (2011-2015)
Berry and Small Fruit	Strawberries	AMRD (2011-2015)
Berry and Small Fruit	Strawberries	AMRD (2011-2015)
Berry and Small Fruit	Strawberries	AMRD (2011-2015)
Tree Nuts	Chestnuts	**
Tree Nuts	Chestnuts	CDPR - CCACR (2011-2015)
Tree Nuts	Macadamia Nut	**
Tree Nuts	Macadamia Nut	CDPR (2011-2015)
Tree Nuts	Pecans	CDPR - CASR (2011-2015)
Tree Nuts	Pecans	AMRD (2011-2015)
Tree Nuts	Pecans	AMRD (2011-2015)
Tree Nuts	Pecans	AMRD (2011-2015)
Tree Nuts	Pecans	AMRD (2011-2015)
Tree Nuts	Pecans	AMRD (2011-2015)
Tree Nuts	Pecans	AMRD (2011-2015)
Tree Nuts	Pecans	AMRD (2011-2015)
Tree Nuts	Walnuts	CDPR - CASR (2011-2015)
Cereal Grains	Barley	CDPR - CCACR (2011-2015)
Cereal Grains	Corn, Field	CDPR - CASR (2011-2015)
Cereal Grains	Corn, Field	AMRD (2011-2015)
Cereal Grains	Corn, Field	AMRD (2011-2015)
Cereal Grains	Corn, Field	AMRD (2011-2015)
Cereal Grains	Corn, Field	AMRD (2011-2015)

Cereal Grains	Corn, Field	AMRD (2011-2015)
Cereal Grains	Corn, Field	AMRD (2011-2015)
Cereal Grains	Corn, Field	AMRD (2011-2015)
Cereal Grains	Corn, Field	AMRD (2011-2015)
Cereal Grains	Corn, Field	AMRD (2011-2015)
Cereal Grains	Corn, Field	AMRD (2011-2015)
Cereal Grains	Corn, Field	AMRD (2011-2015)
Cereal Grains	Corn, Field	AMRD (2011-2015)
Cereal Grains	Corn, Field	AMRD (2011-2015)
Cereal Grains	Corn, Field	AMRD (2011-2015)
Cereal Grains	Corn, Field	AMRD (2011-2015)
Cereal Grains	Corn, Field	AMRD (2011-2015)
Cereal Grains	Corn, Field	AMRD (2011-2015)
Cereal Grains	Corn, Field	AMRD (2011-2015)
Cereal Grains	Corn, Field	AMRD (2011-2015)
Cereal Grains	Corn, Field	AMRD (2011-2015)
Cereal Grains	Corn, Field	AMRD (2011-2015)
Cereal Grains	Corn, Field	AMRD (2011-2015)
Cereal Grains	Corn, Field	AMRD (2011-2015)
Cereal Grains	Corn, Field	AMRD (2011-2015)
Cereal Grains	Corn, Field	AMRD (2011-2015)
Cereal Grains	Corn, Field	AMRD (2011-2015)
Cereal Grains	Corn, Field	AMRD (2011-2015)
Cereal Grains	Corn, Field	AMRD (2011-2015)
Cereal Grains	Corn, Field	AMRD (2011-2015)
Cereal Grains	Corn, Field	AMRD (2011-2015)
Cereal Grains	Corn, Field	AMRD (2011-2015)

Cereal Grains	Corn, Field	AMRD (2011-2015)
Cereal Grains	Corn, Field	AMRD (2011-2015)
Cereal Grains	Corn, Pop	**
Cereal Grains	Corn, Sweet	CDPR - CASR (2011-2015)
Cereal Grains	Corn, Sweet	AMRD (2011-2015)
Cereal Grains	Corn, Sweet	AMRD (2011-2015)
Cereal Grains	Corn, Sweet	AMRD (2011-2015)
Cereal Grains	Corn, Sweet	AMRD (2011-2015)
Cereal Grains	Corn, Sweet	AMRD (2011-2015)
Cereal Grains	Corn, Sweet	AMRD (2011-2015)
Cereal Grains	Corn, Sweet	AMRD (2011-2015)
Cereal Grains	Corn, Sweet	AMRD (2011-2015)
Cereal Grains	Corn, Sweet	AMRD (2011-2015)
Cereal Grains	Corn, Sweet	AMRD (2011-2015)
Cereal Grains	Corn, Sweet	AMRD (2011-2015)
Cereal Grains	Corn, Sweet	AMRD (2011-2015)
Cereal Grains	Oats	**
Cereal Grains	Oats	CDPR - CASR (2011-2015)
Cereal Grains	Rice	CDPR - CASR (2011-2015)
Cereal Grains	Rice	AMRD (2011-2015)
Cereal Grains	Rice	AMRD (2011-2015)
Cereal Grains	Rice	AMRD (2011-2015)
Cereal Grains	Rice	AMRD (2011-2015)
Cereal Grains	Rice	AMRD (2011-2015)
Cereal Grains	Rye	**
Cereal Grains	Rye	CDPR - CCACR (2011-2015)
Cereal Grains	Sorghum (Milo)	CDPR - CCACR (2011-2015)
Cereal Grains	Sorghum (Milo)	AMRD (2011-2015)

Cereal Grains	Sorghum (Milo)	AMRD (2011-2015)
Cereal Grains	Sorghum (Milo)	AMRD (2011-2015)
Cereal Grains	Sorghum (Milo)	AMRD (2011-2015)
Cereal Grains	Sorghum (Milo)	AMRD (2011-2015)
Cereal Grains	Sorghum (Milo)	AMRD (2011-2015)
Cereal Grains	Sorghum (Milo)	AMRD (2011-2015)
Cereal Grains	Sorghum (Milo)	AMRD (2011-2015)
Cereal Grains	Sorghum (Milo)	AMRD (2011-2015)
Cereal Grains	Sorghum (Milo)	AMRD (2011-2015)
Cereal Grains	Sorghum (Milo)	AMRD (2011-2015)
Cereal Grains	Sorghum (Milo)	AMRD (2011-2015)
Cereal Grains	Wheat	CDPR - CASR (2011-2015)
Cereal Grains	Wheat	AMRD (2011-2015)
Cereal Grains	Wheat	AMRD (2011-2015)
Cereal Grains	Wheat	AMRD (2011-2015)
Cereal Grains	Wheat	AMRD (2011-2015)
Cereal Grains	Wheat	AMRD (2011-2015)
Cereal Grains	Wheat	AMRD (2011-2015)
Cereal Grains	Wheat	AMRD (2011-2015)
Cereal Grains	Wheat	AMRD (2011-2015)
Cereal Grains	Wheat	AMRD (2011-2015)
Cereal Grains	Wheat	AMRD (2011-2015)
Cereal Grains	Wheat	AMRD (2011-2015)
Cereal Grains	Wheat	AMRD (2011-2015)
Cereal Grains	Wheat	AMRD (2011-2015)
Cereal Grains	Wheat	AMRD (2011-2015)
Cereal Grains	Wheat	AMRD (2011-2015)

Cereal Grains	Wheat	AMRD (2011-2015)
Cereal Grains	Wheat	AMRD (2011-2015)
Cereal Grains	Wheat	AMRD (2011-2015)
Cereal Grains	Wheat	AMRD (2011-2015)
Cereal Grains	Wheat	AMRD (2011-2015)
Cereal Grains	Wheat	AMRD (2011-2015)
Cereal Grains	Wheat	AMRD (2011-2015)
Cereal Grains	Wheat	AMRD (2011-2015)
Cereal Grains	Wheat	AMRD (2011-2015)
Cereal Grains	Wheat	AMRD (2011-2015)
Cereal Grains	Wheat	AMRD (2011-2015)
Cereal Grains	Wild rice	**
Cereal Grains	Wild rice	CDPR - CCACR (2011-2015)
Grass Forage, Fodder, and Hay	Alfalfa	CDPR - CASR (2011-2015)
Grass Forage, Fodder, and Hay	Alfalfa	AMRD (2011-2015)
Grass Forage, Fodder, and Hay	Alfalfa	AMRD (2011-2015)
Grass Forage, Fodder, and Hay	Alfalfa	AMRD (2011-2015)
Grass Forage, Fodder, and Hay	Alfalfa	AMRD (2011-2015)
Grass Forage, Fodder, and Hay	Alfalfa	AMRD (2011-2015)
Grass Forage, Fodder, and Hay	Alfalfa	AMRD (2011-2015)
Grass Forage, Fodder, and Hay	Alfalfa	AMRD (2011-2015)
Grass Forage, Fodder, and Hay	Alfalfa	AMRD (2011-2015)
Grass Forage, Fodder, and Hay	Alfalfa	AMRD (2011-2015)
Grass Forage, Fodder, and Hay	Alfalfa	AMRD (2011-2015)
Grass Forage, Fodder, and Hay	Alfalfa	AMRD (2011-2015)
Grass Forage, Fodder, and Hay	Alfalfa	AMRD (2011-2015)
Grass Forage, Fodder, and Hay	Alfalfa	AMRD (2011-2015)
Grass Forage, Fodder, and Hay	Alfalfa	AMRD (2011-2015)

Grass Forage, Fodder, and Hay	Alfalfa	AMRD (2011-2015)
Grass Forage, Fodder, and Hay	Alfalfa	AMRD (2011-2015)
Grass Forage, Fodder, and Hay	Alfalfa	AMRD (2011-2015)
Grass Forage, Fodder, and Hay	Alfalfa	AMRD (2011-2015)
Grass Forage, Fodder, and Hay	Alfalfa	AMRD (2011-2015)
Grass Forage, Fodder, and Hay	Alfalfa	AMRD (2011-2015)
Grass Forage, Fodder, and Hay	Alfalfa	AMRD (2011-2015)
Grass Forage, Fodder, and Hay	Alfalfa	AMRD (2011-2015)
Grass Forage, Fodder, and Hay	Alfalfa	AMRD (2011-2015)
Grass Forage, Fodder, and Hay	Alfalfa	AMRD (2011-2015)
Grass Forage, Fodder, and Hay	Alfalfa	AMRD (2011-2015)
Grass Forage, Fodder, and Hay	Alfalfa	AMRD (2011-2015)
Grass Forage, Fodder, and Hay	Alfalfa	AMRD (2011-2015)
Grass Forage, Fodder, and Hay	Alfalfa	AMRD (2011-2015)
Grass Forage, Fodder, and Hay	Birdsfoot Trefoil	**
Grass Forage, Fodder, and Hay	Bermudagrass	**
Grass Forage, Fodder, and Hay	Bermudagrass	CDPR - CCACR (2011-2015)
Grass Forage, Fodder, and Hay	Barnyardgrass	**
Grass Forage, Fodder, and Hay	Canarygrass	**
Grass Forage, Fodder, and Hay	Fescue	**
Grass Forage, Fodder, and Hay	Orchardgrass	**
Grass Forage, Fodder, and Hay	Orchardgrass	CDPR (2011-2015)
Grass Forage, Fodder, and Hay	Red top	**
Grass Forage, Fodder, and Hay	Timothy	**
Grass Forage, Fodder, and Hay	Timothy	CDPR (2011-2015)
Grass Forage, Fodder, and Hay	Yellow foxtail	**
Grass Forage, Fodder, and Hay	Pasture and Rangeland	**
Grass Forage, Fodder, and Hay	Pasture and Rangeland	CDPR - CCACR (2011-2015)
Nongrass Animal Feeds	Clover (seed)	**
Nongrass Animal Feeds	Clover (seed)	CDPR - CCACR (2011-2015)
Nongrass Animal Feeds	Lespedeza	**

Nongrass Animal Feeds	Lupine	**
Nongrass Animal Feeds	Vetch (seed)	**
Nongrass Animal Feeds	Vetch (seed)	CDPR - CCACR (2011-2015)
Oilseed Crops	Cotton	CDPR - CASR (2011-2015)
Oilseed Crops	Cotton	AMRD (2011-2015)
Oilseed Crops	Cotton	AMRD (2011-2015)
Oilseed Crops	Cotton	AMRD (2011-2015)
Oilseed Crops	Cotton	AMRD (2011-2015)
Oilseed Crops	Cotton	AMRD (2011-2015)
Oilseed Crops	Cotton	AMRD (2011-2015)
Oilseed Crops	Cotton	AMRD (2011-2015)
Oilseed Crops	Cotton	AMRD (2011-2015)
Oilseed Crops	Cotton	AMRD (2011-2015)
Oilseed Crops	Cotton	AMRD (2011-2015)
Oilseed Crops	Cotton	AMRD (2011-2015)
Oilseed Crops	Cotton	AMRD (2011-2015)
Oilseed Crops	Cotton	AMRD (2011-2015)
Oilseed Crops	Cotton	AMRD (2011-2015)
Oilseed Crops	Flax	**
Edible Fungi	Mushrooms	**
Edible Fungi	Mushrooms	CDPR - CASR (2011-2015)
Stalk Stem and Leaf Petiole Vegetables	Asparagus	CDPR - CCACR (2011-2015)
Stalk Stem and Leaf Petiole Vegetables	Asparagus	AMRD (2011-2015)
Stalk Stem and Leaf Petiole Vegetables	Asparagus	AMRD (2011-2015)
Tropical and Subtropical Fruit	Avocados	CDPR - CCACR (2011-2015)
Tropical and Subtropical Fruit	Figs	CDPR - CASR (2011-2015)
Tropical and Subtropical Fruit	Guava	**
Tropical and Subtropical Fruit	Guava	CDPR - CCACR (2011-2015)
Tropical and Subtropical Fruit	Mangos	**

Tropical and Subtropical Fruit	Papaya	**
Tropical and Subtropical Fruit	Passion Fruit	**
Tropical and Subtropical Fruit	Pineapples	**
Misc. Crops	Hops	**
Misc. Crops	Hops	CDPR (2011-2015)
Misc. Crops	Mint	**
Misc. Crops	Peppermint	**
Misc. Crops	Peppermint	CDPR - CASR (2011-2015)
Misc. Crops	Spearmint	**

AMRD (YEAR-YEAR)	
NASS (YEAR)	Surveyed by U
CDPR (YEAR-YEAR)	Surveyed by the Cali
CDPR - CASR (YEAR-YEAR)	Surveyed by the Calif
CDPR - CCACR (YEAR-YEAR)	Surveyed by the California
+	
†	Crop Acres Grown (CAG) i of treatment with any pesti estimate, NASS comes fror Agricultural Statistics Revie
a	The pounds AI displayed i
*	Surv
**	
(S)	Insufficient number of rep
--	

ed Years).

State	Avg. Annual Crop Acres Grown [†]	Avg. Annual Total Lbs. AI Applied [a]	Min. Annual PCT	Max. Annual PCT	Avg. Annual PCT
**	**	**	**	**	**
California (21.1%)[+]	1,067	99	3.3	20.8	7.8
California (65.9%)[+]	64,600	162	0.0	0.4	0.1
Michigan	3,000	*	*	*	*
Texas	1,700	*	*	*	*
Washington	7,000	*	*	*	*
Wisconsin	4,800	*	*	*	*
**	**	**	**	**	**
California (--%)[+]	7	*	*	*	*
**	**	**	**	**	**
California (19.0%)[+]	432	*	*	*	*
California (--%)[+]	15	0	0.0	6.7	1.2
California (3.7%)[+]	35,540	353	0.0	1.5	0.6
Idaho	324,800	300	0.0	0.4	0.1
Minnesota	47,000	800	0.0	7.1	1.4
Montana	11,500	20	0.0	0.8	0.2
Colorado	58,600	*	*	*	*
Florida	35,000	*	*	*	*
Maine	54,400	*	*	*	*
Michigan	45,800	*	*	*	*
Nebraska	18,500	*	*	*	*
New York	17,400	*	*	*	*
North Carolina	15,700	*	*	*	*
North Dakota	82,800	*	*	*	*

Oregon	40,000	*	*	*	*
Pennsylvania	7,700	*	*	*	*
Texas	18,100	*	*	*	*
Washington	164,000	*	*	*	*
Wisconsin	63,300	*	*	*	*
**	**	**	**	**	**
California (13.0%)+	1,499	183	0.5	1.0	0.8
**	**	**	**	**	**
California (--%)+	128	*	*	*	*
**	**	**	**	**	**
**	**	**	**	**	**
California (14.5%)+	18,620	1	0.0	0.03	0.01
**	**	**	**	**	**
California (1.0%)+	235	77	13.3	86.6	26.4
**	**	**	**	**	**
California (86.4%)+	23,780	137	0.0	2.4	0.7
**	**	**	**	**	**
California (--%)+	625	476	0.0	100.0	57.6
California (25.4%)+	47,382	1,834	0.4	3.1	2.0
Idaho	8,400	500	0.0	16.5	5.4
Oregon	19,700	6,000	4.7	26.0	12.9
Washington	23,900	2,900	0.0	16.6	3.3
Colorado	5,700	*	*	*	*
Georgia	12,700	*	*	*	*
New York	8,900	*	*	*	*
Texas	9,800	*	*	*	*
**	**	**	**	**	**
California (55.7%)+	1,452	153	0.0	23.6	6.1
**	**	**	**	**	**

California (--%)+	--	34	--	--	--
**	**	**	**	**	**
California (83.0%)+	27,660	10,204	16.0	27.4	22.0
**	**	**	**	**	**
**	**	**	**	**	**
**	**	**	**	**	**
California (--%)+	53	1	0.0	1.2	0.9
**	**	**	**	**	**
California (--%)+	--	*	*	*	*
**	**	**	**	**	**
California (--%)+	--	9	--	--	--
**	**	**	**	**	**
**	**	**	**	**	**
California (62.0%)+	489	45	1.6	12.6	6.7
**	**	**	**	**	**
California (--%)+	818	75	0.0	13.0	4.0
**	**	**	**	**	**
California (26.9%)+	4,671	488	4.5	10.7	7.1
**	**	**	**	**	**
California (--%)+	58	23	0.0	1.0	0.5
California (72.0%)+	205,920	33,025	6.7	11.3	8.4
Arizona	12,800	100	0.0	0.6	0.1
**	**	**	**	**	**
California (10.6%)+	2,432	1	0.0	0.2	0.1
**	**	**	**	**	**
**	**	**	**	**	**
**	**	**	**	**	**
California (48.1%)+	3,391	40	0.2	2.3	0.8
**	**	**	**	**	**
**	**	**	**	**	**
California (--%)+	1,743	19	0.0	2.6	0.8
**	**	**	**	**	**

California (--%)+	1,468	42	1.7	5.6	2.7
**	**	**	**	**	**
California (31.2%)+	--	354	--	--	--
California (80.9%)+	120,800	14,513	4.3	7.9	5.9
**	**	**	**	**	**
California (--%)+	2,866	366	1.3	8.8	3.7
California (96.4%)+	2,342	2,102	22.9	70.2	52.2
California (14.9%)+	13,300	2,007	8.5	14.6	10.3
Florida	9,400	1,200	0.0	9.5	2.8
Michigan	3,200	200	0.0	23.3	5.3
Arizona	2,900	*	*	*	*
Colorado	1,800	*	*	*	*
Georgia	5,400	*	*	*	*
New York	9,900	*	*	*	*
North Carolina	4,000	*	*	*	*
Texas	6,800	*	*	*	*
Wisconsin	3,000	*	*	*	*
California (81.7%)+	32,660	1,995	1.7	6.3	4.5
**	**	**	**	**	**
**	**	**	**	**	**
California (--%)+	--	21	--	--	--
**	**	**	**	**	**
California (42.7%)+	6,910	1,749	8.8	30.0	14.5
**	**	**	**	**	**
California (--%)+	--	7	--	--	--
**	**	**	**	**	**
California (5.0%)+	299	41	2.1	21.9	11.4
**	**	**	**	**	**
**	**	**	**	**	**

**	**	**	**	**	**
California (--%)+	--	17	--	--	--
California (3.5%)+	5,120	148	0.0	3.8	1.3
Florida	42,200	*	*	*	*
Georgia	10,300	*	*	*	*
Illinois	8,300	*	*	*	*
Indiana	3,600	*	*	*	*
Michigan	19,000	*	*	*	*
New York	26,200	*	*	*	*
North Carolina	6,600	*	*	*	*
Oregon	13,200	*	*	*	*
Pennsylvania	12,800	*	*	*	*
Tennessee	8,700	*	*	*	*
Texas	2,600	*	*	*	*
Wisconsin	71,200	*	*	*	*
California (2.4%)+	51,800	1,010	0.5	3.0	1.5
Idaho	188,800	300	0.0	0.8	0.2
Texas	23,600	500	0.0	8.9	2.2
Colorado	45,400	*	*	*	*
Michigan	206,000	*	*	*	*
Minnesota	156,000	*	*	*	*
Montana	602,000	*	*	*	*
Nebraska	131,000	*	*	*	*
New York	9,200	*	*	*	*
North Dakota	970,000	*	*	*	*
Washington	245,000	*	*	*	*
Wisconsin	37,200	*	*	*	*

California (37.2%)	7,600	*	*	*	*
Delaware	11,700	*	*	*	*
Georgia	1,600	*	*	*	*
Illinois	2,400	*	*	*	*
Maryland	400	*	*	*	*
South Carolina	500	*	*	*	*
Washington	2,500	*	*	*	*
Wisconsin	4,200	*	*	*	*
California (1.6%)+	2,309	626	12.7	30.6	22.5
Minnesota	70,800	*	*	*	*
Oregon	16,700	*	*	*	*
Washington	39,300	*	*	*	*
Wisconsin	37,900	*	*	*	*
**	**	**	**	**	**
California (23.1%)+	1,202	11	0.2	0.7	0.4
**	**	**	**	**	**
California (17.5%)+	662	*	*	*	*
California (32.1%)+	26,740	353	0.3	1.7	1.1
Texas	3,000	10	0.0	1.6	0.3
Arizona	1,300	*	*	*	*
Florida	16,200	*	*	*	*
Georgia	3,700	*	*	*	*
New Jersey	3,300	*	*	*	*
New Mexico	9,200	*	*	*	*
North Carolina	2,400	*	*	*	*
Ohio	3,000	*	*	*	*
**	**	**	**	**	**

California (--%)+	33	*	*	*	*
California (74.2%)+	308,180	10,672	0.3	4.1	1.7
Florida	32,000	3,700	0.0	8.7	3.5
California (52.0%)+	35,240	131	0.0	0.8	0.3
Texas	2,500	40	0.0	7.6	1.5
Arizona	16,600	*	*	*	*
Florida	2,900	*	*	*	*
Georgia	3,400	*	*	*	*
Indiana	1,900	*	*	*	*
North Carolina	1,700	*	*	*	*
South Carolina	200	*	*	*	*
California (7.0%)+	3,760	59	0.1	1.6	0.7
Florida	26,000	1,100	0.0	4.0	0.8
Delaware	4,800	*	*	*	*
Georgia	10,400	*	*	*	*
Maryland	2,600	*	*	*	*
Michigan	33,700	*	*	*	*
Missouri	4,700	*	*	*	*
New Jersey	700	*	*	*	*
North Carolina	11,900	*	*	*	*
South Carolina	2,900	*	*	*	*
Washington	1,800	*	*	*	*
Wisconsin	5,600	*	*	*	*
**	**	**	**	**	**
**	**	**	**	**	**
**	**	**	**	**	**
California (80.8%)+	13,958	24	0.0	0.8	0.2
**	**	**	**	**	**

**	**	**	**	**	**
California (6.8%)+	4,297	1,192	15.7	37.2	27.2
Illinois	17,300	10	0.0	0.3	0.1
Indiana	3,400	100	0.0	12.5	2.5
New Jersey	2,400	200	0.0	26.7	5.3
New York	6,000	90	0.0	7.7	1.5
Ohio	7,100	1,100	0.0	38.4	7.8
Pennsylvania	6,600	30	0.0	2.3	0.6
Tennessee	1,800	80	0.0	23.3	4.9
Virginia	2,200	60	0.0	7.5	1.9
Connecticut	1,400	*	*	*	*
Maryland	1,100	*	*	*	*
Massachusetts	2,000	*	*	*	*
Michigan	6,500	*	*	*	*
Minnesota	2,300	*	*	*	*
Missouri	1,300	*	*	*	*
New Mexico	2,000	*	*	*	*
North Carolina	500	*	*	*	*
Oregon	2,100	*	*	*	*
Texas	2,200	*	*	*	*
Washington	2,100	*	*	*	*
Wisconsin	3,300	*	*	*	*
California (13.3%)+	6,160	343	0.3	4.0	2.7
Georgia	3,300	10	0.0	1.3	0.3
Ohio	1,900	900	0.0	91.8	32.5
Texas	1,900	700	0.0	29.2	6.9
Connecticut	800	*	*	*	*

Florida	9,100	*	*	*	*
Massachusetts	1,700	*	*	*	*
Michigan	6,100	*	*	*	*
New Jersey	3,100	*	*	*	*
New York	4,600	*	*	*	*
North Carolina	3,300	*	*	*	*
Oregon	2,600	*	*	*	*
Pennsylvania	900	*	*	*	*
South Carolina	2	*	*	*	*
Tennessee	300	*	*	*	*
Wisconsin	1	*	*	*	*
California (10.4%)+	10,680	143	0.0	1.3	0.5
Mississippi	2,200	1,000	0.0	62.1	12.4
Oklahoma	4,800	4	0.0	0.4	0.1
South Carolina	7,900	90	0.0	6.2	1.6
Texas	27,000	1,400	0.0	5.8	2.3
Alabama	3,400	*	*	*	*
Arizona	2,800	*	*	*	*
Florida	24,300	*	*	*	*
Georgia	23,300	*	*	*	*
Indiana	7,400	*	*	*	*
Maryland	700	*	*	*	*
Missouri	2,300	*	*	*	*
North Carolina	7,300	*	*	*	*
California (11.8%)+	9,780	266	0.3	2.0	1.0
Florida	52,400	6,300	4.8	12.9	9.4
Texas	18,300	*	*	*	*

California (71.3%)+	93	*	*	*	*
California (79.6%)+	45,600	145	0.0	0.4	0.2
**	**	**	**	**	**
California (56.1%)+	354	*	*	*	*
California (28.8%)+	171,400	7,683	2.0	3.5	2.6
Florida	481,800	172,700	12.1	33.2	24.5
California (42.5%)+	1,379	102	0.4	12.9	3.0
Florida	4,925	550	7.0	41.0	20.3
California (79.1%)+	54,347	6,816	1.5	3.5	2.7
Florida	31,238	3,400	12.0	12.0	12.0
**	**	**	**	**	**
California (21.0%)+	11,860	10	0	0.1	0.0
Oregon	17,100	*	*	*	*
Washington	23,000	*	*	*	*
California (83.6%)+	9,560	2	0.0	0.1	0.1
California (49.0%)+	32,000	23,399	24.9	48.2	32.6
Oregon	17,000	11,000	3.0	71.1	21.3
Washington	42,200	17,000	6.9	29.4	16.2
Michigan	45,900	*	*	*	*
California (87.4%)+	19,400	8	0.0	0.0	0.0
California (40.4%)+	45,500	13	0.0	0.4	0.1
Michigan	4,100	20	0.0	3.0	0.6
Texas	5,100	1,600	0.0	55.0	13.6
Washington	2,600	700	0.0	67.2	13.4
Alabama	2,300	*	*	*	*
Colorado	700	*	*	*	*

Georgia	10,500	*	*	*	*
Illinois	1,400	*	*	*	*
New Jersey	5,300	*	*	*	*
New York	400	*	*	*	*
Pennsylvania	5,000	*	*	*	*
South Carolina	18,000	*	*	*	*
California (5.0%)+	4,740	776	4.0	20.2	11.3
Georgia	44,171	23,450	54.0	55.0	54.5
Michigan	28,381	9,100	13.0	26.0	19.5
New Jersey	13,239	3,750	4.0	27.0	15.5
North Carolina	35,709	17,800	55.0	60.0	57.5
Oregon	17,775	11,750	32.0	58.0	45.0
Washington	15,592	10,050	32.0	75.0	53.5
**	**	**	**	**	**
California (--%)+	--	0	--	--	--
**	**	**	**	**	**
California (17.6%)+	9,715	10,175	21.1	50.9	40.2
Oregon	11,800	9,500	5.1	59.8	39.0
Washington	9,300	26,800	0.0	87.1	51.9
California (7.8%)+	1,708	1,519	6.0	60.6	25.1
California (13.8%)+	6	1	0.0	27.4	4.9
California (8.8%)+	--	*	*	*	*
California (24.1%)+	8,001	8,656	30.4	51.1	43.5
California (82.5%)+	917,000	2,041	0.0	0.2	0.1
California (--%)+	317,400	1,162	0.1	0.3	0.2
California (--%)+	599,600	879	0.0	0.2	0.1

**	**	**	**	**	**
California (60.7%)+	40,100	85,260	45.0	65.4	57.5
Florida	9,700	700	0.0	9.8	2.5
Michigan	700	20	0.0	5.7	1.4
New York	1,400	50	0.0	6.0	1.4
Oregon	2,100	1,000	16.8	60.2	37.5
Pennsylvania	800	10	0.0	3.6	1.0
Washington	1,200	400	0.0	39.3	10.9
**	**	**	**	**	**
California (13.4%)+	24	*	*	*	*
**	**	**	**	**	**
California (1.4%)+	230	*	*	*	*
California (0.6%)+	3,166	459	0.0	13.2	2.8
Arizona	14,200	2,200	0.0	4.9	1.8
Georgia	117,900	800	0.0	1.3	0.3
Texas	170,900	4,900	0.4	2.0	1.0
Alabama	14,100	*	*	*	*
Louisiana	3,200	*	*	*	*
New Mexico	40,100	*	*	*	*
Oklahoma	126,700	*	*	*	*
California (99.1%)+	329,000	14,333	1.1	2.0	1.5
California (2.5%)+	32,552	1,337	0.9	10.5	3.6
California (0.2%)+	564,000	541	0.1	0.1	0.1
Michigan	2,600,000	500	0.0	0.2	0.0
Alabama	296,000	*	*	*	*
Arkansas	648,000	*	*	*	*
Colorado	1,268,000	*	*	*	*

Delaware	186,000	*	*	*	*
Georgia	379,000	*	*	*	*
Idaho	352,000	*	*	*	*
Illinois	12,300,000	*	*	*	*
Indiana	5,980,000	*	*	*	*
Iowa	542,000	*	*	*	*
Kansas	4,490,000	*	*	*	*
Kentucky	1,520,000	*	*	*	*
Louisiana	542,000	*	*	*	*
Maryland	490,000	*	*	*	*
Minnesota	8,440,000	*	*	*	*
Mississippi	748,000	*	*	*	*
Missouri	3,400,000	*	*	*	*
Nebraska	9,740,000	*	*	*	*
New Mexico	122,000	*	*	*	*
New York	1,150,000	*	*	*	*
North Carolina	878,000	*	*	*	*
North Dakota	3,090,000	*	*	*	*
Ohio	3,710,000	*	*	*	*
Oklahoma	358,000	*	*	*	*
Pennsylvania	1,460,000	*	*	*	*
South Carolina	319,000	*	*	*	*
South Dakota	5,640,000	*	*	*	*
Tennessee	890,000	*	*	*	*
Texas	2,150,000	*	*	*	*
Virginia	494,000	*	*	*	*
Washington	196,000	*	*	*	*

Wisconsin	4,200,000	*	*	*	*
Wyoming	95,000	*	*	*	*
**	**	**	**	**	**
California (5.7%)+	32,340	1,633	2.8	3.6	3.2
Florida	46,100	*	*	*	*
Georgia	21,500	*	*	*	*
Illinois	17,200	*	*	*	*
Michigan	9,900	*	*	*	*
Minnesota	124,000	*	*	*	*
New Jersey	7,500	*	*	*	*
New York	32,900	*	*	*	*
Ohio	16,000	*	*	*	*
Oregon	23,600	*	*	*	*
Pennsylvania	14,700	*	*	*	*
Washington	93,300	*	*	*	*
Wyoming	78,300	*	*	*	*
**	**	**	**	**	**
California (2.3%)+	150,000	371	0.0	0.5	0.3
California (20.9%)+	516,400	678	0.0	0.7	0.1
Arkansas	1,262,000	700	0.0	0.4	0.1
Louisiana	424,400	6,000	0.0	2.7	1.4
Texas	144,400	100	0.0	0.6	0.1
Mississippi	152,000	*	*	*	*
Missouri	178,200	*	*	*	*
**	**	**	**	**	**
California (0.7%)+	6,762	4	0.0	1.3	0.1
California (0.3%)+	28,462	112	0.0	0.8	0.4
Texas	2,510,000	1,100	0.0	0.1	0.0

Arkansas	191,600	*	*	*	*
Colorado	316,000	*	*	*	*
Georgia	48,000	*	*	*	*
Illinois	28,600	*	*	*	*
Kansas	2,850,000	*	*	*	*
Louisiana	112,000	*	*	*	*
Missouri	88,000	*	*	*	*
Nebraska	201,000	*	*	*	*
New Mexico	112,000	*	*	*	*
Oklahoma	344,000	*	*	*	*
South Dakota	226,000	*	*	*	*
California (1.0%)+	646,000	8,985	0.3	2.3	1.2
Idaho	1,341,000	1,500	0.0	1.2	0.2
Kansas	9,280,000	12,900	0.0	0.4	0.1
Oklahoma	5,360,000	2,400	0.0	0.4	0.1
Texas	5,830,000	1,100	0.0	0.1	0.0
Washington	2,290,000	60	0.0	0.1	0.0
Arizona	89,600	*	*	*	*
Arkansas	508,000	*	*	*	*
Colorado	2,490,000	*	*	*	*
Georgia	300,000	*	*	*	*
Illinois	718,000	*	*	*	*
Indiana	406,000	*	*	*	*
Kentucky	590,000	*	*	*	*
Michigan	594,000	*	*	*	*
Minnesota	1,410,000	*	*	*	*
Missouri	870,000	*	*	*	*

Montana	5,560,000	*	*	*	*
Nebraska	1,480,000	*	*	*	*
New Mexico	433,000	*	*	*	*
North Carolina	814,000	*	*	*	*
North Dakota	7,460,000	*	*	*	*
Ohio	650,000	*	*	*	*
Oregon	890,000	*	*	*	*
South Dakota	2,670,000	*	*	*	*
Tennessee	470,000	*	*	*	*
Virginia	286,000	*	*	*	*
**	**	**	**	**	**
California (25.4%)+	8,228	784	1.3	5.2	3.5
California (5.39%)+	845,000	103,831	6.1	11.6	8.7
Arizona	258,000	49,900	0.0	21.1	7.9
Colorado	742,000	4,900	0.0	1.4	0.5
Illinois	318,000	700	0.0	2.0	0.4
Indiana	268,000	2,500	0.0	4.4	0.9
Kansas	640,000	4,200	0.0	1.7	0.5
Kentucky	201,000	1,900	0.0	2.4	0.9
Michigan	662,000	50	0.0	0.1	0.0
Nebraska	772,000	200	0.0	0.2	0.0
North Dakota	1,498,000	1,800	0.0	0.7	0.1
Ohio	330,000	50	0.0	0.1	0.0
Oklahoma	260,000	200	0.0	0.4	0.1
Pennsylvania	394,000	200	0.0	0.3	0.1
South Dakota	2,010,000	15,700	0.0	4.7	1.2
Utah	538,000	7,500	0.1	3.2	1.2

Wyoming	520,000	3,700	0.0	4.2	1.2
Idaho	1,060,000	*	*	*	*
Iowa	756,000	*	*	*	*
Minnesota	1,020,000	*	*	*	*
Missouri	250,000	*	*	*	*
Montana	1,870,000	*	*	*	*
Nevada	241,000	*	*	*	*
New Mexico	212,000	*	*	*	*
New York	380,000	*	*	*	*
Oregon	388,000	*	*	*	*
Texas	126,000	*	*	*	*
Virginia	79,000	*	*	*	*
Washington	416,000	*	*	*	*
Wisconsin	520,000	*	*	*	*
**	**	**	**	**	**
**	**	**	**	**	**
California (--%)+	20,560	974	0.0	9.6	4.1
**	**	**	**	**	**
**	**	**	**	**	**
**	**	**	**	**	**
**	**	**	**	**	**
California (--%)+	--	86	--	--	--
**	**	**	**	**	**
**	**	**	**	**	**
California (--%)+	--	253	--	--	--
**	**	**	**	**	**
**	**	**	**	**	**
California (--%)+	19,894,197	133	0.0	0.0	0.0
**	**	**	**	**	**
California (--%)+	1,098	61	0.0	13.4	4.2
**	**	**	**	**	**

**	**	**	**	**	**
**	**	**	**	**	**
California (--%)+	2,032	*	*	*	*
California (3.9%)+	295,800	22,334	0.1	14.5	3.6
Arkansas	421,000	5,900	0.0	2.8	1.0
Louisiana	188,000	32,500	0.0	23.0	9.5
Missouri	287,000	3,900	0.0	8.5	1.7
Oklahoma	275,000	700	0.0	1.6	0.3
Texas	6,234,000	3,800	0.0	0.1	0.0
Alabama	377,000	*	*	*	*
Arizona	177,000	*	*	*	*
Florida	111,000	*	*	*	*
Georgia	1,340,000	*	*	*	*
Kansas	41,200	*	*	*	*
Mississippi	451,000	*	*	*	*
North Carolina	535,000	*	*	*	*
South Carolina	271,000	*	*	*	*
Tennessee	310,000	*	*	*	*
**	**	**	**	**	**
**	**	**	**	**	**
California (--%)+	19,339	0	0.0	0.0	0.0
California (37.4%)+	9,704	2,816	3.4	34.3	17.1
Michigan	10,000	*	*	*	*
Washington	5,100	*	*	*	*
California (81.3%)+	58,642	146	0.0	0.2	0.1
California (95.8%)+	7,220	781	1.0	11.1	5.3
**	**	**	**	**	**
California (15.5%)+	7,312	0	0.0	1.6	0.3
**	**	**	**	**	**

**	**	**	**	**	**
**	**	**	**	**	**
**	**	**	**	**	**
**	**	**	**	**	**
California (0.02%)+	--	*	*	*	*
**	**	**	**	**	**
**	**	**	**	**	**
California (3.9%)+	2,400	12	0.0	0.3	0.2
**	**	**	**	**	**

Notes
Agricultural usage surveyed by market research firm(s)
Jnited States Department of Agriculture National Agricultural Statistics Service
fornia Department of Pesticide Regulation. Over than 80% of crop grown in California
ornia Department of Pesticide Regulation and California Agricultural Statistics Service.
Department of Pesticide Regulation and California Count Agricultural Commsioner' Report
Percent of crop grown in California
represents the total number of acres that are grown of the crop in each state. It is independent cide. CAG values for the data sources come from the following: AMRD comes from AMRD n the 2012 Census of Agriculture, and CDPR estimates come from the 2015-2016 California w (CASR) or 2011-2015 California County Agricultural Commissioners' Reports (CCACR).
n this document may differ from those displayed in the SLUA and other BEAD documents, because different calculation methods were used.
eyed by the indicated source in the years listed, but no usage reported.
Site not surveyed at national level
orts to establish an estimate. This indicates that the chemical is only periodically used by a small number of users.
Estimate not available

Table 1. National Malathion Agricultural Usage by Crop (Data A

Crop Group	Crop	Data Source	States with Reported Usage
Root and Tuber Vegetables (including leaves)	Beets (Garden/Table)	**	**
Root and Tuber Vegetables (including leaves)	Carrots	AMRD (2011-2015)	CA
Root and Tuber Vegetables (including leaves)	Chayote roots	**	**
Root and Tuber Vegetables (including leaves)	Horseradish	**	**
Root and Tuber Vegetables (including leaves)	Parsnip	**	**
Root and Tuber Vegetables (including leaves)	Potatoes	AMRD (2011-2015)	ID, MN, MT
Root and Tuber Vegetables (including leaves)	Radish	**	**
Root and Tuber Vegetables (including leaves)	Rutabagas	**	**
Root and Tuber Vegetables (including leaves)	Salsify	**	**
Root and Tuber Vegetables (including leaves)	Sweet Potato	**	**
Root and Tuber Vegetables (including leaves)	Turnips	**	**
Root and Tuber Vegetables (including leaves)	Yams	**	**
Bulb Vegetable	Garlic	CDPR (2011-2015)	CA
Bulb Vegetable	Leeks	**	**
Bulb Vegetable	Onions (Bulb/Green)	AMRD (2011-2015)	CA, ID, OR, WA
Bulb Vegetable	Shallots	**	**
Leafy Vegetables (except Brassica)	Amaranth	**	**
Leafy Vegetables (except Brassica)	Arugula	**	**
Leafy Vegetables (except Brassica)	Celery	CDPR (2011-2015)	CA
Leafy Vegetables (except Brassica)	Celtuce	**	**
Leafy Vegetables (except Brassica)	Chrysanthemum (Edible)	**	**
Leafy Vegetables (except Brassica)	Corn Salad	**	**
Leafy Vegetables (except Brassica)	Dandelion	**	**
Leafy Vegetables (except Brassica)	Dock (Sorrel)	**	**
Leafy Vegetables (except Brassica)	Endive	**	**
Leafy Vegetables (except Brassica)	Florence fennel (Finnochio)	**	**
Leafy Vegetables (except Brassica)	Kale	**	**
Leafy Vegetables (except Brassica)	Kohlrabi	**	**
Leafy Vegetables (except Brassica)	Lettuce (Leaf and Head)	AMRD (2011-2015)	AZ, CA
Leafy Vegetables (except Brassica)	Mustard Greens	**	**
Leafy Vegetables (except Brassica)	Mustard Spinach	**	**
Leafy Vegetables (except Brassica)	Orach	**	**
Leafy Vegetables (except Brassica)	Parsley	**	**
Leafy Vegetables (except Brassica)	Purslan (Garden/Winter)	**	**
Leafy Vegetables (except Brassica)	Radicchio	**	**
Leafy Vegetables (except Brassica)	Spinach	AMRD (2011-2015)	CA

Leafy Vegetables (except Brassica)	Swiss Chard	**	**
Leafy Vegetables (except Brassica)	Watercress	**	**
Brassica (Cole) Leafy Vegetables	Broccoli	CDPR (2011-2015)	CA
Brassica (Cole) Leafy Vegetables	Broccoli Raab/Rappini	CDPR (2011-2015)	CA
Brassica (Cole) Leafy Vegetables	Brussels sprout	CDPR (2011-2015)	CA
Brassica (Cole) Leafy Vegetables	Cabbage	AMRD (2011-2015)	CA, FL, MI
Brassica (Cole) Leafy Vegetables	Cabbage, Chinese (Bok Choy/Napa)	**	**
Brassica (Cole) Leafy Vegetables	Cauliflower	CDPR (2011-2015)	CA
Brassica (Cole) Leafy Vegetables	Cavalo Broccolo	**	**
Brassica (Cole) Leafy Vegetables	Collards	**	**
Brassica (Cole) Leafy Vegetables	Chinese broccoli (Gai lon)	**	**
Brassica (Cole) Leafy Vegetables	Chinese mustard (Gai choy)	**	**
Brassica (Cole) Leafy Vegetables	Mizuna	**	**
Brassica (Cole) Leafy Vegetables	Mustard spinach	**	**
Brassica (Cole) Leafy Vegetables	Rape greens	**	**
Legume Vegetables	Beans (Succulent)	AMRD (2011-2015)	*
Legume Vegetables	Dry Beans/Peas	AMRD (2011-2015)	CA, ID, TX
Legume Vegetables	Lima Beans	AMRD (2011-2015)	*
Legume Vegetables	Peas (Fresh/Green/ Sweet)	AMRD (2011-2015)	*
Fruiting Vegetable	Eggplant	**	**
Fruiting Vegetable	Okra	**	**
Fruiting Vegetable	Peppers	AMRD (2011-2015)	CA, TX
Fruiting Vegetable	Tomatillos	**	**
Fruiting Vegetable	Tomatoes	AMRD (2011-2015)	CA, FL
Cucurbit Vegetables	Chayote fruit	**	**
Cucurbit Vegetables	Cucumbers	AMRD (2011-2015)	FL
Cucurbit Vegetables	Melons, Cantaloupe	AMRD (2011-2015)	CA, TX
Cucurbit Vegetables	Melons, Casabas	**	**
Cucurbit Vegetables	Melons, Crenshaw	**	**
Cucurbit Vegetables	Melons, Honey Balls	**	**
Cucurbit Vegetables	Melons, Honeydews	NASS (2014)	*
Cucurbit Vegetables	Melons, Muskmelons	**	**
Cucurbit Vegetables	Melons, Persians	**	**
Cucurbit Vegetables	Pumpkins	AMRD (2011-2015)	CA, IL, IN, NJ, NY, OH, PA, TN, VA

Cucurbit Vegetables	Squash (Summer/Winter)	AMRD (2011-2015)	CA, GA, OH, TX
Cucurbit Vegetables	Watermelons	AMRD (2011-2015)	MS, OK, SC, TX
Citrus	Grapefruit	AMRD (2011-2015)	FL
Citrus	Kumquats	**	**
Citrus	Lemons	CDPR (2011-2015)	CA
Citrus	Limes	**	**
Citrus	Oranges	AMRD (2011-2015)	CA, FL
Citrus	Tangelos	NASS (2011, 2015)	FL
Citrus	Tangerines	NASS (2011, 2015)	FL, CA
Citrus	Other citrus hybrids	**	**
Pome Fruit	Pears	AMRD (2011-2015)	*
Stone Fruits	Apricots*	CDPR (2011-2015)	CA
Stone Fruits	Cherries (Sweet/Tart)	AMRD (2011-2015)	CA, OR, WA
Stone Fruits	Nectarines	CDPR (2011-2015)	CA
Stone Fruits	Peaches	AMRD (2011-2015)	MI, TX, WA
Berry and Small Fruit	Bushberries, Blueberries	NASS (2011, 2015)	GA, MI, NJ, NC, OR, WA
Berry and Small Fruit	Bushberries, Currants	**	**
Berry and Small Fruit	Bushberries, Gooseberries	**	**
Berry and Small Fruit	Caneberries[1]	AMRD (2011-2015)	CA, OR, WA
Berry and Small Fruit	Grapes (All)	CDPR (2011-2015)	CA
Berry and Small Fruit	Grapes (Raisin/Table)	CDPR (2011-2015)	CA
Berry and Small Fruit	Grapes (Wine)	CDPR (2011-2015)	CA
Berry and Small Fruit	Grape vines	**	**
Berry and Small Fruit	Strawberries	AMRD (2011-2015)	CA, FL, MI, NY, OR, PA, WA
Tree Nuts	Chestnuts	**	**
Tree Nuts	Macadamia Nut	**	**
Tree Nuts	Pecans	AMRD (2011-2015)	AL, GA, TX
Tree Nuts	Walnuts	CDPR (2011-2015)	CA

Cereal Grains	Barley	NASS (2011)	CA
Cereal Grains	Corn, Field	AMRD (2011-2015)	MI
Cereal Grains	Corn, Pop	**	**
Cereal Grains	Corn, Sweet	AMRD (2011-2015)	CA
Cereal Grains	Oats	NASS (2015)	*
Cereal Grains	Rice	AMRD (2011-2015)	AR, LA, TX
Cereal Grains	Rye	**	**
Cereal Grains	Sorghum (Milo)	AMRD (2011-2015)	TX
Cereal Grains	Wheat	AMRD (2011-2015)	CA, ID, KS, OK, TX, WY
Cereal Grains	Spring Wheat	AMRD (2011-2015)	CA, ID
Cereal Grains	Winter Wheat	AMRD (2011-2015)	CA, KS, OK, TX, WA
Cereal Grains	Wild rice	**	**
Grass Forage, Fodder, and Hay	Alfalfa	AMRD (2011-2015)	AZ, CA, CO, IL, IN, KS, KY, MI, NE, ND, OH, OK, PA, SD, UT, WY
Grass Forage, Fodder, and Hay	Birdsfoot Trefoil	**	**
Grass Forage, Fodder, and Hay	Grasses[2]	**	**
Grass Forage, Fodder, and Hay	Pasture and Rangeland	**	**
Nongrass Animal Feeds	Clover	**	**
Nongrass Animal Feeds	Lespedeza	**	**
Nongrass Animal Feeds	Lupine	**	**
Nongrass Animal Feeds	Vetch	**	**
Oilseed Crops	Cotton	AMRD (2011-2015)	AR, LA, MO, OK, TX
Oilseed Crops	Flax	**	**
Edible Fungi	Mushrooms	**	**
Stalk Stem and Leaf Petiole Vegetables	Asparagus	AMRD (2011-2015)	CA
Tropical and Subtropical Fruit	Avocados	CDPR (2011-2015)	CA
Tropical and Subtropical Fruit	Figs	CDPR (2011-2015)	CA
Tropical and Subtropical Fruit	Guava	**	**
Tropical and Subtropical Fruit	Mangos	**	**
Tropical and Subtropical Fruit	Papaya	**	**
Tropical and Subtropical Fruit	Passion Fruit	**	**
Tropical and Subtropical Fruit	Pineapples	**	**
Misc. Crops	Hops	**	**
Misc. Crops	Mint	**	**
Misc. Crops	Mint, Peppermint	**	**

Misc. Crops	Mint, Spearmint	**	**

[1] Caneberries include blackberries, boysenberries, dewberries, loganbe

[2] Grasses include Bermuda, barnyard grass, canary grass, fescue, orcha

Notes	
AMRD (YEAR-YEAR)	Agricultural usage surveyed by market research fi
NASS (YEAR)	Surveyed by United States Department of Agriculture National Agric
Cal DPR (YEAR)	Surveyed by the California Department of Pesticide Regulation. Over in California
a	The pounds AI displayed in this document may differ from those dis other BEAD documents, because different calculation meth
b	Total Acres Treated accounts for multiple applications to a single area the number of acres treated as some acres are treated mor
c	Malathion underwent re-registration, which culminated in a registrati 2009. As a result of that decision the maximum single application r "Max Labeled Single AI Rate" listed for this crop. Because the mal application rate for this crop was immediately prior to the sampling products bearing the reduced application rate may not have entered th the beginning of the sampling interval and/or existing stocks of prod reregistration application rate were likely still being exhausted by gro interval. For these reasons, use rate averages during the sampling i current labeled maximum use rates are not unexpected. The obser current "Max Labeled Single AI Rate" for this crop during the sa suggestive of any exceedances of the maximum application rate by a due to the application rate change near or during the sampling inter application rate average cannot be considered a reliable estimate of t application rate for this crop. The "Max Labeled Single AI Rate" s application rate for this crop.
d	Max labeled rate from 2017 EFED 1-3 Master Use
*	Surveyed by the indicated source in the years listed, but no
**	Site not surveyed at national level
(S)	Insufficient number of reports to establish an estimate. This indicates periodically used by a small number of users.

veraged Over Reported Years).

Avg. Annual Pounds AI Applied [a]	Avg. Annual Total Acres Treated [b]	% Applied by Air	Avg. Single AI Rate	Max Single Labeled Rate lb/a [d]
**	**	**	**	1.25
500	300	19	1.600[c]	1.25
**	**	**	**	1.56
**	**	**	**	1.25
**	**	**	**	1.25
1,200	1,100	98	1.03	1.56
**	**	**	**	1
**	**	**	**	1
**	**	**	**	1.25
**	**	**	**	1.56
**	**	**	**	1.25
**	**	**	**	1.56
137	206	65	0.663	1.56
**	**	**	**	1.56
10,300	6,900	9	1.468	1.56
**	**	**	**	1.56
**	**	**	**	1.25
**	**	**	**	1.25
10,204	6,983	23	1.461	1.5
**	**	**	**	1.25
**	**	**	**	1.25
**	**	**	**	1.25
**	**	**	**	1.25
**	**	**	**	1.25
**	**	**	**	1.25
**	**	**	**	1.25
**	**	**	**	1
**	**	**	**	1.25
17,500	9,900	1	1.772	1.88
**	**	**	**	1
**	**	**	**	1.25
**	**	**	**	1.25
**	**	**	**	1.5
**	**	**	**	1.25
**	**	**	**	0.188
800	600	0	1.368[c]	1

**	**	**	**	1
**	**	**	**	1.25
14,513	10,558	30	1.375[c]	1.25
366	205	51	1.778[c]	1.25
2,102	1,484	4	1.416[c]	1.25
2,000	1,700	0	1.162	1.25
**	**	**	**	1.25
1,995	1,543	18	1.293[c]	1.25
**	**	**	**	1.25
**	**	**	**	1
**	**	**	**	1.25
**	**	**	**	1.25
**	**	**	**	1.25
**	**	**	**	1.25
**	**	**	**	1.25
*	*	*	*	0.61
1,000	1,000	0	1.000[c]	0.61
*	*	*	*	0.61
*	*	*	*	1
**	**	**	**	1.56
**	**	**	**	1.2
700	500	0	1.297	1.56
**	**	**	**	1.56
5,600	4,900	0	1.141	1.56
**	**	**	**	1.75
1,100	900	0	1.25	1.75
400	400	91	0.994	1
**	**	**	**	1
**	**	**	**	1
**	**	**	**	1
*	*	*	*	1
**	**	**	**	1
**	**	**	**	1
1,700	1,900	6	0.91	1

1,800	1,900	0	0.95	1.750 (summer) 1.000 (winter)
2,400	2,400	0	1.031	1.5
6,300	5,700	41	1.098	7.5 (CA) 4.5 (non-CA)
**	**	**	**	7.5 (CA) 4.5 (non-CA)
145	92	0	1.571	7.5 (CA) 4.5 (non-CA)
**	**	**	**	7.5 (CA) 4.5 (non-CA)
174,400	148,600	24	1.173	7.5 (CA) 4.5 (non-CA)
550	1,000	--	0.55	7.5 (CA) 4.5 (non-CA)
16,150	19,918	--	0.811	7.5 (CA) 4.5 (non-CA)
**	**	**	**	7.5 (CA) 4.5 (non-CA)
*	*	*	*	1.25
2	6	0	0.415	1.5
66,500	49,200	55	1.351	1.75
8	2	0	3.921[c]	3
2,300	1,600	0	1.49	3
75,950	70,209	NA	1.082	1.25
**	**	**	**	1.25
**	**	**	**	2
53,600	28,300	5	1.892	2
2,041	1,288	0	1.584	1.88
1,162	776	0	1.498	1.88
879	512	0	1.716	1.88
**	**	**	**	1.888
91,800	48,800	0	1.883	2
**	**	**	**	2.5
**	**	**	**	0.94
7,800	2,900	21	2.701[c]	2.5
14,333	6,228	16	2.301	2.5

(S)	(S)	(S)	(S)	1.25
500	800	0	0.625	1
**	**	**	**	1
6,200	6,100	35	1.005	1
*	*	*	*	1
7,100	7,500	100	0.944	1.25
**	**	**	**	1
1,100	1,200	0	0.938	1
100,700	85,700	71	1.175[c]	1
8,800	9,000	0	0.979	1
91,900	76,700	79	1.198[c]	1
**	**	**	**	1.25
155,300	166,100	17	0.935	1.25
**	**	**	**	1.25
**	**	**	**	1.25
**	**	**	**	1.25
**	**	**	**	1.25
**	**	**	**	1.25
**	**	**	**	0.61
**	**	**	**	1.25
46,800	39,600	17	1.182	2.5
**	**	**	**	0.5
**	**	**	**	1.7
2,100	2,000	0	1.037	1.25
146	38	65	3.805	4.7
781	455	59	1.716	2
**	**	**	**	1.25
**	**	**	**	0.94
**	**	**	**	1.25
**	**	**	**	1
**	**	**	**	2
**	**	**	**	0.63
**	**	**	**	0.94
**	**	**	**	0.94

**	**	**	**	0.94

rries, and raspberries.

ırdgrass, red top, timothy, and yellow foxtail

rm(s)
ultural Statistics Service
than 80% of crop grown
ɔlayed in the SLUA and ods were used.
ı. This may overestimate e than once.
on eligibility decision in ate was reduced to the athion maximum single interval (2011-2015), e marketplace until after uct with the higher pre- wers during the sampling nterval that exceed the ved exceedance of the npling interval is not ɔplicators. Furthermore, val, the reported single ıe current average single ıould be considered the
Table.
ısage reported.
that the chemical is only

Message

From: Sims, Diann [Sims.Diann@epa.gov]
Sent: 6/26/2018 5:41:29 PM
To: Garber, Kristina [Garber.Kristina@epa.gov]
Subject: FW: Use/usage comments on the BEs
Attachments: 3 OPs.use.usage.comments.docx

From: Miller, Wynne
Sent: Tuesday, June 26, 2018 1:41 PM
To: Sims, Diann <Sims.Diann@epa.gov>; Suarez, Mark <Suarez.Mark@epa.gov>
Cc: Perry, Tracy <Perry.Tracy@epa.gov>
Subject: FW: Use/usage comments on the BEs

From: Perry, Tracy
Sent: Monday, April 09, 2018 5:38 PM
To: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>
Cc: Miller, Wynne <Miller.Wynne@epa.gov>; Sherman, Kelly <Sherman.Kelly@epa.gov>
Subject: RE: Use/usage comments on the BEs

Yu-Ting,

I looked through the Response to Comments document on the draft BEs and put together the attached table. It is a subset of the substantive comments that were received – the ones that pertain to use/usage. The first 5 entries (see yellow highlights) may have information we could use in refined assessments. The comments below these 5 are from grower groups, mosquito use districts, etc. and contain information on benefits (some with specific crop/chemical/pest information). USDA/IR-4 recommended formation of an expert mosquito workgroup, to include EPA, Services, IR-4 and AMCA, to review mosquito usage data, modeling assumptions, etc.

Tracy L. Perry
Senior Regulatory Advisor
Risk Management and Implementation Branch III
Pesticide Re-evaluation Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
(703) 308-0128

From: Guilaran, Yu-Ting
Sent: Monday, April 09, 2018 8:28 AM
To: Perry, Tracy <Perry.Tracy@epa.gov>

Cc: Miller, Wynne <Miller.Wynne@epa.gov>
Subject: Use/usage comments on the BEs

Tracy
When you get a chance, can you see what use/usage comments EPA received on the three BEs? I know it is probably pretty tedious....let me know if it's possible. Thanks.

Regards,

Yu-Ting Guilaran, P.E.
Director

Pesticide Re-evaluation Division (PRD)
Office of Pesticide Programs
Office of Chemical Safety and Pollution Prevention
(tel) 703 308 0052
(fax)703 308 8005
Mail code 7508P
Room number PY S9623

Message

From: Anderson, Brian [Anderson.Brian@epa.gov]
Sent: 6/8/2018 2:04:35 PM
To: Panger, Melissa [Panger.Melissa@epa.gov]; Garber, Kristina [Garber.Kristina@epa.gov]
Subject: FW: AMCA meeting follow-up: list of additional useful information

---

From: Perry, Tracy
Sent: Tuesday, June 05, 2018 5:34 PM
To: Ruckert, Edward <eruckert@mwe.com>
Cc: Anderson, Brian <Anderson.Brian@epa.gov>
Subject: RE: AMCA meeting follow-up: list of additional useful information

Ed,

Thank you for the confirmation. Please be sure to keep Brian in the loop. I will be on vacation starting this Friday (8th) and won't be back in the office until June 25.

Tracy L. Perry
Senior Regulatory Advisor
Risk Management and Implementation Branch III
Pesticide Re-evaluation Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
(703) 308-0128

From: Ruckert, Edward [mailto:eruckert@mwe.com]
Sent: Monday, June 04, 2018 10:16 AM
To: Perry, Tracy <Perry.Tracy@epa.gov>
Cc: Anderson, Brian <Anderson.Brian@epa.gov>
Subject: RE: AMCA meeting follow-up: list of additional useful information

Tracy,
My apologies for not confirming with you earlier that I had received your email with the list of information needs. I had sent it to the client, and there will be a conference call internally regarding what information can be provided more readily and what information may be more difficult to collect. I will keep you posted. Regards, Ed

Edward M. Ruckert
Partner

McDermott Will & Emery LLP | The McDermott Building | 500 North Capitol Street, N.W. | Washington, DC 20001
Tel +1 202 756 8214 | Fax +1 202 756 8087

Biography | Website | vCard | Email | Twitter | LinkedIn | Blog

---

From: Perry, Tracy [mailto:Perry.Tracy@epa.gov]
Sent: Friday, June 01, 2018 6:16 PM

To: Ruckert, Edward
Cc: Anderson, Brian
Subject: RE: AMCA meeting follow-up: list of additional useful information

Hi Ed,

Just checking in to see if you received this email?

-Tracy

From: Perry, Tracy
Sent: Tuesday, May 29, 2018 5:54 PM
To: 'Ruckert, Edward' <eruckert@mwe.com>
Cc: Anderson, Brian <Anderson.Brian@epa.gov>
Subject: AMCA meeting follow-up: list of additional useful information

Hello Ed,

Following up on our May 16 meeting with the American Mosquito Control Association, we have put together a list of the types of information that would be useful for refining our risk assessments on mosquito adulticides. In general, information that identifies where specific chemicals are or are not used, how they are applied (application method, frequency, quantity), and information that will allow us to refine our modeling (e.g., wind speed, deposition data, etc.) would be helpful. Additional details are below. Please contact Brian Anderson if you have any questions.

- Information regarding the following per year (separated by larvicides and adulticides; and by chemical):
  - What pesticide active ingredients/products are being used
  - How often (applications per year; application intervals)
  - Application method (truck, airplane, backpack, etc.)
  - When is it being used (time of year)
  - How much is being used (areas covered; lb a.i./acre)
  - Where is it being used (the more specific, the more useful) – it will also be useful to know where they are **not** being used.

- Data on deposition of adulticides on non-target areas (terrestrial and aquatic) after applications (separated by aerial vs ground applications).

- Usage and/or sales data to help determine where specific pesticides have been used (last ~5 years)
  - The finest spatial resolution available is of interest

- Description of any factors that would help exclude areas where treatments wouldn't occur (with reasonable confidence), for example:
  - Temperature, Elevation, Agricultural areas
  - Any mappable features that can be excluded as potential use sites

- Information on the mosquito species found in different areas of the US and biological information on the species (limited to those species which are actively treated either as a known disease vector and/or a nuisance pest), for example:
  - Daily activity period, flight distances

    - Which species vector what diseases, what species are the targets and where
    - Mosquito range data, mosquito habitat information

- Information on consultations between mosquito districts/states and other federal agencies (*e.g.*, FL – FEMA MOU example)
    - List of mosquito control districts that are coordinating with the Services on ESA

- Description of the GPS-based application technology (*e.g.*, ability to maintain droplet size and exclude areas b/c of sensitivity, elevation, *etc.*)

- Information on states using private applicators vs states with mosquito control districts (or states that use both)? (how are they similar/different?)

- Ideas on label language that would minimize impacts to species while maintaining vector control; in particular, practices that are already being implemented that should be added to or clarified on existing labels

Regards,

Tracy L. Perry
Senior Regulatory Advisor
Risk Management and Implementation Branch III
Pesticide Re-evaluation Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
(703) 308-0128

From: Perry, Tracy
Sent: Friday, May 18, 2018 11:49 AM
To: 'eruckert@mwe.com' <eruckert@mwe.com>
Cc: Anderson, Brian <Anderson.Brian@epa.gov>
Subject: AMCA participant list

Ed,

Attached is the participant list from the AMCA/EPA/Services/USDA meeting on May 16. Please let me know if I've left anyone off the list from the AMCA group that may have been on the phone.

As I mentioned, I will be putting a short summary of this meeting (along with the agenda and participant list) in the NMFS BiOp docket as well as the chlorpyrifos and malathion dockets.

Regards,

Tracy L. Perry
Senior Regulatory Advisor
Risk Management and Implementation Branch III
Pesticide Re-evaluation Division
Office of Pesticide Programs
U.S. Environmental Protection Agency

(703) 308-0128

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****

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Message

From: Paisley-Jones, Claire [Paisley-Jones.Claire@epa.gov]
Sent: 4/23/2018 7:58:29 PM
To: Garber, Kristina [Garber.Kristina@epa.gov]; Panger, Melissa [Panger.Melissa@epa.gov]
CC: Sims, Diann [Sims.Diann@epa.gov]
Subject: Chlorpyrifos SUUM draft
Attachments: Chlorpyrifos SUUM_update (for efed review).xlsx

Hi Kris and Melissa,

Attached is the draft SUUM for Chlorpyrifos. We're still reviewing internally, and waiting to hear from Kline on a few things, but I wanted to give you as much time as possible to review. Let me know if you have any comments!

Please forward this to whoever on the team should review.

Thanks,

Claire

Message

From: Peck, Charles [Peck.Charles@epa.gov]
Sent: 3/1/2018 10:45:01 PM
To: Garber, Kristina [Garber.Kristina@epa.gov]
Subject: Thoughts on Workgroup Etiquette
Attachments: working group guidelines.docx

Flowery language for guidelines. Feel free to edit and share

Chuck Peck
OPP/EFED/ERB VI
Potomac Yard South
Crystal City, VA
Room 12314
(703) 347-8064
peck.charles@epa.gov

Message

From: White, Katrina [White.Katrina@epa.gov]
Sent: 3/1/2018 5:22:27 AM
To: Garber, Kristina [Garber.Kristina@epa.gov]
CC: Panger, Melissa [Panger.Melissa@epa.gov]
Subject: RE: Notes from ESA use workshop attached and final charge

I am not sure, I was on the other side of the room. I will go ahead and update the notes.

Katrina White
Senior Fate Scientist
Environmental Risk Branch IV
Environmental Fate & Effects Division

703-308-4536
White.katrina@epa.gov

From: Garber, Kristina
Sent: Wednesday, February 28, 2018 4:06 PM
To: White, Katrina <White.Katrina@epa.gov>
Cc: Panger, Melissa <Panger.Melissa@epa.gov>
Subject: RE: Notes from ESA use workshop attached and final charge

I wonder if some of the "Nancy FWS" comments were really Nancy Beck (EPA)? I'm thinking of p. 17:

Deliberative Process / Ex. 5

From: White, Katrina
Sent: Tuesday, February 27, 2018 11:13 AM
To: Anderson, Brian <Anderson.Brian@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>; Connolly, Jennifer <Connolly.Jennifer@epa.gov>; Lennartz, Steven <Lennartz.Steven@epa.gov>; Eckel, William <Eckel.William@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Donovan, Elizabeth <Donovan.Elizabeth@epa.gov>
Cc: Holmes, Jean <Holmes.Jean@epa.gov>; Harwood, Douglas <harwood.douglas@epa.gov>; Mastrota, Nicholas <Mastrota.Nicholas@epa.gov>; Blankinship, Amy <Blankinship.Amy@epa.gov>; Lin, James <lin.james@epa.gov>; Perry, Tracy <Perry.Tracy@epa.gov>
Subject: Notes from ESA use workshop attached and final charge

Attached are notes from the ESA workshop and the final charge below. Please pass this on to anyone I missed, who should receive these. Chuck's shorter version of the notes are at the bottom of the document.

PESTICIDE USAGE WORK GROUP (2/26/18)

Form working group with decision-makers and experts from each agency to find a path forward; decisions will be elevated to the policy team members as needed.
Team members and a team lead will be identified by this Thursday.
Check in on methodologies and next steps in 2 weeks.
EPA will provide the malathion agricultural and non-agricultural usage report by March 31.

CHARGE:
The group will determine where malathion is likely to be applied, consistent with the best available scientific and commercial data, and how those data can be incorporated into the risk assessment method.

- Use the most granular data available.
- Explain the limitations to providing more granular data as needed.
- Include assumptions about the data used to determine areas where usage is likely.
- Step 1: Come up with a timeline and tasks for developing a methodology.

Katrina White
Senior Fate Scientist
Environmental Risk Branch IV
Environmental Fate & Effects Division

703-308-4536
White.katrina@epa.gov

PESTICIDE USAGE WORK GROUP (2/26/18)

Form working group with decision-makers and experts from each agency to find a path forward; decisions will be elevated to the policy team members as needed.
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- Use the most granular data available.
- Explain the limitations to providing more granular data as needed.
- Include assumptions about the data used to determine areas where usage is likely.
- Step 1: Come up with a timeline and tasks for developing a methodology.

Message

From: Paisley-Jones, Claire [Paisley-Jones.Claire@epa.gov]
Sent: 2/21/2018 10:13:31 PM
To: Garber, Kristina [Garber.Kristina@epa.gov]
Subject: RE: for your review: draft of applying usage slide deck for meeting with services
Attachments: Pesticide Usage Data Sources Overview (bead+efed).pptx

Let me know if anything needs tweaking.

From: Garber, Kristina
Sent: Wednesday, February 21, 2018 4:55 PM
To: Paisley-Jones, Claire <Paisley-Jones.Claire@epa.gov>
Subject: RE: for your review: draft of applying usage slide deck for meeting with services

Thank you. I think that the examples will help a lot. Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Do you want me to comment on your slides?

From: Paisley-Jones, Claire
Sent: Wednesday, February 21, 2018 4:53 PM
To: Garber, Kristina <Garber.Kristina@epa.gov>
Subject: RE: for your review: draft of applying usage slide deck for meeting with services

It looks really great! Much clearer than before ☺

I'll send you mine and Melissa's in a few

From: Garber, Kristina
Sent: Wednesday, February 21, 2018 4:52 PM
To: Paisley-Jones, Claire <Paisley-Jones.Claire@epa.gov>
Subject: RE: for your review: draft of applying usage slide deck for meeting with services

Thanks. I'll work on incorporating your comments.

From: Paisley-Jones, Claire
Sent: Wednesday, February 21, 2018 4:45 PM
To: Garber, Kristina <Garber.Kristina@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>; Donovan, Elizabeth <Donovan.Elizabeth@epa.gov>; Anderson, Brian <Anderson.Brian@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Connolly, Jennifer <Connolly.Jennifer@epa.gov>; White, Katrina <White.Katrina@epa.gov>; Blankinship, Amy <Blankinship.Amy@epa.gov>; Lennartz, Steven <Lennartz.Steven@epa.gov>; Eckel, William <Eckel.William@epa.gov>
Cc: Sims, Diann <Sims.Diann@epa.gov>; Becker, Jonathan <Becker.Jonathan@epa.gov>
Subject: RE: for your review: draft of applying usage slide deck for meeting with services

I do think you could add rows to your table for total unregistered, since you need that to get to the pie chart.

Deliberative Process / Ex. 5

My comments are attached. Overall it looks really good!
Claire

From: Garber, Kristina
Sent: Wednesday, February 21, 2018 4:29 PM
To: Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>; Donovan, Elizabeth <Donovan.Elizabeth@epa.gov>; Anderson, Brian <Anderson.Brian@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Connolly, Jennifer <Connolly.Jennifer@epa.gov>; White, Katrina <White.Katrina@epa.gov>; Blankinship, Amy <Blankinship.Amy@epa.gov>; Lennartz, Steven <Lennartz.Steven@epa.gov>; Eckel, William <Eckel.William@epa.gov>
Cc: Paisley-Jones, Claire <Paisley-Jones.Claire@epa.gov>; Sims, Diann <Sims.Diann@epa.gov>; Becker, Jonathan <Becker.Jonathan@epa.gov>
Subject: RE: for your review: draft of applying usage slide deck for meeting with services

Yes, the table is pretty big when we consider all the uses that were not surveyed. I will list them off in my talking points and refer to the pie chart to indicate that they are a small part of the footprint.

From: Rossmeisl, Colleen
Sent: Wednesday, February 21, 2018 4:08 PM
To: Donovan, Elizabeth <Donovan.Elizabeth@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>; Anderson, Brian <Anderson.Brian@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Connolly, Jennifer <Connolly.Jennifer@epa.gov>; White, Katrina <White.Katrina@epa.gov>; Blankinship, Amy <Blankinship.Amy@epa.gov>; Lennartz, Steven <Lennartz.Steven@epa.gov>; Eckel, William <Eckel.William@epa.gov>
Cc: Paisley-Jones, Claire <Paisley-Jones.Claire@epa.gov>; Sims, Diann <Sims.Diann@epa.gov>; Becker, Jonathan <Becker.Jonathan@epa.gov>
Subject: RE: for your review: draft of applying usage slide deck for meeting with services

Kris –

I don't have anything additional to Elizabeth's edits. I do think you will have to cover the talking points (could add all those non-registered crops in your table with the example to show the real totals for acres, but I think it will get too busy, so maybe just discuss as it looks like you are proposing.)

Chuck – I think it **Deliberative Process / Ex. 5**
Deliberative Process / Ex. 5

Thanks-
Colleen

From: Donovan, Elizabeth
Sent: Wednesday, February 21, 2018 4:01 PM
To: Garber, Kristina <Garber.Kristina@epa.gov>; Anderson, Brian <Anderson.Brian@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Connolly, Jennifer <Connolly.Jennifer@epa.gov>; White, Katrina <White.Katrina@epa.gov>; Blankinship, Amy <Blankinship.Amy@epa.gov>; Lennartz, Steven <Lennartz.Steven@epa.gov>; Eckel, William <Eckel.William@epa.gov>
Cc: Paisley-Jones, Claire <Paisley-Jones.Claire@epa.gov>; Sims, Diann <Sims.Diann@epa.gov>; Becker, Jonathan <Becker.Jonathan@epa.gov>
Subject: RE: for your review: draft of applying usage slide deck for meeting with services

I made a few edits in red throughout (sorry I didn't realize you had red text in there until the end).

e

From: Garber, Kristina
Sent: Wednesday, February 21, 2018 3:46 PM
To: Anderson, Brian <Anderson.Brian@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Connolly, Jennifer <Connolly.Jennifer@epa.gov>; White, Katrina <White.Katrina@epa.gov>; Blankinship, Amy <Blankinship.Amy@epa.gov>; Donovan, Elizabeth <Donovan.Elizabeth@epa.gov>; Lennartz, Steven <Lennartz.Steven@epa.gov>; Eckel, William <Eckel.William@epa.gov>
Cc: Paisley-Jones, Claire <Paisley-Jones.Claire@epa.gov>; Sims, Diann <Sims.Diann@epa.gov>; Becker, Jonathan <Becker.Jonathan@epa.gov>
Subject: for your review: draft of applying usage slide deck for meeting with services

Internal deliberative, do not cite or distribute

Please review the attached slide deck. Jen and I are still working on the examples, but there's enough here to see where we are going.

Please forward this on to others I accidentally forgot that should review the slides.

Kris Garber, Senior Science Advisor
Environmental Fate and Effects Division
Office of Pesticide Programs
US Environmental Protection Agency
Mail Code: 7507P
1200 Pennsylvania Ave NW
Washington DC 20460-0001

Phone: 703-347-8940

PESTICIDE USAGE DATA SOURCES OVERVIEW

The Importance of Pesticide Usage Statistics

- Reflects actual observed agricultural and non-agricultural practices

- Helps to *realistically* characterize risk
  - ***Compare*** *label use to actual usage practices*
  - ***Refine*** *risks and benefits*
  - ***Support*** *decision making*

Who Collects Usage Data?

- USDA NASS
- CADPR
- Kynetec
- Kline

- BLM
- Few Other States
- Some crop associations
- Some chemical producers

Sources below line, can provide data, but metadata is limited

Primary Sources for National Level Pesticide Usage Data

- **Agricultural**
  - *USDA Census of Agriculture (public)*
  - *USDA NASS (public)*
  - *CADPR (public)*
    - *(for Crops >80% grown in CA)*
  - *Kynetec (proprietary)*
- **Non-agricultural**
  - *Kline (proprietary)*

4

almost all of the country's almonds, apricots, dates, figs, kiwi fruit, nectarines, olives, pistachios, prunes, and walnuts are produced in California

Deliberative Process / Ex. 5

Quality Requirements

- **EPA Quality System Policy (CIO 2106.0)**
- Documented quality assurance procedures
  - Statistical methodologies
  - Sampling plan (survey design)
  - ***Credibility, Comparability, Relevance, Completeness***
- USDA NASS
  - https://www.nass.usda.gov/Publications/Methodology_and_Data_Quality/index.php
- CADPR
  - www.cdpr.ca.gov/docs/pur/purmain.htm
- Proprietary Sources (Kynetec, Kline)
  - *Quality Management Plans*
  - *Statement of Data Quality*

5

The Agency-wide Quality System (CIO 2106.0) designed to ensure that decisions are supported by data of the type and quality needed and expected for their intended use. The policy covers a broad range of environmental data and information, including secondary data. Consistent with this policy, OPP uses best available data for its work.

Deliberative Process / Ex. 5

Publicly Available Sources

- **USDA**
  - Census of Agriculture
  - National Agricultural Statistics Service
- **CADPR**

CENSUS OF AGRICULTURE
YOUR VOICE. YOUR FUTURE. YOUR OPPORTUNITY.

Census of Ag Survey

- **Scope:**
  - Complete count of U.S. ag operations
    - *everyone who produces >$1000 of commodity in a survey year*
  - Conducted very five years
  - For each commodity
    - Who produces
    - Where produced
    - How much produced
    - How is it produced
- **Benefits:**
  - It's publicly available
  - Uniform, comprehensive, impartial
- **Limitations:**
  - Does not include pesticide information
  - Only conducted every five years

NASS Survey

- **Scope:**
  - *90 Use Sites*
  - *42 States*
  - *731 AIs*
- **Program state selection:**
  - *Ensure ≥ 80% coverage of target commodity acreage*
  - *Top-producing states, to minimize the total number of states selected.*
- **Reporting**
  - *~ Every other year (depending on study)*
  - *At program state level*
  - *Aggregated across all program states*

8

Methodology documented on its website.

BENEFITS of NASS Data

- **It's publicly available**
- Crops only surveyed by NASS on the national level
  - *blueberries, eggplant, honeydew, oats, and specific caneberries (blackberries and raspberries)*
- Helps to validate data from other sources

9

In addition to EPA, information is used by:
* Other Federal Agencies
* Academia
* Industry
* Farming/Grower Community

Deliberative Process / Ex. 5

CADPR Survey

- **Scope:**
  - Census
  - Complete reporting from
    - Agricultural applicators
    - Professional pesticide applicators
  - All AIs used
  - All use sites treated
- **Reporting:**
  - *Reported continually*
  - *Published annually*

11

CADPR receives monthly reports from county ag commissioners who require growers to report their use of pesticides.

BENEFITS of Using CADPR Data

- **It's publicly available**
- The full use reporting program =
  - *comprehensive state usage data*
- Many crops grown almost exclusively in CA =
  - *comprehensive crop usage data*
- Helps to validate data from other sources

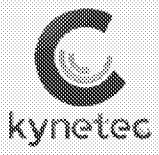
12

In addition to EPA, information is used by:
* Other Federal Agencies
* Industry
* Grower Groups
* Chemical Manufacturers

Deliberative Process / Ex. 5

Proprietary Data Sources

- Kynetec



Kynetec Survey

- **Scope:**
  - *Cover 60 unique crops (grown on 390+ Mill acres)*
    - Herbicides –60 crops
    - Insecticides - 57 crops
    - Fungicides - 53 crops
    - Nematicides - 45 crops
    - Growth Regulators - 11 crops
  - *Reports usage for 389 AIs*
- **States:**
  - *45 States surveyed for one or more*
  - *Ensure ≥ 80% coverage of target commodity acreage*
- **Reporting:**
  - *Updated annually*

Deliberative Process / Ex. 5

BENEFITS of Using Kynetec Data

- Our most complete and adaptable data set
- Provides:
  - Major crop usage
  - Major growing state usage
  - Only source for pest specific information
- *Huge* database in iMap.
  - Can be dynamically analyzed/aggregated
  - VS NASS and CDPR's static data elements
    - *must be combined and manipulated in excel/access,*
    - *can be difficult because of how the data is reported.*

Deliberative Process / Ex. 5

High Consumption Crops not Surveyed at the National Level

- Beets
- Cactus
- Cashew
- Cassava
- Collards
- Cranberries
- Endive
- Honey
- Hops
- Limes
- Maple syrup
- Mushroom
- Okra
- Onion, green
- Papaya
- Pineapple
- Plantain
- Radishes
- Rye
- Safflower
- Spices, other
- Sweet Potatoes
- Tomatillo
- Turnips
- *Many other lower consumption crops*

18

+++++ more

Non-Agricultural Data

- Kline

eKline

©Kline

Kline Survey

- **Scope:**
  - Non-ag market information
  - Combo of structured survey and unstructured expert interviews
  - We purchase 9 studies
- **Location selection:**
  - Sampling frame influenced by market size and share information
- **Reporting**
  - *~ Every other year (depending on study)*
  - *National level*
  - *Regional available for some studies*

Data items published include:
Consumer Pesticides and Fertilizers
Professional Turf and Ornamental Markets for Pesticides and Fertilizers
Professional Pest Management Markets for Pesticides
Pest Control in Food Handling
Industrial Vegetation Management
Mosquito Control Market
Stored Grains Market
Biopesticides
Specialty Biocides

Kline

Benefits of Kline's Data

- **Our only source of non-agricultural data**
- Variables include acres treated, pounds a.i. applied, sample size, sales data by a.i. and product
- Narrative descriptions of data

In addition to EPA, information is used by:
* Commodity Groups and Chemical Manufacturers
* USGS
* NGOs
* Universities
* Other Gov't Groups

Deliberative Process / Ex. 5

Usage Data Applications

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Message

From: Peck, Charles [Peck.Charles@epa.gov]
Sent: 2/20/2018 12:07:25 PM
To: Garber, Kristina [Garber.Kristina@epa.gov]
Subject: RE: summary - part two.

Thanks!

From: Garber, Kristina
Sent: Tuesday, February 20, 2018 7:06 AM
To: Peck, Charles <Peck.Charles@epa.gov>
Cc: Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>; Anderson, Brian <Anderson.Brian@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Lennartz, Steven <Lennartz.Steven@epa.gov>; Connolly, Jennifer <Connolly.Jennifer@epa.gov>; Eckel, William <Eckel.William@epa.gov>
Subject: Re: summary - part two.

I am asking Brian why we have this set of bullets. Who is the audience? Is this for BEAD or for the services?

On Feb 20, 2018, at 7:02 AM, Peck, Charles <Peck.Charles@epa.gov> wrote:

> I think Brian/Mel's write-up captures the method we're using. I think the questions of how drift and non-ag acres will be handled will come up. Not sure I understand Kris' question about another write-up.
>
> ---
>
> **From:** Garber, Kristina
> **Sent:** Tuesday, February 20, 2018 6:54 AM
> **To:** Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>
> **Cc:** Anderson, Brian <Anderson.Brian@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Lennartz, Steven <Lennartz.Steven@epa.gov>; Connolly, Jennifer <Connolly.Jennifer@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Eckel, William <Eckel.William@epa.gov>
> **Subject:** Re: summary - part two.
>
> **Deliberative Process / Ex. 5**
>
> Why do we need another write up?
>
> On Feb 20, 2018, at 6:33 AM, Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov> wrote:
>
> **Deliberative Process / Ex. 5**
>
> Thanks!
> Colleen

From: Anderson, Brian
Sent: Friday, February 16, 2018 1:30 PM
To: Panger, Melissa <Panger.Melissa@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>; Lennartz, Steven <Lennartz.Steven@epa.gov>; Connolly, Jennifer <Connolly.Jennifer@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>; Eckel, William <Eckel.William@epa.gov>
Subject: RE: summary - part two.

Just got make sure that was everyone's understanding and that BEAD can get us all the acres from their data sources on the crops within the CDL.

Thanks guys,

Brian

From: Panger, Melissa
Sent: Friday, February 16, 2018 12:45 PM
To: Anderson, Brian <Anderson.Brian@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>; Lennartz, Steven <Lennartz.Steven@epa.gov>; Connolly, Jennifer <Connolly.Jennifer@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>; Eckel, William <Eckel.William@epa.gov>
Subject: RE: summary - part two.

Clap… clap… clap! I think we're getting there. A few minor clarifications (see green text below):

From: Anderson, Brian
Sent: Friday, February 16, 2018 11:56 AM
To: Panger, Melissa <Panger.Melissa@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>; Lennartz, Steven <Lennartz.Steven@epa.gov>; Connolly, Jennifer <Connolly.Jennifer@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>; Eckel, William <Eckel.William@epa.gov>
Subject: summary - part two.

Just had a conversation with Steve and Mel - It seems to jive with what I heard at the Tuesday meeting with Bead – parts of what I was just overhearing. I just want to summarize for the group and make sure it's consistent with our plan. Is it?

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Message

From: Anderson, Brian [Anderson.Brian@epa.gov]
Sent: 2/19/2018 2:35:29 PM
To: Panger, Melissa [Panger.Melissa@epa.gov]; Garber, Kristina [Garber.Kristina@epa.gov]; Eckel, William [Eckel.William@epa.gov]; Peck, Charles [Peck.Charles@epa.gov]; Rossmeisl, Colleen [Rossmeisl.Colleen@epa.gov]; Connolly, Jennifer [Connolly.Jennifer@epa.gov]; Lennartz, Steven [Lennartz.Steven@epa.gov]; Sims, Diann [Sims.Diann@epa.gov]
Subject: Workshop agenda - for Tomorrow's discussion
Attachments: Draft agenda for pesticide use meeting_2_16_2018b.docx

Flag: Flag for follow up

Hey Guys,

I spoke with FWS on Friday –

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Table 1. National Diazinon Agricultural Usage by Crop. Data Averaged Over Reported Years.

Crop
Almonds
Apples
Broccoli
Cabbage
Caneberries
Cantaloupes
Carrots
Cauliflower
Cherries
Cucumbers
Lettuce
Onions
Peaches
Pears
Peppers
Plums/Prunes
Pumpkins
Spinach
Squash
Strawberries
Tomatoes
Watermelons
Blueberries

Survey Status	Avg. Annual Pounds AI Applied*	Avg. Annual Total Acres Treated*	Avg. AI Rate	Max Labeled Rate
MRD (2010-2014)	3,780	2,016	1.87	3
MRD (2010-2014)	26,030	22,900	1.14	2
MRD (2010-2014)	4,070	3,660	1.11	4
MRD (2010-2014)	8,820	6,320	1.4	4
MRD (2010-2014)	7,150	6,650	1.08	2
MRD (2010-2014)	3,900	5,450	0.72	4
MRD (2010-2014)	9,970	4,700	2.12	4
MRD (2010-2014)	640	680	0.94	4
MRD (2010-2014)	13,490	8,500	1.59	2
MRD (2010-2014)	1,590	1,130	1.41	4
MRD (2010-2014)	11,120	16,000	0.69	2
MRD (2010-2014)	25,800	15,360	1.68	4
MRD (2010-2014)	5,370	3,890	1.38	2
MRD (2010-2014)	1,430	870	1.64	2
MRD (2010-2014)	1,080	370	2.92	4
MRD (2010-2014)	3,470	380	1.46	2
MRD (2010-2014)	610	380	1.6	4
MRD (2010-2014)	7,850	5,460	1.44	4
MRD (2010-2014)	1,270	1,150	1.1	4
MRD (2010-2014)	2,980	4,740	0.63	1
MRD (2010-2014)	23,420	11,750	1.99	4
MRD (2010-2014)	4,660	4,530	1.03	4
NASS (2011)	7,100	--	0.62	1

Brussels Sprouts
Figs
Nectarines
Cranberries
Apricots
Beans (Snap, Bush, Pole, String)
Dry Beans/Peas
Lima Beans
Potatoes
Garlic
Honeydew Melon
Hazelnut
Beets (Red And Table)
Casaba Mellon
Chinese Broccoli
Chinese Cabbage
Chinese Mustard
Chinese Radish Daikon
Collards
Crenshaw Melon
Endive
Ginseng
Kale
Leeks
Muskmelons
Mustard Greens
Parsley
Parsnips
Persian Melons
Pineapple
Radishes
Rutabagas
Shallots
Sweet Potatoes
Swiss Chard
Turnips

CalDPR (2010-2014)	24	32	1.32	4
CalDPR (2010-2014)	155	208	1.45	0.5
CalDPR (2010-2014)	266	140	1.98	2
Cranberry Institute (2015)	41,000	--	--	3
MRD (2010-2014)	Insufficient number of reports to establish an estimate.			2
MRD (2010-2014)	Insufficient number of reports to establish an estimate.			4
MRD (2010-2014)	Insufficient number of reports to establish an estimate.			4
MRD (2010-2014)	Insufficient number of reports to establish an estimate.			4
MRD (2010-2014)	Insufficient number of reports to establish an estimate.			4
CalDPR (2010-2014)	Insufficient number of reports to establish an estimate.			4
NASS (2010,2014)	Surveyed but no usage reported			4
MRD (2010-2014)	Surveyed but no usage reported			0.5
Not Surveyed at National Level				4
Not Surveyed at National Level				4
Not Surveyed at National Level				4
Not Surveyed at National Level				4
Not Surveyed at National Level				4
Not Surveyed at National Level				4
Not Surveyed at National Level				4
Not Surveyed at National Level				4
Not Surveyed at National Level				4
Not Surveyed at National Level				0.5
Not Surveyed at National Level				4
Not Surveyed at National Level				4
Not Surveyed at National Level				4
Not Surveyed at National Level				4
Not Surveyed at National Level				4
Not Surveyed at National Level				0.996
Not Surveyed at National Level				4
Not Surveyed at National Level				1
Not Surveyed at National Level				4
Not Surveyed at National Level				4
Not Surveyed at National Level				4
Not Surveyed at National Level				4
Not Surveyed at National Level				4
Not Surveyed at National Level				4

Table 2. Diazinon Agricultural Usage by Crop and State.

Crop	State
Almonds	California
Apples	Virginia
Apples	California
Apples	Pennsylvania
Apples	Washington
Apples	Oregon
Apples	New York
Apples	Ohio
Apples	Michigan
Apples	NC, WV
Apricots	California
Beans (Snap, Bush, Pole, String	California
Beans (Snap, Bush, Pole, String	New York
Beans (Snap, Bush, Pole, String	FL. GA, IL, ID, MI, NC, OR, PA, TN, TX, WI
Broccoli	California
Cabbage	Texas
Cabbage	New York
Cabbage	Florida
Cabbage	California
Cabbage	Michigan
Cabbage	AZ, CO, GA, NC, WI
Caneberries	Oregon
Caneberries	Washington
Caneberries	California
Cantaloupes	Texas
Cantaloupes	California
Cantaloupes	Florida
Cantaloupes	AZ, GA, ID, NC
Carrots	Washington
Carrots	California
Carrots	Texas
Carrots	MI, WI
Cauliflower	Arizona
Cauliflower	California
Cherries	California
Cherries	Oregon
Cherries	Washington
Cherries	MI

Avg. Annual Crop Acres Grown (Kynetec)	Avg. Annual Total Lbs. AI Applied	Min. Annual PCT	Max. Annual PCT	Avg. Annual PCT
947,400	3776	0	0.5	**0.2**
13,200	1,670	0	53	**21**
18,700	4,117	7	31	**16**
23,600	4,612	5	24	**15**
164,000	14,627	1	10	**5**
5,400	(S)	0	38	**8**
49,200	724	0.2	3	**2**
5,000	(S)	0	2	**0.3**
44,000	(S)	0	0.2	**0.05**
SNUR				
10,800	(S)	0	11	**3**
9,500	(S)	0	3	**0.6**
26,800	(S)	0	0.9	**0.2**
SNUR				
121,500	4067	0.4	8	**3**
7,200	6,253	23	74	**52**
10,400	1,954	0	33	**20**
9,700	(S)	0	12	**2**
13,000	(S)	0	3	**2**
3,100	(S)	0	4	**0.9**
SNUR				
11,800	1,595	0	15	**8**
11,600	5,019	0	10	**3**
6,800	(S)	0	10	**3**
2,700	(S)	0	57	**18**
37,300	2,884	4	35	**13**
2,800	(S)	0	6	**1**
SNUR				
7,500	(S)	0	49	**10**
64,900	8,956	0	22	**6**
2,900	(S)	0	33	**7**
SNUR				
3,400	(S)	0	28	**8**
33,600	(S)	0	4	**1**
35,800	10,814	3	45	**18**
17,600	704	0.3	8	**3**
43,000	1,972	1	5	**3**
SNUR				

SNUR = surveyed but no usage reported	**NS = Not Surveyed**	**(S) = data witheld due to small sample size**

Cucumbers	California
Cucumbers	Florida
Cucumbers	GA, MD, MI, MO, NC, SC, TX, WA, WI
Dry Beans/Peas	California
Dry Beans/Peas	CO, ID, MI, MN, MO, NE, NY, ND, TX, WA, WY
Hazelnut	OR
Lettuce	California
Lettuce	AZ
Lima Beans	California
Lima Beans	DE, GA, IL, SC, WA, WI
Onions	Texas
Onions	California
Onions	New York
Onions	Washington
Onions	Colorado
Onions	Oregon
Onions	GA, ID
Peaches	California
Peaches	Pennsylvania
Peaches	Texas
Peaches	AL, GA, IL, MI, NJ, SC, WA
Pears	California
Pears	Oregon
Pears	WA
Peppers	New Jersey
Peppers	California
Peppers	Florida
Peppers	AZ, GA, NM, NC, OH, TX
Plums/Prunes	California
Potatoes	Idaho
Potatoes	CA, C0, FL, ME, MI,MN, MT, NE, NY, NC, ND, OR, PA, TX, VA, WA, WI
Pumpkins	New Jersey
Pumpkins	California
Pumpkins	Michigan

8,300	400	0	11	**7**
24,500	(S)	0	5	**2**
SNUR				
53,300	(S)	0	6	**1**
SNUR				
SNUR				
204,400	11,115	0.9	20	**6**
SNUR				
8,400	(S)	0	21	**4**
SNUR				
11,900	14,644	31	82	**58**
44,600	5,511	0	27	**11**
9,500	797	1	12	**7**
24,300	4,430	0	10	**5**
6,400	165	0	8	**3**
19,900	(S)	0	1	**0.4**
SNUR				
54,200	4,706	1	11	**5**
5,100	(S)	0	19	**8**
5,500	(S)	0	19	**4**
SNUR				
14,100	1,398	0	16	**6**
17,800	(S)	0	0.4	**0.1**
SNUR				
3,500	(S)	0	21	**6**
29,100	(S)	0	2	**0.4**
17,500	(S)	0	1	**0.2**
SNUR				
88,200	3,472	0.6	4	**2**
318,800	(S)	0	0.4	**0.1**
SNUR				
2,400	(S)	0	23	**8**
5,900	(S)	0	2	**0.4**
6,900	(S)	0	1	**0.2**

Pumpkins	CT, IL, ID, IN, MD, MA, NM, MO, NM, NY, OH, OR, PA, TN, TX, VA, WA, WI
Spinach	Texas
Spinach	New Jersey
Spinach	California
Spinach	Oklahoma
Spinach	AZ, CO
Squash	California
Squash	Florida
Squash	Pennsylvania
Squash	Georgia
Squash	Texas
Squash	New Jersey
Squash	New York
Squash	CT,MA, MI, NC, OH, OR, SC,TN, WI
Strawberries	California
Strawberries	Florida
Strawberries	Washington
Strawberries	Pennsylvania
Strawberries	MI, NY, OR
Tomatoes	Florida
Tomatoes	
Tomatoes	California
Watermelons	Texas
Watermelons	California
Watermelons	Florida
Watermelons	AL, AZ, GA, ID, MS, MO, NC, OK, SC
Blueberries	Washington
Blueberries	Georgia
Blueberries	Oregon
Blueberries	New Jersey
Blueberries	Michigan
Blueberries	North Carolina
Honeydew Melon	AZ, CA, TX
Beets (Red And Table)	California
Beets (Red And Table)	Other states
Chinese Cabbage	California
Chinese Cabbage	Other states
Chinese Radish Daikon	California
Chinese Radish Daikon	Other states
Collards	California

SNUR				
2,300	2,759	69	100	**88**
3,200	2,373	0	78	**47**
30,800	2,632	0	10	**3**
1,800	(S)	0	34	**12**
SNUR				
6,300	385	3	16	**8**
9,800	(S)	0	12	**5**
800	(S)	0	9	**2**
3,500	(S)	0	6	**1**
1,900	(S)	0	5	**1**
3,100	(S)	0	3	**0.5**
4,900	(S)	0	1	**0.3**
SNUR				
39,100	2,024	3	14	**9**
9,300	(S)	0	35	**9**
1,800	(S)	0	24	**7**
1,000	(S)	0	4	**1**
SNUR				
31,800	713	0	27	**11**
302,000	22,704	2	3	**3**
26,800	3,255	0	33	**13**
10,600	1,378	5	19	**11**
25,200	(S)	0	2	**0.3**
SNUR				
--	2,600	34	47	**41**
--	3,350	23	25	**24**
--	950	16	17	**17**
--	800	8	19	**14**
--	(S)	(S)	(S)	(S)
--	(S)	(S)	(S)	(S)
SNUR				
--	160	0.4	1.6	**0.8**
NS				
--	86	0	1	**0.4**
NS				
--	38	0	1.1	**1**
NS				
--	5	0	0.3	**0.1**

Collards	Other states
Endive	California
Endive	Other states
Kale	California
Kale	Other states
Mustard Greens	California
Mustard Greens	Other states
Radishes	California
Radishes	Other states
Swiss Chard	California
Swiss Chard	Other states
Brussels Sprouts	California (93%)
Figs	California (96%)
Garlic	California (85%)
Nectarines	California (89%)
Casaba Mellon	NS
Chinese Broccoli	NS
Chinese Mustard	NS
Crenshaw Melon	NS
Ginseng	NS
Leeks	NS
Muskmelons	NS
Parsley	NS
Parsnips	NS
Persian Melons	NS
Pineapple	NS
Rutabagas	NS
Shallots	NS
Sweet Potatoes	NS

NS				
--	18	0	0.1	**0.02**
NS				
--	41	0.03	0.2	**0.1**
NS				
--	(S)	0	0.1	**0.012**
NS				
--	56	0.1	1.6	**0.5**
NS				
--	(S)	0	0.002	**0.0003**
NS				
--	24	0	1.5	**0.4**
--	208	0	7	**2.7**
--	(S)	0	0.3	**0.1**
--	140	0.3	0.8	**0.6**
NS	NS	NS	NS	NS
NS	NS	NS	NS	NS
NS	NS	NS	NS	NS
NS	NS	NS	NS	NS
NS	NS	NS	NS	NS
NS	NS	NS	NS	NS
NS	NS	NS	NS	NS
NS	NS	NS	NS	NS
NS	NS	NS	NS	NS
NS	NS	NS	NS	NS
NS	NS	NS	NS	NS
NS	NS	NS	NS	NS
NS	NS	NS	NS	NS
NS	NS	NS	NS	NS

Crops growns in florida

Reg Status	Census of Ag	
	Crop	State
reg	Cabbage	Florida
reg	Cantaloupes	Florida
reg	Cucumbers	Florida
reg	Peppers	Florida
reg	Squash	Florida
reg	Strawberries	Florida
reg	Tomatoes	Florida
reg	Watermelons	Florida
reg	Beans (Snap, Bush, Pole, String	FL
reg	Potatoes	FL
reg	BROCCOLI	FL
reg	GREENS, MUSTARD	FL
reg	PARSLEY	FL
reg	SWEET POTATOES	FL
reg	VEGETABLES, OTHER	FL
not reg	BEANS, DRY EDIBLE, (EXCL LIMA)	FL
not reg	BEANS, DRY EDIBLE, LIMA	FL
not reg	BEETS	FL
not reg	BERRIES, OTHER	FL
not reg	BLACKBERRIES, INCL DEWBERRIES & MARIONBERRIES	FL
not reg	BLUEBERRIES, TAME	FL
not reg	BLUEBERRIES, WILD	FL
not reg	CABBAGE, CHINESE	FL
not reg	CARROTS	FL
not reg	CORN, GRAIN	FL
not reg	CORN, SILAGE	FL
not reg	COTTON	FL
not reg	DAIKON	FL
not reg	EGGPLANT	FL
not reg	FIELD CROPS, OTHER	FL
not reg	GARLIC	FL
not reg	GRASSES & LEGUMES TOTALS, SEED	FL
not reg	GREENS, COLLARD	FL
not reg	GREENS, KALE	FL
not reg	GREENS, TURNIP	FL
not reg	HAY	FL
not reg	HAYLAGE	FL
not reg	HERBS, FRESH CUT	FL
not reg	LETTUCE	FL
not reg	LOGANBERRIES	FL
not reg	MILLET, PROSO	FL
not reg	OATS	FL

	SUUM		
Crop Acres Harvested_ Ag Census 2012	Avg. Annual Crop Acres Grown[1]	Avg. Annual Total Lbs. AI Applied	Min. Annual PCT
7,338	9,700	(S)	0
3,010	2,800	(S)	0
20,057	24,500	(S)	0
12,377	17,500	(S)	0
5,901	9,800	(S)	0
11,350	9,300	(S)	0
39,807	31,800	713	0
20,693	25,200	(S)	0
33,338	SNUR		
35,251	SNUR		
983	NS		
109	NS		
232	NS		
5,988	NS		
1,066	NS		
28	NS		
361	NS		
14	NS		
44	NS		
190	NS		
6,179	NS		
203	NS		
2,387	NS		
2,208	NS		
39,330	NS		
27,715	NS		
105,420	NS		
1	NS		
694	NS		
335	NS		
3	NS		
8,924	NS		
274	NS		
205	NS		
114	NS		
354,127	NS		
48,078	NS		
680	NS		
9,827	NS		
47	NS		
1,475	NS		
4,631	NS		

Max. Annual PCT	Avg. Annual PCT
12	**2**
6	**1**
5	**2**
1	**0.2**
12	**5**
35	**9**
27	**11**
2	**0.3**

not reg	OKRA	FL
not reg	ONIONS, DRY	FL
not reg	ONIONS, GREEN	FL
not reg	PEANUTS	FL
not reg	PEAS, DRY EDIBLE	FL
not reg	PEAS, DRY, SOUTHERN (COWPEAS)	FL
not reg	PEAS, GREEN, (EXCL SOUTHERN)	FL
not reg	PEAS, GREEN, SOUTHERN (COWPEAS)	FL
not reg	PEPPERS, CHILE	FL
not reg	PUMPKINS	FL
not reg	RADISHES	FL
not reg	RYE	FL
not reg	SORGHUM, GRAIN	FL
not reg	SORGHUM, SILAGE	FL
not reg	SOYBEANS	FL
not reg	SPINACH	FL
not reg	SUGARCANE, SEED	FL
not reg	SUGARCANE, SUGAR	FL
not reg	SUNFLOWER	FL
not reg	SWEET CORN	FL
not reg	TOBACCO	FL
not reg	TURNIPS	FL
not reg	WHEAT	FL

316	NS		
141	NS		
66	NS		
196,320	NS		
74	NS		
3,765	NS		
629	NS		
1,568	NS		
1,188	NS		
43	NS		
6,061	NS		
2,942	NS		
3,541	NS		
8,385	NS		
19,409	NS		
104	NS		
15,840	NS		
401,491	NS		
16	NS		
35,225	NS		
482	NS		
12	NS		
15,456	NS		

Crops grown in Colorado

Reg Status	Census of Ag		
	Crop	State	Avg. Annual Crop Acres Grown[†]
reg	Onions	Colorado	6,400
reg	Cabbage	CO	SNUR
reg	Dry Beans/Peas	CO	SNUR
reg	Spinach	CO	SNUR
not reg	ASPARAGUS	CO	NS
not reg	BARLEY	CO	NS
not reg	BEANS, SNAP	CO	NS
not reg	BEETS	CO	NS
not reg	BERRIES, OTHER	CO	NS
not reg	BLACKBERRIES, INCL DEWBERRIES & MARIONBERRIES	CO	NS
not reg	BLUEBERRIES, TAME	CO	NS
reg	BROCCOLI	CO	NS
reg	VEGETABLES, OTHER	CO	NS
not reg	BRUSSELS SPROUTS	CO	NS
not reg	CANOLA	CO	NS
not reg	CARROTS	CO	NS
not reg	CAULIFLOWER	CO	NS
not reg	CORN, GRAIN	CO	NS
not reg	CORN, SILAGE	CO	NS
not reg	CUCUMBERS	CO	NS
not reg	EGGPLANT	CO	NS
not reg	EMMER & SPELT	CO	NS
not reg	GRASSES & LEGUMES TOTALS, SEED	CO	NS
not reg	GRASSES, WHEATGRASS, SEED	CO	NS
not reg	GREENS, KALE	CO	NS
not reg	HAY	CO	NS
not reg	HAYLAGE	CO	NS
not reg	HERBS, DRY	CO	NS
not reg	HERBS, FRESH CUT	CO	NS
not reg	HOPS	CO	NS
not reg	LEGUMES, ALFALFA, SEED	CO	NS
not reg	LETTUCE, ROMAINE	CO	NS
not reg	MELONS, CANTALOUP	CO	NS
not reg	MELONS, HONEYDEW	CO	NS
not reg	MELONS, WATERMELON	CO	NS
not reg	MILLET, PROSO	CO	NS
not reg	OATS	CO	NS
not reg	OKRA	CO	NS
not reg	PEAS, CHINESE (SUGAR & SNOW)	CO	NS
not reg	PEAS, DRY EDIBLE	CO	NS
not reg	PEAS, GREEN, (EXCL SOUTHERN)	CO	NS
not reg	PEPPERS, BELL	CO	NS

SUUM				
Crop Acres Harvested_ Ag Census 2012	Avg. Annual Total Lbs. AI Applied	Min. Annual PCT	Max. Annual PCT	Avg. Annual PCT
6,432	165	0	8	**3**
1544				
42,573				
1,088				
24				
54,828				
801				
70				
5				
2				
2				
14				
541				
1				
1,618				
1,630				
9				
1,011,151				
157,285				
72				
15				
36				
2,089				
205				
28				
1,248,960				
59,587				
95				
87				
110				
855				
9				
438				
23				
698				
119,910				
5,936				
3				
1				
1,260				
100				
69				

not reg	PEPPERS, CHILE	CO	NS
not reg	POTATOES	CO	NS
not reg	PUMPKINS	CO	NS
not reg	RADISHES	CO	NS
not reg	RASPBERRIES	CO	NS
not reg	RHUBARB	CO	NS
not reg	RYE	CO	NS
not reg	SAFFLOWER	CO	NS
not reg	SORGHUM, GRAIN	CO	NS
not reg	SORGHUM, SILAGE	CO	NS
not reg	SOYBEANS	CO	NS
not reg	SQUASH	CO	NS
not reg	STRAWBERRIES	CO	NS
not reg	SUGARBEETS	CO	NS
not reg	SUNFLOWER	CO	NS
not reg	SWEET CORN	CO	NS
not reg	TOMATOES, IN THE OPEN	CO	NS
not reg	TRITICALE	CO	NS
not reg	WHEAT	CO	NS

333				
59,281				
1,824				
76				
24				
3				
1,094				
1,745				
147,955				
11,209				
12,602				
1,092				
23				
30,553				
69,307				
4,885				
341				
3,173				
2,181,967				

OPTIONS FOR CROPS (choose one from each type, suggestions in green):

Table 1. National Diazinon Agricultural Usage by Crop. Data Averaged Over Reported Years.

Crop	Survey Status	Avg. Annual Pounds AI Applied*	Avg. Annual Total Acres Treated*	Avg. AI Rate	Max Labeled Rate
Almonds	MRD (2010-2014)	3,780	2,016	1.87	3
Apples	MRD (2010-2014)	26,030	22,900	1.14	2
Broccoli	MRD (2010-2014)	4,070	3,660	1.11	4
Cabbage	MRD (2010-2014)	8,820	6,320	1.4	4
Caneberries	MRD (2010-2014)	7,150	6,650	1.08	2
Cantaloupes	MRD (2010-2014)	3,900	5,450	0.72	4
Carrots	MRD (2010-2014)	9,970	4,700	2.12	4
Cauliflower	MRD (2010-2014)	640	680	0.94	4
Cherries	MRD (2010-2014)	13,490	8,500	1.59	2
Cucumbers	MRD (2010-2014)	1,590	1,130	1.41	4
Lettuce	MRD (2010-2014)	11,120	16,000	0.69	2
Onions	MRD (2010-2014)	25,800	15,360	1.68	4
Peaches	MRD (2010-2014)	5,370	3,890	1.38	2
Pears	MRD (2010-2014)	1,430	870	1.64	2
Peppers	MRD (2010-2014)	1,080	370	2.92	4
Plums/Prunes	MRD (2010-2014)	3,470	380	1.46	2
Pumpkins	MRD (2010-2014)	610	380	1.6	4
Spinach	MRD (2010-2014)	7,850	5,460	1.44	4
Squash	MRD (2010-2014)	1,270	1,150	1.1	4
Strawberries	MRD (2010-2014)	2,980	4,740	0.63	1
Tomatoes	MRD (2010-2014)	23,420	11,750	1.99	4
Watermelons	MRD (2010-2014)	4,660	4,530	1.03	4

Blueberries	NASS (2011)	7,100	--	0.62	1
Brussels Sprouts	CalDPR (2010-2014)	24	32	1.32	4
Figs	CalDPR (2010-2014)	155	208	1.45	0.5
Nectarines	CalDPR (2010-2014)	266	140	1.98	2
Apricots	MRD (2010-2014)	Insufficient number of reports to establish an estimate.			2
Beans (Snap, Bush, Pole, String)	MRD (2010-2014)	Insufficient number of reports to establish an estimate.			4
Dry Beans/Peas	MRD (2010-2014)	Insufficient number of reports to establish an estimate.			4
Lima Beans	MRD (2010-2014)	Insufficient number of reports to establish an estimate.			4
Potatoes	MRD (2010-2014)	Insufficient number of reports to establish an estimate.			4
Garlic	CalDPR (2010-2014)	Insufficient number of reports to establish an estimate.			4
Honeydew Melon	NASS (2010,2014)	Surveyed but no usage reported			4
Hazelnut	MRD (2010-2014)	Surveyed but no usage reported			0.5
Beets (Red and Table)	Not Surveyed at National Level				4
Casaba Mellon	Not Surveyed at National Level				4
Chinese Broccoli	Not Surveyed at National Level				4
Chinese Cabbage	Not Surveyed at National Level				4
Chinese Mustard	Not Surveyed at National Level				4
Chinese Radish Daikon	Not Surveyed at National Level				4
Collards	Not Surveyed at National Level				4
Crenshaw Melon	Not Surveyed at National Level				4
Endive	Not Surveyed at National Level				4
Ginseng	Not Surveyed at National Level				0.5
Kale	Not Surveyed at National Level				4
Leeks	Not Surveyed at National Level				4
Muskmelons	Not Surveyed at National Level				4
Mustard Greens	Not Surveyed at National Level				4
Parsley	Not Surveyed at National Level				4
Parsnips	Not Surveyed at National Level				0.996
Persian Melons	Not Surveyed at National Level				4
Pineapple	Not Surveyed at National Level				1
Radishes	Not Surveyed at National Level				4
Rutabagas	Not Surveyed at National Level				4
Shallots	Not Surveyed at National Level				4
Sweet Potatoes	Not Surveyed at National Level				4
Swiss Chard	Not Surveyed at National Level				4
Turnips	Not Surveyed at National Level				4

OPTIONS FOR MRD CROP/STATE COMBOS (suggestions in green):

Table 2. Diazinon Agricultural Usage by Crop and State.

Crop	Survey Status	State	Avg. Annual Crop Acres Grown†	Avg. Annual Total Lbs. AI Applied	Min. Annual PCT	Max. Annual PCT	Avg. Annual PCT
Apples	MRD (2010-2014)	Virginia	13,200	1,670	0	53	**21**
		California	18,700	4,117	7	31	**16**
		Pennsylvania	23,600	4,612	5	24	**15**
		Washington	164,000	14,627	1	10	**5**
		Oregon	5,400	(S)	0	38	**8**
		New York	49,200	724	0.2	3	**2**
		Ohio	5,000	(S)	0	2	**0.3**
		Michigan	44,000	(S)	0	0.2	**0.05**
		NC, WV	Surveyed but no usage reported				
Cabbage	MRD (2010-2014)	Texas	7,200	6,253	23	74	**52**
		New York	10,400	1,954	0	33	**20**
		Florida	9,700	(S)	0	12	**2**
		California	13,000	(S)	0	3	**2**
		Michigan	3,100	(S)	0	4	**0.9**
		AZ, CO, GA, NC, WI	Surveyed but no usage reported				
Cantaloupes	MRD (2010-2014)	Texas	2,700	(S)	0	57	**18**
		California	37,300	2,884	4	35	**13**
		Florida	2,800	(S)	0	6	**1**
		AZ, GA, ID, NC	Surveyed but no usage reported				
Carrots	MRD (2010-2014)	Washington	7,500	(S)	0	49	**10**
		California	64,900	8,956	0	22	**6**
		Texas	2,900	(S)	0	33	**7**
		MI, WI	Surveyed but no usage reported				
Cucumbers	MRD (2010-2014)	California	8,300	400	0	11	**7**
		Florida	24,500	(S)	0	5	**2**
		GA, MD, MI, MO, NC, SC, TX, WA, WI	Surveyed but no usage reported				
Onions	MRD (2010-2014)	Texas	11,900	14,644	31	82	**58**
		California	44,600	5,511	0	27	**11**
		New York	9,500	797	1	12	**7**
		Washington	24,300	4,430	0	10	**5**
		Colorado	6,400	165	0	8	**3**
		Oregon	19,900	(S)	0	1	**0.4**
		GA, ID	Surveyed but no usage reported				
Peaches	MRD (2010-2014)	California	54,200	4,706	1	11	**5**
		Pennsylvania	5,100	(S)	0	19	**8**
		Texas	5,500	(S)	0	19	**4**
		AL, GA, IL, MI, NJ, SC, WA	Surveyed but no usage reported				
Pears	MRD (2010-2014)	California	14,100	1,398	0	16	**6**
		Oregon	17,800	(S)	0	0.4	**0.1**
		WA	Surveyed but no usage reported				
Spinach	MRD (2010-2014)	Texas	2,300	2,759	69	100	**88**
		New Jersey	3,200	2,373	0	78	**47**
		California	30,800	2,632	0	10	**3**

Crop	Survey Status	State	Avg. Annual Crop Acres Grown†	Avg. Annual Total Lbs. AI Applied	Min. Annual PCT	Max. Annual PCT	Avg. Annual PCT
		Oklahoma	1,800	(S)	0	34	**12**
		AZ, CO	Surveyed but no usage reported				
Squash	MRD (2010-2014)	California	6,300	385	3	16	**8**
		Florida	9,800	(S)	0	12	**5**
		Pennsylvania	800	(S)	0	9	**2**
		Georgia	3,500	(S)	0	6	**1**
		Texas	1,900	(S)	0	5	**1**
		New Jersey	3,100	(S)	0	3	**0.5**
		New York	4,900	(S)	0	1	**0.3**
		CT, MA, MI, NC, OH, OR, SC,TN, WI	Surveyed but no usage reported				
Strawberries	MRD (2010-2014)	California	39,100	2,024	3	14	**9**
		Florida	9,300	(S)	0	35	**9**
		Washington	1,800	(S)	0	24	**7**
		Pennsylvania	1,000	(S)	0	4	**1**
		MI, NY, OR	Surveyed but no usage reported				
Watermelons	MRD (2010-2014)	Texas	26,800	3,255	0	33	**13**
		California	10,600	1,378	5	19	**11**
		Florida	25,200	(S)	0	2	**0.3**
		AL, AZ, GA, ID, MS, MO, NC, OK, SC	Surveyed but no usage reported				

Table 2. Diazinon Agricultural Usage by Crop and State.

Crop	Survey Status	State	Avg. Annual Crop Acres Grown†	Avg. Annual Total Lbs. AI Applied	Min. Annual PCT	Max. Annual PCT	Avg. Annual PCT
Blueberries NASS	(2011)	Washington	--	2,600	34	47	**41**
		Georgia	--	3,350	23	25	**24**
		Oregon	--	950	16	17	**17**
		New Jersey	--	800	8	19	**14**
		Michigan	--	Insufficient number of reports to establish an estimate.			
		North Carolina	--	Insufficient number of reports to establish an estimate.			
Beets (Red and Table)	CalDPR (2010-2014)	California (12%)	--	160	0.4	1.6	**0.8**
		Other states (88%)	Crop grown in other states, but not surveyed at national level				
Figs	CalDPR (2010-2014)	California (96%)	--	208	0	7	**2.7**

Crop	State	Avg. Annual Crop Acres Grown (Kynetec)	Avg. Annual Total Lbs. AI Applied	Min. Annual PCT
Almonds	California	947,400	3776	0
Apples	Virginia	13,200	1,670	0
Apples	California	18,700	4,117	7
Apples	Pennsylvania	23,600	4,612	5
Apples	Washington	164,000	14,627	1
Apples	Oregon	5,400	(S)	0
Apples	New York	49,200	724	0.2
Apples	Ohio	5,000	(S)	0
Apples	Michigan	44,000	(S)	0
Apples	NC, WV	SNUR		
Apricots	California	10,800	(S)	0
Beans (Snap, Bush, Pole, String	California	9,500	(S)	0
Beans (Snap, Bush, Pole, String	New York	26,800	(S)	0
Beans (Snap, Bush, Pole, String	FL. GA, IL, ID, MI, NC, OR, PA, TN, TX, WI	SNUR		
Broccoli	California	121,500	4067	0.4
Cabbage	Texas	7,200	6,253	23
Cabbage	New York	10,400	1,954	0
Cabbage	Florida	9,700	(S)	0
Cabbage	California	13,000	(S)	0
Cabbage	Michigan	3,100	(S)	0
Cabbage	AZ, CO, GA, NC, WI	SNUR		
Caneberries	Oregon	11,800	1,595	0
Caneberries	Washington	11,600	5,019	0
Caneberries	California	6,800	(S)	0
Cantaloupes	Texas	2,700	(S)	0
Cantaloupes	California	37,300	2,884	4
Cantaloupes	Florida	2,800	(S)	0
Cantaloupes	AZ, GA, ID, NC	SNUR		
Carrots	Washington	7,500	(S)	0
Carrots	California	64,900	8,956	0
Carrots	Texas	2,900	(S)	0
Carrots	MI, WI	SNUR		
Cauliflower	Arizona	3,400	(S)	0
Cauliflower				
Cauliflower	California	33,600	(S)	0
Cherries	California	35,800	10,814	3
Cherries	Oregon	17,600	704	0.3
Cherries	Washington	43,000	1,972	1

Max. Annual PCT	Avg. Annual PCT
0.5	**0.2**
53	**21**
31	**16**
24	**15**
10	**5**
38	**8**
3	**2**
2	**0.3**
0.2	**0.05**
11	**3**
3	**0.6**
0.9	**0.2**
8	**3**
74	**52**
33	**20**
12	**2**
3	**2**
4	**0.9**
15	**8**
10	**3**
10	**3**
57	**18**
35	**13**
6	**1**
49	**10**
22	**6**
33	**7**
28	**8**
4	**1**
45	**18**
8	**3**
5	**3**

SNUR = surveyed but no usage reported

NS = Not Surveyed

(S) = data witheld due to small sample size

Cherries	MI	SNUR		
Cucumbers	California	8,300	400	0
Cucumbers	Florida	24,500	(S)	0
Cucumbers	GA, MD, MI, MO, NC, SC, TX, WA, WI	SNUR		
Dry Beans/Peas	California	53,300	(S)	0
Dry Beans/Peas	CO, ID, MI, MN, MO, NE, NY, ND, TX, WA, WY	SNUR		
Hazelnut	OR	SNUR		
Lettuce	California	204,400	11,115	0.9
Lettuce	AZ	SNUR		
Lima Beans	California	8,400	(S)	0
Lima Beans	DE, GA, IL, SC, WA, WI	SNUR		
Onions	Texas	11,900	14,644	31
Onions	California	44,600	5,511	0
Onions	New York	9,500	797	1
Onions	Washington	24,300	4,430	0
Onions	Colorado	6,400	165	0
Onions	Oregon	19,900	(S)	0
Onions	GA, ID	SNUR		
Peaches	California	54,200	4,706	1
Peaches	Pennsylvania	5,100	(S)	0
Peaches	Texas	5,500	(S)	0
Peaches	AL, GA, IL, MI, NJ, SC, WA	SNUR		
Pears	California	14,100	1,398	0
Pears	Oregon	17,800	(S)	0
Pears	WA	SNUR		
Peppers	New Jersey	3,500	(S)	0
Peppers	California	29,100	(S)	0
Peppers	Florida	17,500	(S)	0
Peppers	AZ, GA, NM, NC, OH, TX	SNUR		
Plums/Prunes	California	88,200	3,472	0.6
Potatoes	Idaho	318,800	(S)	0
Potatoes	CA, C0, FL, ME, MI,MN, MT, NE, NY, NC, ND, OR, PA, TX, VA, WA, WI	SNUR		
Pumpkins	New Jersey	2,400	(S)	0
Pumpkins	California	5,900	(S)	0
Pumpkins	Michigan	6,900	(S)	0

11	**7**
5	**2**
6	**1**
20	**6**
21	**4**
82	**58**
27	**11**
12	**7**
10	**5**
8	**3**
1	**0.4**
11	**5**
19	**8**
19	**4**
16	**6**
0.4	**0.1**
21	**6**
2	**0.4**
1	**0.2**
4	**2**
0.4	**0.1**
23	**8**
2	**0.4**
1	**0.2**

Pumpkins	CT, IL, ID, IN, MD, MA, NM, MO, NM, NY, OH, OR, PA, TN, TX, VA, WA, WI	SNUR		
Spinach	Texas	2,300	2,759	69
Spinach	New Jersey	3,200	2,373	0
Spinach	California	30,800	2,632	0
Spinach	Oklahoma	1,800	(S)	0
Spinach	AZ, CO	SNUR		
Squash	California	6,300	385	3
Squash	Florida	9,800	(S)	0
Squash	Pennsylvania	800	(S)	0
Squash	Georgia	3,500	(S)	0
Squash	Texas	1,900	(S)	0
Squash	New Jersey	3,100	(S)	0
Squash	New York	4,900	(S)	0
Squash	CT,MA, MI, NC, OH, OR, SC,TN, WI	SNUR		
Strawberries	California	39,100	2,024	3
Strawberries	Florida	9,300	(S)	0
Strawberries	Washington	1,800	(S)	0
Strawberries	Pennsylvania	1,000	(S)	0
Strawberries	MI, NY, OR	SNUR		
Tomatoes	Florida	31,800	713	0
Tomatoes				
Tomatoes	California	302,000	22,704	2
Watermelons	Texas	26,800	3,255	0
Watermelons	California	10,600	1,378	5
Watermelons	Florida	25,200	(S)	0
Watermelons	AL, AZ, GA, ID, MS, MO, NC, OK, SC	SNUR		
Blueberries	Washington	--	2,600	34
Blueberries	Georgia	--	3,350	23
Blueberries	Oregon	--	950	16
Blueberries	New Jersey	--	800	8
Blueberries	Michigan	--	(S)	(S)
Blueberries	North Carolina	--	(S)	(S)
Honeydew Melon	AZ, CA, TX	SNUR		
Beets (Red And Table)	California	--	160	0.4
Beets (Red And Table)	Other states	NS		
Chinese Cabbage	California	--	86	0
Chinese Cabbage	Other states	NS		
Chinese Radish Daikon	California	--	38	0
Chinese Radish Daikon	Other states	NS		

100	**88**
78	**47**
10	**3**
34	**12**
16	**8**
12	**5**
9	**2**
6	**1**
5	**1**
3	**0.5**
1	**0.3**
14	**9**
35	**9**
24	**7**
4	**1**
27	**11**
3	**3**
33	**13**
19	**11**
2	**0.3**
47	**41**
25	**24**
17	**17**
19	**14**
(S)	(S)
(S)	(S)
1.6	**0.8**
1	**0.4**
1.1	**1**

Collards	California	--	5	0
Collards	Other states	NS		
Endive	California	--	18	0
Endive	Other states	NS		
Kale	California	--	41	0.03
Kale	Other states	NS		
Mustard Greens	California	--	(S)	0
Mustard Greens	Other states	NS		
Radishes	California	--	56	0.1
Radishes	Other states	NS		
Swiss Chard	California	--	(S)	0
Swiss Chard	Other states	NS		
Brussels Sprouts	California (93%)	--	24	0
Figs	California (96%)	--	208	0
Garlic	California (85%)	--	(S)	0
Nectarines	California (89%)	--	140	0.3
Casaba Mellon	NS	NS	NS	NS
Chinese Broccoli	NS	NS	NS	NS
Chinese Mustard	NS	NS	NS	NS
Crenshaw Melon	NS	NS	NS	NS
Ginseng	NS	NS	NS	NS
Leeks	NS	NS	NS	NS
Muskmelons	NS	NS	NS	NS
Parsley	NS	NS	NS	NS
Parsnips	NS	NS	NS	NS
Persian Melons	NS	NS	NS	NS
Pineapple	NS	NS	NS	NS
Rutabagas	NS	NS	NS	NS
Shallots	NS	NS	NS	NS
Sweet Potatoes	NS	NS	NS	NS

0.3	**0.1**
0.1	**0.02**
0.2	**0.1**
0.1	**0.012**
1.6	**0.5**
0.002	**0.0003**
1.5	**0.4**
7	**2.7**
0.3	**0.1**
0.8	**0.6**
NS	NS
NS	NS
NS	NS
NS	NS
NS	NS
NS	NS
NS	NS
NS	NS
NS	NS
NS	NS
NS	NS
NS	NS
NS	NS
NS	NS

Crop	State	Avg. Annual Crop Acres Grown†
Cabbage	Florida	9,700
Cantaloupes	Florida	2,800
Cucumbers	Florida	24,500
Peppers	Florida	17,500
Squash	Florida	9,800
Strawberries	Florida	9,300
Tomatoes	Florida	31,800
Watermelons	Florida	25,200
Beans (Snap, Bush, Pole, String	FL	SNUR
Potatoes	FL	SNUR
BEANS, DRY EDIBLE, (EXCL LIMA)	FL	NS
BEANS, DRY EDIBLE, LIMA	FL	NS
BEETS	FL	NS
BERRIES, OTHER	FL	NS
BLACKBERRIES, INCL DEWBERRIES & MARIONBERRIES	FL	NS
BLUEBERRIES, TAME	FL	NS
BLUEBERRIES, WILD	FL	NS
BROCCOLI	FL	NS
CABBAGE, CHINESE	FL	NS
CARROTS	FL	NS
CORN, GRAIN	FL	NS
CORN, SILAGE	FL	NS
COTTON	FL	NS
DAIKON	FL	NS
EGGPLANT	FL	NS
FIELD CROPS, OTHER	FL	NS
GARLIC	FL	NS
GRASSES & LEGUMES TOTALS, SEED	FL	NS
GREENS, COLLARD	FL	NS
GREENS, KALE	FL	NS
GREENS, MUSTARD	FL	NS
GREENS, TURNIP	FL	NS
HAY	FL	NS
HAYLAGE	FL	NS
HERBS, FRESH CUT	FL	NS
LETTUCE	FL	NS
LOGANBERRIES	FL	NS
MILLET, PROSO	FL	NS
OATS	FL	NS
OKRA	FL	NS
ONIONS, DRY	FL	NS
ONIONS, GREEN	FL	NS
PARSLEY	FL	NS
PEANUTS	FL	NS

Crop Acres Harvested_ Ag Census 2012	Avg. Annual Total Lbs. AI Applied	Min. Annual PCT	Max. Annual PCT	Avg. Annual PCT
7,338	(S)	0	12	**2**
3,010	(S)	0	6	**1**
20,057	(S)	0	5	**2**
12,377	(S)	0	1	**0.2**
5,901	(S)	0	12	**5**
11,350	(S)	0	35	**9**
39,807	713	0	27	**11**
20,693	(S)	0	2	**0.3**
33,338				
35,251				
28				
361				
14				
44				
190				
6,179				
203				
983				
2,387				
2,208				
39,330				
27,715				
105,420				
1				
694				
335				
3				
8,924				
274				
205				
109				
114				
354,127				
48,078				
680				
9,827				
47				
1,475				
4,631				
316				
141				
66				
232				
196,320				

PEAS, DRY EDIBLE	FL	NS
PEAS, DRY, SOUTHERN (COWPEAS)	FL	NS
PEAS, GREEN, (EXCL SOUTHERN)	FL	NS
PEAS, GREEN, SOUTHERN (COWPEAS)	FL	NS
PEPPERS, CHILE	FL	NS
PUMPKINS	FL	NS
RADISHES	FL	NS
RYE	FL	NS
SORGHUM, GRAIN	FL	NS
SORGHUM, SILAGE	FL	NS
SOYBEANS	FL	NS
SPINACH	FL	NS
SUGARCANE, SEED	FL	NS
SUGARCANE, SUGAR	FL	NS
SUNFLOWER	FL	NS
SWEET CORN	FL	NS
SWEET POTATOES	FL	NS
TOBACCO	FL	NS
TURNIPS	FL	NS
VEGETABLE TOTALS, IN THE OPEN	FL	NS
VEGETABLES, OTHER	FL	NS
WHEAT	FL	NS

74				
3,765				
629				
1,568				
1,188				
43				
6,061				
2,942				
3,541				
8,385				
19,409				
104				
15,840				
401,491				
16				
35,225				
5,988				
482				
12				
251,011				
1,066				
15,456				

Crop	State	Avg. Annual Crop Acres Grown[†]
Onions	Colorado	6,400
Cabbage	CO	SNUR
Dry Beans/Peas	CO	SNUR
Spinach	CO	SNUR
ASPARAGUS	CO	NS
BARLEY	CO	NS
BEANS, SNAP	CO	NS
BEETS	CO	NS
BERRIES, OTHER	CO	NS
BLACKBERRIES, INCL DEWBERRIES & MARIONBERRIES	CO	NS
BLUEBERRIES, TAME	CO	NS
BROCCOLI	CO	NS
BRUSSELS SPROUTS	CO	NS
CANOLA	CO	NS
CARROTS	CO	NS
CAULIFLOWER	CO	NS
CORN, GRAIN	CO	NS
CORN, SILAGE	CO	NS
CUCUMBERS	CO	NS
EGGPLANT	CO	NS
EMMER & SPELT	CO	NS
GRASSES & LEGUMES TOTALS, SEED	CO	NS
GRASSES, WHEATGRASS, SEED	CO	NS
GREENS, KALE	CO	NS
HAY	CO	NS
HAYLAGE	CO	NS
HERBS, DRY	CO	NS
HERBS, FRESH CUT	CO	NS
HOPS	CO	NS
LEGUMES, ALFALFA, SEED	CO	NS
LETTUCE, ROMAINE	CO	NS
MELONS, CANTALOUP	CO	NS
MELONS, HONEYDEW	CO	NS
MELONS, WATERMELON	CO	NS
MILLET, PROSO	CO	NS
OATS	CO	NS
OKRA	CO	NS
PEAS, CHINESE (SUGAR & SNOW)	CO	NS
PEAS, DRY EDIBLE	CO	NS
PEAS, GREEN, (EXCL SOUTHERN)	CO	NS
PEPPERS, BELL	CO	NS
PEPPERS, CHILE	CO	NS
POTATOES	CO	NS
PUMPKINS	CO	NS

Crop Acres Harvested_ Ag Census 2012	Avg. Annual Total Lbs. AI Applied	Min. Annual PCT	Max. Annual PCT	Avg. Annual PCT
6,432	165	0	8	**3**
1544				
42,573				
1,088				
24				
54,828				
801				
70				
5				
2				
2				
14				
1				
1,618				
1,630				
9				
1,011,151				
157,285				
72				
15				
36				
2,089				
205				
28				
1,248,960				
59,587				
95				
87				
110				
855				
9				
438				
23				
698				
119,910				
5,936				
3				
1				
1,260				
100				
69				
333				
59,281				
1,824				

RADISHES	CO	NS
RASPBERRIES	CO	NS
RHUBARB	CO	NS
RYE	CO	NS
SAFFLOWER	CO	NS
SORGHUM, GRAIN	CO	NS
SORGHUM, SILAGE	CO	NS
SOYBEANS	CO	NS
SQUASH	CO	NS
STRAWBERRIES	CO	NS
SUGARBEETS	CO	NS
SUNFLOWER	CO	NS
SWEET CORN	CO	NS
TOMATOES, IN THE OPEN	CO	NS
TRITICALE	CO	NS
VEGETABLE TOTALS, IN THE OPEN	CO	NS
VEGETABLES, OTHER	CO	NS
WHEAT	CO	NS

76				
24				
3				
1,094				
1,745				
147,955				
11,209				
12,602				
1,092				
23				
30,553				
69,307				
4,885				
341				
3,173				
83,266				
541				
2,181,967				

Message

From: Panger, Melissa [Panger.Melissa@epa.gov]
Sent: 2/20/2018 12:50:49 PM
To: Garber, Kristina [Garber.Kristina@epa.gov]
Subject: RE: summary - part two.

Sounds good. Thanks!

Melissa Panger, Ph.D.
Senior Advisor, ERB2
Environmental Fate and Effects Division
Office of Pesticide Programs
USEPA

703-305-6136
panger.melissa@epa.gov

From: Garber, Kristina
Sent: Tuesday, February 20, 2018 7:10 AM
To: Panger, Melissa <Panger.Melissa@epa.gov>
Subject: Re: summary - part two.

On the train. I can stop by your cube when I get in. Should be a bit before 8.

On Feb 20, 2018, at 7:08 AM, Panger, Melissa <Panger.Melissa@epa.gov> wrote:

> Are you in or on the train?
>
> Melissa Panger, Ph.D.
> Senior Advisor, ERB2
> Environmental Fate and Effects Division
> Office of Pesticide Programs
> USEPA
>
> 703-305-6136
> panger.melissa@epa.gov
>
> **From:** Garber, Kristina
> **Sent:** Tuesday, February 20, 2018 7:08 AM
> **To:** Peck, Charles <Peck.Charles@epa.gov>
> **Cc:** Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>; Anderson, Brian <Anderson.Brian@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Lennartz, Steven <Lennartz.Steven@epa.gov>; Connolly, Jennifer <Connolly.Jennifer@epa.gov>; Eckel, William <Eckel.William@epa.gov>
> **Subject:** Re: summary - part two.
>
> I saw two sets of bullets circulated for comment on Friday (I am digging through those emails now) and we have the bullets that are in the slide deck.
>
> On Feb 20, 2018, at 7:02 AM, Peck, Charles <Peck.Charles@epa.gov> wrote:

I think Brian/Mel's write-up captures the method we're using. I think the questions of how drift and non-ag acres will be handled will come up. Not sure I understand Kris' question about another write-up.

From: Garber, Kristina
Sent: Tuesday, February 20, 2018 6:54 AM
To: Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>
Cc: Anderson, Brian <Anderson.Brian@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Lennartz, Steven <Lennartz.Steven@epa.gov>; Connolly, Jennifer <Connolly.Jennifer@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Eckel, William <Eckel.William@epa.gov>
Subject: Re: summary - part two.

Deliberative Process / Ex. 5

Why do we need another write up?

On Feb 20, 2018, at 6:33 AM, Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov> wrote:

Deliberative Process / Ex. 5

Thanks!
Colleen

From: Anderson, Brian
Sent: Friday, February 16, 2018 1:30 PM
To: Panger, Melissa <Panger.Melissa@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>; Lennartz, Steven <Lennartz.Steven@epa.gov>; Connolly, Jennifer <Connolly.Jennifer@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>; Eckel, William <Eckel.William@epa.gov>
Subject: RE: summary - part two.

Just got make sure that was everyone's understanding and that BEAD can get us all the acres from their data sources on the crops within the CDL.

Thanks guys,

Brian

From: Panger, Melissa
Sent: Friday, February 16, 2018 12:45 PM
To: Anderson, Brian <Anderson.Brian@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>; Lennartz, Steven <Lennartz.Steven@epa.gov>; Connolly, Jennifer <Connolly.Jennifer@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>; Eckel, William <Eckel.William@epa.gov>
Subject: RE: summary - part two.

Clap… clap… clap! I think we're getting there. A few minor clarifications (see green text below):

From: Anderson, Brian
Sent: Friday, February 16, 2018 11:56 AM
To: Panger, Melissa <Panger.Melissa@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>; Lennartz, Steven <Lennartz.Steven@epa.gov>; Connolly, Jennifer <Connolly.Jennifer@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>; Eckel, William <Eckel.William@epa.gov>
Subject: summary - part two.

Just had a conversation with Steve and Mel - It seems to jive with what I heard at the Tuesday meeting with Bead – parts of what I was just overhearing. I just want to summarize for the group and make sure it's consistent with our plan. Is it?

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Message

From: Eckel, William [Eckel.William@epa.gov]
Sent: 2/14/2018 3:36:58 PM
To: Garber, Kristina [Garber.Kristina@epa.gov]
Subject: RE: discuss draft method for incorporating usage data (PCT) into ESA method
Attachments: Usage_step2_2-14-18(BEAD scientists)_wpe.pptx

Kris:

Here are some edits suggested for clarity, or where I couldn't follow the logic.

Bill

From: Garber, Kristina
Sent: Wednesday, February 14, 2018 9:51 AM
To: Panger, Melissa <Panger.Melissa@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Lennartz, Steven <Lennartz.Steven@epa.gov>; Connolly, Jennifer <Connolly.Jennifer@epa.gov>; Blankinship, Amy <Blankinship.Amy@epa.gov>; Donovan, Elizabeth <Donovan.Elizabeth@epa.gov>; Eckel, William <Eckel.William@epa.gov>; White, Katrina <White.Katrina@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>; Thurman, Nelson <Thurman.Nelson@epa.gov>; Barrett, Dena <Barrett.Dena@epa.gov>; Bohaty, Rochelle <Bohaty.Rochelle@epa.gov>; Khan, Faruque <Khan.Faruque@epa.gov>; Corbin, Mark <Corbin.Mark@epa.gov>; Anderson, Brian <Anderson.Brian@epa.gov>; Odenkirchen, Edward <Odenkirchen.Edward@epa.gov>; Sappington, Keith <Sappington.Keith@epa.gov>; Steeger, Thomas <Steeger.Thomas@epa.gov>
Subject: discuss draft method for incorporating usage data (PCT) into ESA method

Thank you all for your input yesterday. Attached is a revised slide deck that reflects changes to the method that account for the discussions. Please let me know if you have any additional comments or concerns. This slide deck will be presented to BEAD tomorrow.

Kris Garber, Senior Science Advisor
Environmental Fate and Effects Division
Office of Pesticide Programs
US Environmental Protection Agency
Mail Code: 7507P
1200 Pennsylvania Ave NW
Washington DC 20460-0001

Phone: 703-347-8940

Proposed method for incorporating usage data into revised ESA method

2/14/18 DRAFT

Presentation to BEAD scientists

Internal deliberative, do not cite or distribute

1

SAM approach is similar to maximum scenario for DW

EFED ESA team

- Amy Blankinship
- Jen Connolly
- Elizabeth Donovan
- Bill Eckel
- Kris Garber
- Ethan Harwood
- Steve Lennartz
- Melissa Panger
- Chuck Peck
- Colleen Rossmeisl
- Christina Wendel
- Katrina White

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

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Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Message

From: Connolly, Jennifer [Connolly.Jennifer@epa.gov]
Sent: 2/12/2018 4:09:14 PM
To: Garber, Kristina [Garber.Kristina@epa.gov]
Subject: RE: new concept to potentially add to ESA usage method (sales data)

Deliberative Process / Ex. 5

From: Garber, Kristina
Sent: Monday, February 12, 2018 11:02 AM
To: Connolly, Jennifer <Connolly.Jennifer@epa.gov>
Subject: RE: new concept to potentially add to ESA usage method (sales data)

Deliberative Process / Ex. 5

From: Connolly, Jennifer
Sent: Monday, February 12, 2018 10:16 AM
To: Garber, Kristina <Garber.Kristina@epa.gov>
Subject: RE: new concept to potentially add to ESA usage method (sales data)

Deliberative Process / Ex. 5

From: Garber, Kristina
Sent: Monday, February 12, 2018 10:03 AM
To: Connolly, Jennifer <Connolly.Jennifer@epa.gov>
Subject: RE: new concept to potentially add to ESA usage method (sales data)

Deliberative Process / Ex. 5

From: Connolly, Jennifer
Sent: Monday, February 12, 2018 10:01 AM
To: Garber, Kristina <Garber.Kristina@epa.gov>
Subject: RE: new concept to potentially add to ESA usage method (sales data)

Deliberative Process / Ex. 5

From: Garber, Kristina
Sent: Monday, February 12, 2018 9:54 AM
To: Eckel, William <Eckel.William@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>; White, Katrina <White.Katrina@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Lennartz, Steven <Lennartz.Steven@epa.gov>; Connolly, Jennifer <Connolly.Jennifer@epa.gov>; Blankinship, Amy <Blankinship.Amy@epa.gov>; Donovan, Elizabeth <Donovan.Elizabeth@epa.gov>; Kanarek, Andrew

<Kanarek.Andrew@epa.gov>; Harwood, Douglas <harwood.douglas@epa.gov>; Wendel, Christina <Wendel.Christina@epa.gov>
Cc: Anderson, Brian <Anderson.Brian@epa.gov>
Subject: new concept to potentially add to ESA usage method (sales data)

At one of our many meetings last week, the topic of sales data came up. this did not get incorporated into the slides; however, I would like to do so. I am putting forward the following proposal for the team's consideration:

Deliberative Process / Ex. 5

If folks don't have any major objections, I would like to get this into the slide deck for BEAD so that we can get their thoughts on how to incorporate the sales data. I would also like to ask them some questions: what spatial scale do sales data come in (e.g., state level)? Will data be available for all of US or just 48 conterminous states?

So, what do you all think of this?

Message

From: Connolly, Jennifer [Connolly.Jennifer@epa.gov]
Sent: 2/9/2018 3:41:53 PM
To: Garber, Kristina [Garber.Kristina@epa.gov]
Subject: RE: More computer woes/ usage slides
Attachments: Usage_step2_2-7-18_JC.pptx

Deliberative Process / Ex. 5

Jen

From: Garber, Kristina
Sent: Friday, February 9, 2018 8:54 AM
To: Connolly, Jennifer <Connolly.Jennifer@epa.gov>
Subject: Re: More computer woes/ usage slides

Sorry that your computer problems are continuing to make life difficult for you. Thank you for the update and for working on the diazinon example. Please let me know if I can do anything to help. I'm on flexiplace today and Monday (571-249-9977).

On Feb 9, 2018, at 8:04 AM, Connolly, Jennifer <Connolly.Jennifer@epa.gov> wrote:

> I'm in the office right now because when I went back to add in the slides we talked about, I noticed everything was being deleted from my OneDrive. The person in EzTech that I spoke with last night thinks they deleted my OneDrive folder from my old computer without halting the syncing. Around 9 last night EZ Tech decided I needed to let everything delete then it could be restored.
>
> Needless to say, I didn't get to the slides, and I couldn't get the diazinon tables to Colleen to work on today. But I will see what I can do today while Ez Tech tries to restore all of my files.

Proposed method for incorporating usage data into revised ESA method

2/7/18 DRAFT
Presentation to BEAD and EFED scientists

Internal deliberative, do not cite or distribute

EFED ESA team

- Amy Blankinship
- Jen Connolly
- Elizabeth Donovan
- Bill Eckel
- Kris Garber
- Ethan Harwood
- Steve Lennartz
- Melissa Panger
- Chuck Peck
- Colleen Rossmeisl
- Christina Wendel
- Katrina White

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

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Deliberative Process / Ex. 5

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Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Message

From: Khan, Faruque [Khan.Faruque@epa.gov]
Sent: 2/14/2018 1:44:02 PM
To: Garber, Kristina [Garber.Kristina@epa.gov]
Subject: RE: discuss draft method for incorporating usage data (PCT) into ESA method
Attachments: Usage_step2_2-13-18_FK.pptx

Hi Kris,
Slides are great and no major comments. I added my comments what we talked about this morning. Comments are minor and please see slides # 5, 9 and 19 in the attached file,

---

From: Garber, Kristina
Sent: Tuesday, February 13, 2018 4:36 PM
To: Panger, Melissa <Panger.Melissa@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Lennartz, Steven <Lennartz.Steven@epa.gov>; Connolly, Jennifer <Connolly.Jennifer@epa.gov>; Blankinship, Amy <Blankinship.Amy@epa.gov>; Donovan, Elizabeth <Donovan.Elizabeth@epa.gov>; Eckel, William <Eckel.William@epa.gov>; White, Katrina <White.Katrina@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>; Thurman, Nelson <Thurman.Nelson@epa.gov>; Barrett, Dena <Barrett.Dena@epa.gov>; Bohaty, Rochelle <Bohaty.Rochelle@epa.gov>; Khan, Faruque <Khan.Faruque@epa.gov>; Corbin, Mark <Corbin.Mark@epa.gov>; Anderson, Brian <Anderson.Brian@epa.gov>; Odenkirchen, Edward <Odenkirchen.Edward@epa.gov>; Sappington, Keith <Sappington.Keith@epa.gov>; Steeger, Thomas <Steeger.Thomas@epa.gov>
Subject: discuss draft method for incorporating usage data (PCT) into ESA method

Here are the slides that were presented. I will modify based on our conversation and send a new version out ASAP.

Kris Garber, Senior Science Advisor
Environmental Fate and Effects Division
Office of Pesticide Programs
US Environmental Protection Agency
Mail Code: 7507P
1200 Pennsylvania Ave NW
Washington DC 20460-0001

Phone: 703-347-8940

Proposed method for incorporating usage data into revised ESA method

2/13/18 DRAFT

Presentation to EFED scientists

Internal deliberative, do not cite or distribute

1

Deliberative Process / Ex. 5

EFED ESA team

- Amy Blankinship
- Jen Connolly
- Elizabeth Donovan
- Bill Eckel
- Kris Garber
- Ethan Harwood
- Steve Lennartz
- Melissa Panger
- Chuck Peck
- Colleen Rossmeisl
- Christina Wendel
- Katrina White

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

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Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Message

From: Anderson, Brian [Anderson.Brian@epa.gov]
Sent: 2/2/2018 1:55:08 PM
To: Rossmeisl, Colleen [Rossmeisl.Colleen@epa.gov]; Garber, Kristina [Garber.Kristina@epa.gov]; Panger, Melissa [Panger.Melissa@epa.gov]; Connolly, Jennifer [Connolly.Jennifer@epa.gov]; Lennartz, Steven [Lennartz.Steven@epa.gov]; Peck, Charles [Peck.Charles@epa.gov]
CC: Spatz, Dana [Spatz.Dana@epa.gov]; Sankula, Sujatha [Sankula.Sujatha@epa.gov]; Villanueva, Philip [Villanueva.Philip@epa.gov]; Kyle, Lee [Kyle.Lee@epa.gov]
Subject: use and usage workshop presentation
Attachments: Draft agenda for pesticide use meeting_1_12_18.docx

Hi Guys,

Attached is the draft agenda for the use and usage workshop.

Deliberative Process / Ex. 5

Thanks

Brian

Message

From: Connolly, Jennifer [Connolly.Jennifer@epa.gov]
Sent: 1/4/2018 4:27:15 PM
To: Lennartz, Steven [Lennartz.Steven@epa.gov]
CC: Kyle, Lee [Kyle.Lee@epa.gov]; Garber, Kristina [Garber.Kristina@epa.gov]; Panger, Melissa [Panger.Melissa@epa.gov]; Peck, Charles [Peck.Charles@epa.gov]; Rossmeisl, Colleen [Rossmeisl.Colleen@epa.gov]
Subject: RE: ESA overlap and use layer information

<!--[if lte mso 15 || CheckWebRef]-->

Connolly, Jennifer has shared a OneDrive for Business file with you. To view it, click the link below.

UseLayers_20180103.xlsx

<!--[endif]-->

I've attached my tracking sheet for the use layers. This isn't complete yet but there are couple of things I wanted to bring up soon rather than later.

Deliberative Process / Ex. 5

Jen

From: Lennartz, Steven
Sent: Thursday, January 04, 2018 9:41 AM
To: Connolly, Jennifer <Connolly.Jennifer@epa.gov>
Cc: Kyle, Lee <Kyle.Lee@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>
Subject: RE: ESA overlap and use layer information

Deliberative Process / Ex. 5

-Steve

From: Connolly, Jennifer
Sent: Wednesday, January 03, 2018 7:02 PM
To: Lennartz, Steven <Lennartz.Steven@epa.gov>
Cc: Kyle, Lee <Kyle.Lee@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>
Subject: RE: ESA overlap and use layer information

This is great Steve, thanks!

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Jen

From: Lennartz, Steven
Sent: Wednesday, January 03, 2018 8:59 AM
To: Connolly, Jennifer <Connolly.Jennifer@epa.gov>
Cc: Kyle, Lee <Kyle.Lee@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>
Subject: RE: ESA overlap and use layer information

The data and documentation are complete, a slim 200GB. I've posted a line by line response to your list on SharePoint. I've copied it below, my comments are in blue.

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Steve Lennartz, *MSc, CMS-RS*
Mapping Scientist, EISB
Environmental Fate and Effects Division, PYS12923
Office of Pesticide Programs
U.S. Environmental Protection Agency
Phone: (703) 347-0145
Email: lennartz.steven@epa.gov

From: Connolly, Jennifer
Sent: Thursday, December 21, 2017 7:26 PM
To: Lennartz, Steven <Lennartz.Steven@epa.gov>
Cc: Kyle, Lee <Kyle.Lee@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>
Subject: ESA overlap and use layer information

Hey Steve,

Here is the final list of layer and tables we've been discussing. If I'm unavailable the first week in January please cc Kris, Melissa, Chuck and Colleen as you send me finalized information. Also, please let them know if you run into any issues.

Deliberative Process / Ex. 5

Happy Holidays! And if I don't see you, good luck with the new little one!

Jen

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

****
Jennifer Connolly, GIS Analyst
Environmental Information Support Branch
Environmental Fate and Effects Division
Office of Pesticide Programs, U.S. EPA
1200 Pennsylvania Avenue, NW (7507P)
Washington, DC 20460
phone: (703) 347-0405
fax: (703) 305-0619
e-mail: connolly.jennifer@epa.gov
****

Message

From: Garber, Kristina [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=C4FBFBF6569041F4BD559765E027AA31-KRISTINA GARBER]
Sent: 4/18/2018 9:28:19 PM
To: Odenkirchen, Edward [Odenkirchen.Edward@epa.gov]
Subject: follow up
Attachments: SETAC_2016_garber.pptx

See slides 12 and 13 Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Kris Garber, Senior Science Advisor
Environmental Fate and Effects Division
Office of Pesticide Programs
US Environmental Protection Agency
Mail Code: 7507P
1200 Pennsylvania Ave NW
Washington DC 20460-0001

Phone: 703-347-8940

Message

From: Garber, Kristina [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=C4FBFBF6569041F4BD559765E027AA31-KRISTINA GARBER]
Sent: 5/24/2018 9:39:11 AM
To: Blankinship, Amy [Blankinship.Amy@epa.gov]
Subject: Re: Diazinon (057801) National and State Summary Use and Usage Summary

Hi Amy,

The SUUM has not been revised for diazinon.

Kris

On May 23, 2018, at 5:15 PM, Blankinship, Amy <Blankinship.Amy@epa.gov> wrote:

> Hi Kris,
>
> Josh is working on some drinking water refinements for diazinon and was asking about usage data. I mentioned BEAD had done some work for us for ESA. I have the data/memo from the email below, but I thought I would check with you to make sure I wasn't missing a more recent update.
>
> Thanks,
> Amy
>
> ---
>
> **From:** Garber, Kristina
> **Sent:** Tuesday, September 05, 2017 2:41 PM
> **To:** Connolly, Jennifer <Connolly.Jennifer@epa.gov>; Lennartz, Steven <Lennartz.Steven@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Blankinship, Amy <Blankinship.Amy@epa.gov>; Donovan, Elizabeth <Donovan.Elizabeth@epa.gov>; Kanarek, Andrew <Kanarek.Andrew@epa.gov>; Eckel, William <Eckel.William@epa.gov>
> **Subject:** FW: Diazinon (057801) National and State Summary Use and Usage Summary
>
> Attached is the refined usage data for diazinon
>
> ---
>
> **From:** Garber, Kristina
> **Sent:** Tuesday, September 05, 2017 2:36 PM
> **To:** Echeverria, Marietta <Echeverria.Marietta@epa.gov>; 'Anderson, Brian' <anderson.brian@epa.gov>; Villanueva, Philip <Villanueva.Philip@epa.gov>; Pease, Anita <Pease.Anita@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>
> **Subject:** FW: Diazinon (057801) National and State Summary Use and Usage Summary

Deliberative Process / Ex. 5

From: Garber, Kristina
Sent: Wednesday, October 26, 2016 10:09 AM
To: Pease, Anita <Pease.Anita@epa.gov>
Subject: Fwd: Diazinon (057801) National and State Summary Use and Usage Summary

Here's the final memo from BEAD on the refined usage analysis for diazinon.

Begin forwarded message:

> **From:** "Russell, CarolynY" <Russell.Carolyny@epa.gov>
> **To:** "Garber, Kristina" <Garber.Kristina@epa.gov>, "Nguyen, Khue" <Nguyen.Khue@epa.gov>
> **Cc:** "Paisley-Jones, Claire" <Paisley-Jones.Claire@epa.gov>, "Miller, Wynne" <Miller.Wynne@epa.gov>, "Jarboe, Stephen" <Jarboe.Steve@epa.gov>, "Doucoure, Cynthia" <Doucoure.Cynthia@epa.gov>, "Prieto, Rafael" <Prieto.Rafael@epa.gov>, "Shah, Aruna" <Shah.Aruna@epa.gov>, "Atwood, Donald" <Atwood.Donald@epa.gov>
> **Subject: Diazinon (057801) National and State Summary Use and Usage Summary**
>
> Your message is ready to be sent with the following file or link attachments:
>
> Diazinon.pdf
>
> Note: To protect against computer viruses, e-mail programs may prevent sending or receiving certain types of file attachments. Check your e-mail security settings to determine how attachments are handled.

Message

From: Garber, Kristina [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=C4FBFBF6569041F4BD559765E027AA31-KRISTINA GARBER]
Sent: 3/15/2019 7:36:12 PM
To: Rossmeisl, Colleen [Rossmeisl.Colleen@epa.gov]
CC: Peck, Charles [Peck.Charles@epa.gov]
Subject: RE: ESA revised probabilistic analysis
Attachments: ESA Probabilistic Analysis_updated_3-14-19_kg.docx

Colleen,

This section looks really good. You did a great job on the revisions. Attached are my comments. Let me know if you have any questions.

Kris

From: Rossmeisl, Colleen
Sent: Friday, March 15, 2019 11:52 AM
To: Garber, Kristina <Garber.Kristina@epa.gov>
Cc: Peck, Charles <Peck.Charles@epa.gov>
Subject: ESA revised probabilistic analysis

Hi Kris –

The revised probabilistic section is attached. I am hoping I captured the revisions you recommended and we discussed yesterday. I think it flows better now and gives some base/background. Chuck has reviewed changes made to the aquatic part. It is a clean version but if you want to be reminded of comments, etc. those are still all in the revised methods version on SharePoint.

I will check email again late today. I will be working on the revised document on Monday and can drop it back into the main body then with any other edits or comments you have. I will also be working on reading through the whole document Monday, making any other edits needed from RTC and adding language on drift adjustments to the usage section. When it does go to Brian review Monday/Tuesday, the team will also have the chance to review again then as well.

Thanks!
Colleen

Message

From: Garber, Kristina [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=C4FBFBF6569041F4BD559765E027AA31-KRISTINA GARBER]
Sent: 12/21/2018 9:27:20 PM
To: Etterson, Matthew [Etterson.Matthew@epa.gov]; Schumaker, Nathan [Schumaker.Nathan@epa.gov]
Subject: RE: FW: revised CAGN ms for your review
Attachments: NHS Edits_kg.docx

Hi Matt,

My comments are attached. As we discussed yesterday, after you revise the manuscript, feel free to share the revised draft with our other co-authors. I suggest that you also provide the supplemental info, so folks can double check the changes to that doc as well.

Kris

From: Etterson, Matthew
Sent: Wednesday, December 19, 2018 9:53 AM
To: Nathan Schumaker <nathan.schumaker@gmail.com>; Garber, Kristina <Garber.Kristina@epa.gov>
Subject: RE: FW: revised CAGN ms for your review

Thanks Nathan,
I'll review before our meeting today.
Talk to you soon.
Matt

From: Nathan Schumaker <Personal Email / Ex. 6>
Sent: Wednesday, December 19, 2018 1:13 AM
To: Etterson, Matthew <Etterson.Matthew@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>
Subject: Re: FW: revised CAGN ms for your review

Hi Matt & Kris,

I'm attaching a version of the draft MS with a few edits.

Deliberative Process / Ex. 5

Talk to you in a few hours...

Nathan Schumaker
541-754-4658
Personal Email / Ex. 6

www.nschumaker.net

On Thu, Dec 13, 2018 at 11:25 AM Schumaker, Nathan <Schumaker.Nathan@epa.gov> wrote:

From: Etterson, Matthew
Sent: Wednesday, December 12, 2018 11:36 AM
To: Schumaker, Nathan <Schumaker.Nathan@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>
Subject: revised CAGN ms for your review

Nathan & Kris,

Attached is a revised version of the CAGN manuscript that removes chemical names. Please read when you have a chance. Maybe we can have a quick call next week to discuss?

Matt

Message

From: Garber, Kristina [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=C4FBFBF6569041F4BD559765E027AA31-KRISTINA GARBER]
Sent: 5/22/2018 2:29:46 PM
To: Eckel, William [Eckel.William@epa.gov]; Blankinship, Amy [Blankinship.Amy@epa.gov]; Donovan, Elizabeth [Donovan.Elizabeth@epa.gov]; Connolly, Jennifer [Connolly.Jennifer@epa.gov]; Lennartz, Steven [Lennartz.Steven@epa.gov]
Subject: RE: malathion uses (question about developed)

Deliberative Process / Ex. 5 but I don't think that t Deliberative Process / Ex. 5

From: Eckel, William
Sent: Tuesday, May 22, 2018 10:29 AM
To: Garber, Kristina <Garber.Kristina@epa.gov>; Blankinship, Amy <Blankinship.Amy@epa.gov>; Donovan, Elizabeth <Donovan.Elizabeth@epa.gov>; Connolly, Jennifer <Connolly.Jennifer@epa.gov>; Lennartz, Steven <Lennartz.Steven@epa.gov>
Subject: RE: malathion uses (question about developed)

Deliberative Process / Ex. 5 ?

From: Garber, Kristina
Sent: Tuesday, May 22, 2018 10:23 AM
To: Blankinship, Amy <Blankinship.Amy@epa.gov>; Eckel, William <Eckel.William@epa.gov>; Donovan, Elizabeth <Donovan.Elizabeth@epa.gov>; Connolly, Jennifer <Connolly.Jennifer@epa.gov>; Lennartz, Steven <Lennartz.Steven@epa.gov>
Subject: malathion uses (question about developed)

Deliberative Process / Ex. 5

Could you please confirm?

Kris Garber, Senior Science Advisor
Environmental Fate and Effects Division
Office of Pesticide Programs
US Environmental Protection Agency
Mail Code: 7507P
1200 Pennsylvania Ave NW
Washington DC 20460-0001

Phone: 703-347-8940

Message

From: Garber, Kristina [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=C4FBFBF6569041F4BD559765E027AA31-KRISTINA GARBER]
Sent: 3/11/2019 8:14:29 PM
To: Peck, Charles [Peck.Charles@epa.gov]
Subject: FW: response to comments
Attachments: Response to comments ESA Revised methods_03.7.19.md.docx

---

From: Anderson, Brian
Sent: Monday, March 11, 2019 4:00 PM
To: Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Connolly, Jennifer <Connolly.Jennifer@epa.gov>; Donovan, Elizabeth <Donovan.Elizabeth@epa.gov>
Subject: FW: response to comments

Got input from Dyner - I have not looked yet.

b

From: Dyner, Mark
Sent: Monday, March 11, 2019 3:45 PM
To: Anderson, Brian <Anderson.Brian@epa.gov>
Cc: Knorr, Michele <knorr.michele@epa.gov>
Subject: RE: response to comments

Brian,

Thanks for the chance to review. A lot of hard work went into this. For the most part, **Deliberative Process / Ex. 5**

Attorney Client / Ex. 5

Happy to talk through any questions you have.

Mark

Mark Dyner
Office of General Counsel
(202) 564-1754

From: Anderson, Brian
Sent: Friday, March 08, 2019 8:32 AM

To: Dyner, Mark <dyner.mark@epa.gov>
Subject: FW: response to comments

Hi Mark,

Attached is our response to comment draft on the Services comments on our proposed revised methods. We'd really appreciate your review of this document to see if you think we addressed any concerns that you had with the comments. We tried to incorporate your thoughts from the NMFS review and our disussions about the USDA comments.

Thanks again Mark!!

Brian

From: Anderson, Brian
Sent: Thursday, March 07, 2019 11:04 PM
To: Echeverria, Marietta (Echeverria.Marietta@epa.gov) <Echeverria.Marietta@epa.gov>
Cc: Garber, Kristina (Garber.Kristina@epa.gov) <Garber.Kristina@epa.gov>; Rossmeisl, Colleen (Rossmeisl.Colleen@epa.gov) <Rossmeisl.Colleen@epa.gov>; Panger, Melissa (Panger.Melissa@epa.gov) <Panger.Melissa@epa.gov>; Peck, Charles (Peck.Charles@epa.gov) <Peck.Charles@epa.gov>; Connolly, Jennifer (Connolly.Jennifer@epa.gov) <Connolly.Jennifer@epa.gov>; Donovan, Elizabeth (Donovan.Elizabeth@epa.gov) <Donovan.Elizabeth@epa.gov>
Subject: response to comments

Marietta,

Attached is the draft response to comments document for your reviewing pleasure.

I want to thank the team for their work on this. They really put together a very thoughtful and thorough response in a short amount of time. I can't say enough about the dedication of this team.

A few things we want to flag for you are below – these are just issues in the comments that we thought are notable. They are not really surprises, but particular comments that are worth noting.

Thanks for your comments,

ESA Team

Comments on Usage Data:

Deliberative Process / Ex. 5

Use of Apical vs Sublethal Endpoints:

Deliberative Process / Ex. 5

Collaboration:

Deliberative Process / Ex. 5

1% and 5% cutoff values for overlap:

Deliberative Process / Ex. 5

Message

From: Garber, Kristina [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=C4FBFBF6569041F4BD559765E027AA31-KRISTINA GARBER]
Sent: 6/26/2018 6:39:46 PM
To: Panger, Melissa [Panger.Melissa@epa.gov]; Lennartz, Steven [Lennartz.Steven@epa.gov]; Atwood, Donald [Atwood.Donald@epa.gov]
CC: Perry, Tracy [Perry.Tracy@epa.gov]; Sims, Diann [Sims.Diann@epa.gov]
Subject: description of public comments submitted on diazinon, chlorpyrifos and malathion usage data
Attachments: 3 OPs.use.usage.comments.docx

Mosquito adulticide team.

Tracy Perry put together the attached description of comments received on the first 3 op BEs, with a focus on those containing usage data. I thought that the first one would be of interest to your efforts.

Kris

Kris Garber, Senior Science Advisor
Environmental Fate and Effects Division
Office of Pesticide Programs
US Environmental Protection Agency
Mail Code: 7507P
1200 Pennsylvania Ave NW
Washington DC 20460-0001

Phone: 703-347-8940

Message

From: Garber, Kristina [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=C4FBFBF6569041F4BD559765E027AA31-KRISTINA GARBER]
Sent: 2/11/2019 8:39:08 PM
To: Rossmeisl, Colleen [Rossmeisl.Colleen@epa.gov]
Subject: RE: EPA ESA Usage Workgroup

OK, I thought so, but just wanted to make sure. Thank you.

From: Rossmeisl, Colleen
Sent: Monday, February 11, 2019 3:38 PM
To: Garber, Kristina <Garber.Kristina@epa.gov>
Subject: RE: EPA ESA Usage Workgroup

Oh yes! I just left them in so you could see responses and edits. ;)

From: Garber, Kristina
Sent: Monday, February 11, 2019 3:36 PM
To: Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>
Subject: RE: EPA ESA Usage Workgroup

Are you going to remove the comments and accept the changes before sending out the doc?

From: Rossmeisl, Colleen
Sent: Monday, February 11, 2019 3:34 PM
To: Lennartz, Steven <Lennartz.Steven@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Anderson, Brian <Anderson.Brian@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Connolly, Jennifer <Connolly.Jennifer@epa.gov>; Kyle, Lee <Kyle.Lee@epa.gov>; Becker, Jonathan <Becker.Jonathan@epa.gov>; Paisley-Jones, Claire <Paisley-Jones.Claire@epa.gov>; Sims, Diann <Sims.Diann@epa.gov>; Perry, Tracy <Perry.Tracy@epa.gov>; Suarez, Mark <Suarez.Mark@epa.gov>
Subject: RE: EPA ESA Usage Workgroup

Thanks Steve! I have made the suggested updates from Kris, as attached. I will send this out in the next hour or so along with meeting reminder for tomorrow and Census crosswalk unless I hear from anyone else. This is still a draft and meant for discussion purposes, so don't feel like this is the last chance to provide comments (there will still be more discussion). But hopefully gets us on the path to wrapping up our malathion AA discussions with Services.

Thanks!
Colleen

From: Lennartz, Steven
Sent: Monday, February 11, 2019 12:58 PM
To: Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Anderson, Brian <Anderson.Brian@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Connolly, Jennifer <Connolly.Jennifer@epa.gov>; Kyle, Lee <Kyle.Lee@epa.gov>; Becker, Jonathan <Becker.Jonathan@epa.gov>; Paisley-Jones, Claire <Paisley-Jones.Claire@epa.gov>; Sims, Diann <Sims.Diann@epa.gov>; Perry, Tracy <Perry.Tracy@epa.gov>; Suarez, Mark <Suarez.Mark@epa.gov>
Subject: RE: EPA ESA Usage Workgroup

I have no additional comments.

-Steve

From: Rossmeisl, Colleen
Sent: Monday, February 11, 2019 10:34 AM
To: Garber, Kristina <Garber.Kristina@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Anderson, Brian <Anderson.Brian@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Connolly, Jennifer <Connolly.Jennifer@epa.gov>; Kyle, Lee <Kyle.Lee@epa.gov>; Becker, Jonathan <Becker.Jonathan@epa.gov>; Paisley-Jones, Claire <Paisley-Jones.Claire@epa.gov>; Sims, Diann <Sims.Diann@epa.gov>; Perry, Tracy <Perry.Tracy@epa.gov>; Lennartz, Steven <Lennartz.Steven@epa.gov>; Suarez, Mark <Suarez.Mark@epa.gov>
Subject: RE: EPA ESA Usage Workgroup

I like that last option – **Deliberative Process / Ex. 5**

From: Garber, Kristina
Sent: Monday, February 11, 2019 10:30 AM
To: Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Anderson, Brian <Anderson.Brian@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Connolly, Jennifer <Connolly.Jennifer@epa.gov>; Kyle, Lee <Kyle.Lee@epa.gov>; Becker, Jonathan <Becker.Jonathan@epa.gov>; Paisley-Jones, Claire <Paisley-Jones.Claire@epa.gov>; Sims, Diann <Sims.Diann@epa.gov>; Perry, Tracy <Perry.Tracy@epa.gov>; Lennartz, Steven <Lennartz.Steven@epa.gov>; Suarez, Mark <Suarez.Mark@epa.gov>
Subject: RE: EPA ESA Usage Workgroup

Deliberative Process / Ex. 5

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Sent: Monday, February 11, 2019 10:25 AM
To: Peck, Charles <Peck.Charles@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>; Anderson, Brian <Anderson.Brian@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Connolly, Jennifer <Connolly.Jennifer@epa.gov>; Kyle, Lee <Kyle.Lee@epa.gov>; Becker, Jonathan <Becker.Jonathan@epa.gov>; Paisley-Jones, Claire <Paisley-Jones.Claire@epa.gov>; Sims, Diann <Sims.Diann@epa.gov>; Perry, Tracy <Perry.Tracy@epa.gov>; Lennartz, Steven <Lennartz.Steven@epa.gov>; Suarez, Mark <Suarez.Mark@epa.gov>
Subject: RE: EPA ESA Usage Workgroup

Thanks for the feedback Kris and Chuck!

Deliberative Process / Ex. 5

From: Peck, Charles
Sent: Monday, February 11, 2019 10:22 AM
To: Garber, Kristina <Garber.Kristina@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>; Anderson, Brian <Anderson.Brian@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Connolly, Jennifer <Connolly.Jennifer@epa.gov>; Kyle, Lee <Kyle.Lee@epa.gov>; Becker, Jonathan <Becker.Jonathan@epa.gov>; Paisley-

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Subject: RE: EPA ESA Usage Workgroup

I have nothing to add to Kris' comments

From: Garber, Kristina
Sent: Monday, February 11, 2019 10:14 AM
To: Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>; Anderson, Brian <Anderson.Brian@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Connolly, Jennifer <Connolly.Jennifer@epa.gov>; Kyle, Lee <Kyle.Lee@epa.gov>; Becker, Jonathan <Becker.Jonathan@epa.gov>; Paisley-Jones, Claire <Paisley-Jones.Claire@epa.gov>; Sims, Diann <Sims.Diann@epa.gov>; Perry, Tracy <Perry.Tracy@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Lennartz, Steven <Lennartz.Steven@epa.gov>; Suarez, Mark <Suarez.Mark@epa.gov>
Subject: RE: EPA ESA Usage Workgroup

I have some comments on the table.

Deliberative Process / Ex. 5

From: Rossmeisl, Colleen
Sent: Monday, February 11, 2019 9:51 AM
To: Anderson, Brian <Anderson.Brian@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Connolly, Jennifer <Connolly.Jennifer@epa.gov>; Kyle, Lee <Kyle.Lee@epa.gov>; Becker, Jonathan <Becker.Jonathan@epa.gov>; Paisley-Jones, Claire <Paisley-Jones.Claire@epa.gov>; Sims, Diann <Sims.Diann@epa.gov>; Perry, Tracy <Perry.Tracy@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Lennartz, Steven <Lennartz.Steven@epa.gov>; Suarez, Mark <Suarez.Mark@epa.gov>
Subject: EPA ESA Usage Workgroup

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Files prepared by EPA and shared:

- Malathion Usage Data (SUUM)
- Crosswalk (ULUT Data Source Summary)
- Census of Ag inclusion/exclusion tables *(I can't seem to find these in my email and I am not sure they have been forwarded yet...)*

Claire and/or Jen if you can let me know if you agree with this list or other documents that should be/have been provided. I think it would be good to just confirm we are all working with the same data set.

Thanks!
Colleen

Message

From: Garber, Kristina [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=C4FBFBF6569041F4BD559765E027AA31-KRISTINA GARBER]
Sent: 7/30/2019 2:35:52 PM
To: Corbin, Mark [Corbin.Mark@epa.gov]
CC: 'Anderson, Brian' [anderson.brian@epa.gov]
Subject: ESA usage method slides
Attachments: caracara_example.pptx; Applying_usage_2-26-18.pptx

Internal deliberative, do not cite or distribute

Hi Mark,

Deliberative Process / Ex. 5

Kris

Kris Garber, Senior Science Advisor
Environmental Fate and Effects Division
Office of Pesticide Programs
US Environmental Protection Agency
Mail Code: 7507P
1200 Pennsylvania Ave NW
Washington DC 20460-0001

Phone: 703-347-8940

Message

From: Garber, Kristina [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=C4FBFBF6569041F4BD559765E027AA31-KRISTINA GARBER]
Sent: 2/11/2019 3:36:21 PM
To: Rossmeisl, Colleen [Rossmeisl.Colleen@epa.gov]
Subject: RE: EPA ESA Usage Workgroup

Ok, thanks.

From: Rossmeisl, Colleen
Sent: Monday, February 11, 2019 10:34 AM
To: Garber, Kristina <Garber.Kristina@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Anderson, Brian <Anderson.Brian@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Connolly, Jennifer <Connolly.Jennifer@epa.gov>; Kyle, Lee <Kyle.Lee@epa.gov>; Becker, Jonathan <Becker.Jonathan@epa.gov>; Paisley-Jones, Claire <Paisley-Jones.Claire@epa.gov>; Sims, Diann <Sims.Diann@epa.gov>; Perry, Tracy <Perry.Tracy@epa.gov>; Lennartz, Steven <Lennartz.Steven@epa.gov>; Suarez, Mark <Suarez.Mark@epa.gov>
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Thanks!
Colleen

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Sent: 6/8/2018 1:11:03 PM
To: Panger, Melissa [Panger.Melissa@epa.gov]; Lennartz, Steven [Lennartz.Steven@epa.gov]; Atwood, Donald [Atwood.Donald@epa.gov]
CC: Sims, Diann [Sims.Diann@epa.gov]
Subject: RE: Usage group (Excel spreadsheet)
Attachments: Malathion Extended SUUM.PDF

I forwarded you all a couple of emails with additional docs that are relevant.

I think it **Deliberative Process / Ex. 5**
Deliberative Process / Ex. 5

Also, I forwarded you BEAD's comments on the catalogue.

Attached is the malathion SUUM that Don developed. Just want to make sure all of you have the final version.

From: Panger, Melissa
Sent: Friday, June 08, 2018 8:59 AM
To: Garber, Kristina <Garber.Kristina@epa.gov>
Cc: Lennartz, Steven <Lennartz.Steven@epa.gov>; Atwood, Donald <Atwood.Donald@epa.gov>; Sims, Diann <Sims.Diann@epa.gov>
Subject: RE: Usage group (Excel spreadsheet)

Thanks… I think that might be it. Are there any other spreadsheets/documents (besides the milestones and timelines) that we should be aware of from the usage group?

Thanks!
Melissa

Melissa Panger, Ph.D.
Senior Advisor, ERB2
Environmental Fate and Effects Division
Office of Pesticide Programs
USEPA

703-305-6136
panger.melissa@epa.gov

From: Garber, Kristina
Sent: Friday, June 08, 2018 8:54 AM
To: Panger, Melissa <Panger.Melissa@epa.gov>
Cc: Lennartz, Steven <Lennartz.Steven@epa.gov>; Atwood, Donald <Atwood.Donald@epa.gov>; Sims, Diann <Sims.Diann@epa.gov>
Subject: RE: Usage group (Excel spreadsheet)

Attached is what FWS calls "the catalogue." I think that this is what you are looking for. I don't know if this is the most current version that FWS is working from. It's from March 14.

Diann – do you have another version of the catalogue? Perhaps the one with BEAD's comments?

From: Panger, Melissa
Sent: Friday, June 08, 2018 7:11 AM
To: Garber, Kristina <Garber.Kristina@epa.gov>
Cc: Lennartz, Steven <Lennartz.Steven@epa.gov>; Atwood, Donald <Atwood.Donald@epa.gov>
Subject: Usage group (Excel spreadsheet)

Hi Kris,

We're gearing up for our first mosquito usage meeting… as I understand it, our first charge is to fill in a cell in a usage data spreadsheet – do you have the latest version of the usage spreadsheet? If so, would you mind sending it?

Thanks,
Melissa

Melissa Panger, Ph.D.
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Environmental Fate and Effects Division
Office of Pesticide Programs
USEPA

703-305-6136
panger.melissa@epa.gov

Message

From: Garber, Kristina [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=C4FBFBF6569041F4BD559765E027AA31-KRISTINA GARBER]
Sent: 6/8/2018 12:54:27 PM
To: Panger, Melissa [Panger.Melissa@epa.gov]
CC: Lennartz, Steven [Lennartz.Steven@epa.gov]; Atwood, Donald [Atwood.Donald@epa.gov]; Sims, Diann [Sims.Diann@epa.gov]
Subject: RE: Usage group (Excel spreadsheet)
Attachments: Usage Data.xlsx

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Printed in the USA
DOI: 10.1002/etc.474

USE OF VEGETATED AGRICULTURAL DRAINAGE DITCHES TO DECREASE PESTICIDE TRANSPORT FROM TOMATO AND ALFALFA FIELDS IN CALIFORNIA, USA

MATTHEW T. MOORE,*† DEBRA L. DENTON,‡ CHARLES M. COOPER,† JEANETTE WRYSINSKI,§ JEFF L. MILLER,‖ INGE WERNER,# GERALD HORNER,†† DAVID CRANE,‡‡ DIANE B. HOLCOMB,§§ and GEORGE M. HUDDLESTON III‖‖

†U.S. Department of Agriculture–Agricultural Research Service, National Sedimentation Laboratory, Oxford, Mississippi
‡U.S. Environmental Protection Agency, Region 9, Sacramento, California
§Yolo County Resource Conservation District, Woodland, California, USA
‖AQUA-Science, Davis, California, USA
#Swiss Centre for Applied Ecotoxicology of Eawag, Dübendorf, Switzerland
††California State Water Resources Control Board, Sacramento, California, USA
‡‡California Department of Fish and Game, Rancho Cordova, California, USA
§§U.S. Department of Agriculture Natural Resources Conservation Service, Davis, California
‖‖Cardno-ENTRIX, Pendleton, South Carolina, USA

(*Submitted* 13 *August* 2010; *Returned for Revision* 28 *September* 2010; *Accepted* 30 *November* 2010)

Abstract—Irrigation and storm water runoff from agricultural fields has the potential to cause impairment to downstream aquatic receiving systems. Over the last several years, scientists have discovered the benefit of using edge-of-field practices, such as vegetated agricultural drainage ditches, in the mitigation of pesticides and sediment. After demonstrating this practice's feasibility in California, field trials were initiated to document irrigation runoff pesticide mitigation in California alfalfa and tomato fields. In the alfalfa field, chlorpyrifos concentration was decreased by 20% from the inflow to the ditch outflow. Thirty-two percent of the measured chlorpyrifos mass was associated with ditch plant material. In the tomato field, permethrin concentration was decreased by 67% and there was a 35% reduction in suspended sediment concentration from inflow to the ditch outflow. When surface water was not present in the ditch systems, the sediment was a significant repository for pesticides. Based on the field trials, vegetated agricultural drainage ditches can be successfully used as part of a suite of management practices to reduce pesticide and sediment runoff into aquatic receiving systems. Environ. Toxicol. Chem. 2011;30:1044–1049. © 2011 SETAC

Keywords—Chlorpyrifos Permethrin Management practice Runoff

INTRODUCTION

Agriculture is the single largest user of freshwater, consuming some 70% of the global surface water supply [1]. Adequately feeding an ever-growing global population requires the use of pest control and fertilizers to maximize yield on a limited amount of production acreage. However, nonpoint source pollution attributed to agriculture has sometimes affected the water quality of both groundwater and surface water resources. Olle et al. ([2]; http: // www.environmentcalifornia. org / uploads / 1A/3S/1A3SQHgTmKwLJ4zdz8kpsg / Water _ Woes.pdf) reported pesticides were detected in 96% of the 133 California waterway locations tested, with the most frequent detections being diuron (CAS 330–54–1), diazinon (CAS 333–41–5), simazine (CAS 122–34–9), chlorpyrifos (CAS 2921–88–2), and molinate (CAS 2212–67–1).

In response to nonpoint source pollution concerns, agriculture has invested heavily in development of management practices to alleviate agricultural runoff. Use of practices individually or in suites depends on physical land factors, as well as the willingness of a farmer to try new methods. Although traditional management practices have focused mainly on tillage, the CORE 4 program, developed for the U.S. Department of Agriculture Natural Resource Conservation Service (USDA NRCS), emphasizes four management components to address reduction of agricultural pollution: conservation tillage, crop nutrient management, pest management, and conservation buffers ([3]; http://www.nrcs.usda.gov/technical/ecs/agronomy/core4.pdf).

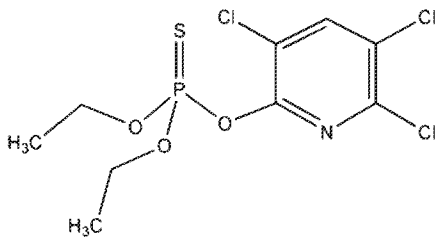
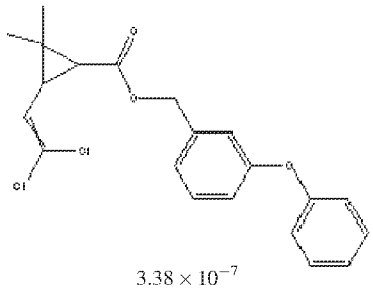
Conservation buffers may include contour buffer strips, field borders, filter strips, windbreaks, or wetlands. In particular, the use of constructed wetlands as an edge-of-field management practice for agricultural runoff has been promoted for the last two decades [4]. Although several studies have reported wetland mitigation efficiency [5–11], removing sufficient land from production acreage for wetland development is not always a viable option. Utilizing in-place, edge-of-field features of the agricultural production landscape, such as drainage ditches, allows for an economically efficient alternative [12–15]. Moore et al. [16] demonstrated that vegetation in similar-shaped ditches in California reduced pesticide half-distances 2 to 3 times as compared to unvegetated ditches. A pesticide half-distance is the distance required in the ditch for the pesticide concentration to be decreased 50% from inflow concentrations.

The objective of the present study was to provide data on the ability of vegetated agricultural drainage ditches (VADD) to mitigate the organophosphate insecticide chlorpyrifos (CAS 2921–88–2) and the pyrethroid insecticide permethrin (CAS 52645–53–1) in runoff from normal crop production settings in California (Table 1). Chlorpyrifos is the most used organophosphate pesticide in California, with nearly 614,000 kg applied in 2008 ([17]; http://www.cdpr.ca.gov/docs/pur/pur08rep/

* To whom correspondence may be addressed (matt.moore@ars.usda.gov).
Published online 1 February 2011 in Wiley Online Library (wileyonlinelibrary.com).

ED_002896_00041398-00001

Table 1. Physical and chemical properties of chlorpyrifos and permethrin

Property	Chlorpyrifos	Permethrin
Structure		
Vapor pressure (mm Hg)[a]	2.0×10^{-5}	3.38×10^{-7}
Water solubility (mg/L)[a]	2.0	0.2
log K_{OW}[b,c]	4.96	6.1
log K_{OC}[a]	3.78–3.93	4.93
Hydrolysis half-life (days)[c]	58.1	37.7
Photolysis half-life (days)[a]	21–28	110
Soil half-life (days)[c,d]		
sandy loam aerobic	113	30
sandy loam anaerobic	136	108

[a] EXTOXNET 1996 ([36]; http://extoxnet.orst.edu/pips/ghindex.html)
[b] Howard 1999 [37]
[c] USDA 1995 ([38]; http://www.ars.usda.gov/Services/docs.htm?docid = 14199)
[d] Kegley et al. 2007 ([39]; http://www.pesticideinfo.org)

tables/table6a.htm). Almonds were the top California crop receiving chlorpyrifos applications in 2008, with 131,140 kg applied ([18]; http://www.cdpr.ca.gov/docs/pur/pur08rep/chmrpt08.pdf). Permethrin, as a representative of the broader pyrethroid pesticide class, has been used to replace the older organophosphates, including chlorpyrifos. As a third-generation pyrethroid, permethrin was the most widely used pyrethroid in California in 2005 [19], and in 2008, approximately 155,700 kg were applied in the state, with pistachio applications (16,168 kg) receiving the most input per crop [18].

MATERIALS AND METHODS

Field information

A 7-ha portion of an alfalfa field and a 6-ha portion of a tomato field in Yolo County, California, were used for farm trials. Each field had existing drainage ditches in place that were reshaped for this research project. The alfalfa field drainage ditch was 1.53 m (width) × 0.61 m (depth) × 402 m (length). Sampling locations were established at 30.8 m (inflow), 185 m (middle), and 326 m (outflow). Tomato field drainage ditch dimensions were 3.29 m (width) × 0.52 m (depth) × 389 m (length). Sampling locations were established at 44.2 m (inflow), 204 m (middle), and 361 m (outflow).

Both ditches, one in each field, were planted with plugs of creeping wildrye, *Leymus triticoides* (Buckley) Pilger, and slender sedge, *Carex praegracilis* W. Boott, on 46-cm centers in 30-m alternating blocks from 13 to 20 December 2006. This allowed approximately five months for the plugs to mature prior to irrigation season. These two native grasses were chosen because of their availability and common use within California ditches. A 10-m unplanted buffer was left between each vegetated block and each block was replicated four times (Fig. 1). One day prior to the irrigation events (11 July 2007), plant density and percent plant cover were recorded in each ditch. Three replicate quadrants (0.25 m^2) were analyzed in each planted section of *Leymus*, *Carex*, as well as the originally unplanted buffers, which subsequently contained various weeds.

Soils data

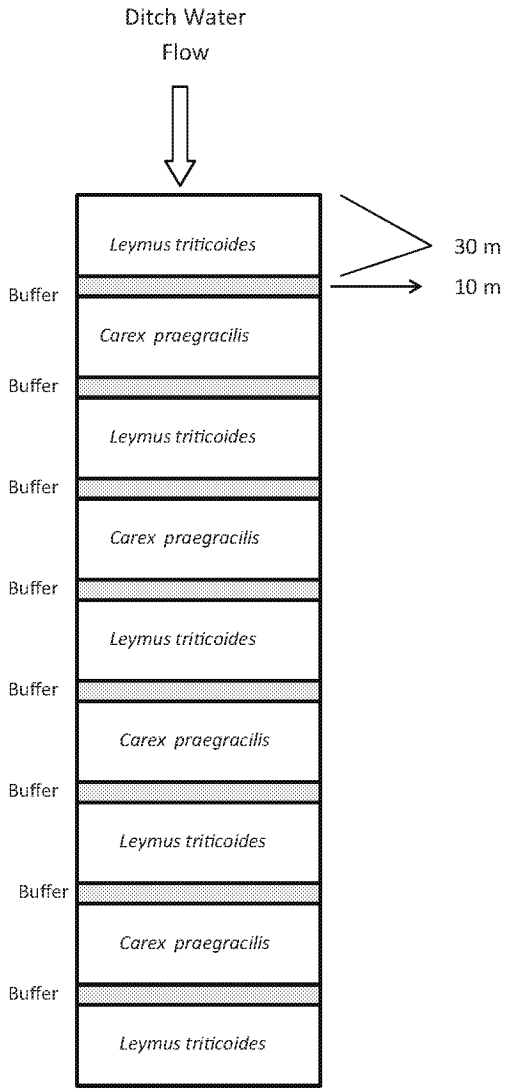
The alfalfa field soil type was Myers clay (fine smectitic, thermic Aridic Haploxererts). Soil analyses conducted by the University of California Agriculture and Natural Resources Analytical Laboratory indicated a soil pH of 7; particle size distribution of 21:35:44 (% sand:silt:clay); cation exchange capacity of 35.5 meq/100 g; 0.6% organic carbon; and 27.6% soil moisture. A mixture of Sycamore silt-clay loam (fine-silty, mixed, superactive, nonacid, thermic Mollic Endoaquepts) and Brentwood silt-clay loam (fine, smectitic, thermic Typic Haploxerepts) were the soil types found in the evaluated tomato field. Soil analyses reported a pH of 7.6; particle size distribution of 13:52:35 (% sand:silt:clay); cation exchange capacity of 31.2 meq/100 g; 0.47% organic carbon; and 32.8% soil moisture.

Pesticides

Lock-On™ (chlorpyrifos; Dow AgroSciences) was applied via an all-terrain vehicle rig to the ground on the alfalfa field at a rate of 1.75 L/ha in July 2007. This corresponded to 0.42 kg of chlorpyrifos/ha. Field (furrow) irrigation was initiated approximately 24 h after application. Although not completely uncommon, irrigation initiation is not usually done within 1 day of pesticide application. However, pest development levels can increase rapidly during summer temperatures under the plant canopy, resulting in the need for insecticide application. Twelve hours after irrigation initiation, runoff water began entering the vegetated ditch, lasting for approximately 14 h. The tomato field was treated aerially with Perm-Up 3.2 EC™ (permethrin; United Phosphorus) at a rate of 0.015 L/L of water dilution per hectare in August 2007. This corresponded to 0.006 kg of permethrin/ha. As with the alfalfa field, furrow irrigation was initiated 24 h after application, with runoff water entering the vegetated ditch 5 h later. Runoff water flowed in the vegetated ditch for a total of 6 h.

Water quality, sediment, and plant samples

Water quality and flow data were collected at each sampling station a minimum of five times during irrigation runoff events



Buffer = originally unplanted; by mid-July consisted of *Polygonum californicum, Convolvulus arvensis, Lolium perenne,* and *Lotus corniculatus* in alfalfa field and *Echinocloa crusgalli* in the tomato field

Fig. 1. Schematic of vegetation planted in drainage ditch. Buffers were originally bare; however, emergent weeds were present by the time irrigation runoff was initiated.

in the VADDs. In addition to flow, parameters measured included pH, dissolved oxygen (mg/L), temperature (°C), conductivity (μS/cm), and total suspended solids (TSS; mg/L). Water quality measurements were collected using calibrated, hand-held YSI™ field meters (Yellow Springs Instruments).

Spatial and temporal water, sediment, and plant samples were collected from VADDs using the same protocol reported by Moore et al. [16]. Briefly, grab samples of water were collected in precleaned, certified 1 L amber Boston round, narrow-mouth glass bottles with Teflon™-lined closures at 0, 0.5, 1, 4, 8, 16, and 24 h after runoff entry into the sampled ditches. Sediment samples were collected in 120-ml wide-mouth glass bottles with Teflon-lined closures using an identical time sequence as water samples. Only the top 1 cm of sediment was collected from ditches sampled, using solvent-rinsed spoons. Plant samples, taken at identical times as water and sediments, were collected from plant material exposed in the water column, using solvent-rinsed scissors, wrapped in aluminum foil, and placed in prelabeled 3-L freezer bags. All samples were preserved on wet ice from collection through transport to the Aquatic Toxicology Laboratory (ATL) at the University of California, Davis. Prior to next-day shipment to the California Department of Fish and Game Water Pollution Control Laboratory (DFG-WPCL), aqueous samples were kept in the dark at 4°C. Sediment and plant samples were frozen immediately upon receipt at the ATL. Sediment samples were shipped next-day to the DFG-WPCL, whereas plant samples were shipped overnight to the USDA Agricultural Research Service National Sedimentation Laboratory (NSL) for sample preparation. After immediate drying and grinding, plant samples were placed in glass vials and shipped overnight to the DFG-WPCL for pesticide analyses. All pesticide analyses followed protocols outlined in Moore et al. [16] using liquid–liquid extraction (water samples) or pressurized fluid extraction (sediment and plants). Final extracts were analyzed using methods U.S. EPA 8141B and U.S. EPA 8081B on a dual column high-resolution gas chromatograph equipped with electron capture detectors.

RESULTS AND DISCUSSION

Alfalfa field ditch

Mean density of *L. triticoides* in plots was 223 ± 42 plants/m^2, with a 46 ± 15% mean cover. In *C. praegracilis* plots, the mean density was 163 ± 15 plants/m^2, with a 93 ± 6% mean cover. By mid-July, the originally bare buffers now had a mixture of *Polygonum californicum*, *Convolvulus arvensis*, *Lolium perenne*, and *Lotus corniculatus* at a mean density of 130 ± 59 plants/m^2, and a 97 ± 5% mean cover.

Water quality variables in the ditch differed slightly through the duration (14 h) of the runoff collection. Ditch pH values were essentially neutral, ranging from 7.6 to 7.7 (± 0.1). These conditions would place the hydrolytic and photolytic half-lives of chlorpyrifos at approximately one month; however, degradation has been known to significantly increase in natural waters, such as in ponds and canals ([20,21]; http://www.dowagro.com/PublishedLiterature/dh_0039/0901b80380039661.pdf). Mean ± standard error (SE) values for temperature, dissolved oxygen, and conductivity were 23.2 ± 5.3°C, 3.4 ± 1.0 mg/L, and 372 ± 76 μS/cm, respectively. Although TSS never exceeded 10 mg/L in any sample, a 5% overall reduction in TSS was noted between the ditch inlet and outlet. The mean flow rate at the top (inlet) of the ditch sampling area was 25 ± 13 L/s, while mean flow rate at the ditch outlet was 27 ± 10 L/s.

Inflow aqueous chlorpyrifos concentrations from irrigation runoff were variable, ranging from 2.8 μg/L (at 0 min) to a maximum of 4.5 μg/L after 90 min of runoff (Fig. 2). Outflow chlorpyrifos concentrations at the bottom of the ditch ranged from 2.6 μg/L (625 min) to a maximum of 3.1 μg/L (265 min). These field concentrations were slightly higher than the 0.22 μg/L to 1.67 μg/L reported by Gill et al. [22]; however, temporal sampling methods between the two studies also differed. Gill et al. [22] collected samples at intervals ranging from 30 min to 1 h, with a target of six collected samples at each site per irrigation set. Using a *t* test, a statistically significant difference ($\alpha = 0.05$) was noted between the mean aqueous chlorpyrifos concentration at the ditch inlet (3.45 μg/L) versus the ditch outlet (2.75 μg/L) in the present study. This represented a 20% mean reduction in aqueous chlorpyrifos concentration through the ditch. Gill et al. [22] reported a 38% chlorpyrifos reduction after traveling through a 200-m

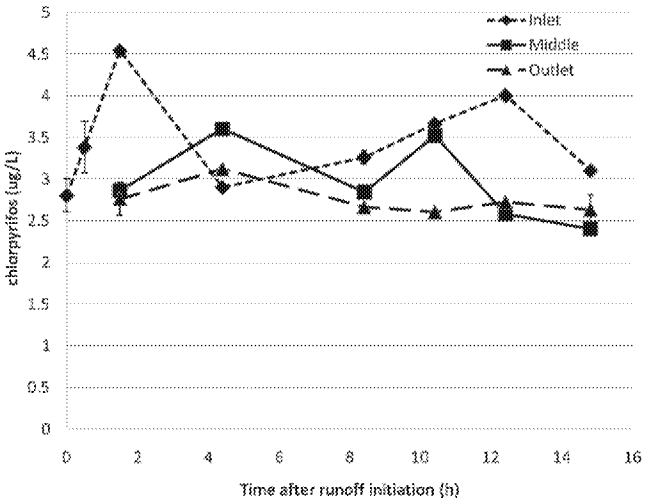


Fig. 2. Mean aqueous chlorpyrifos concentration (μg/L) at ditch inlet, middle, and outlet for a 14-h alfalfa irrigation event.

vegetated ditch, as opposed to only 1% reduction after exposure to a nonvegetated ditch. Chlorpyrifos concentrations measured in the sediment and plants were variable with time and sample location. Inflow sediment concentrations ranged from below detection to 1,700 μg/kg, whereas plant concentrations ranged from 272 to 782 μg/kg. Sediment and plant concentrations at the middle sampling station ranged from 4.35 to 103 μg/kg and 204 to 1,361 μg/kg, respectively. Outflow sediment and plant chlorpyrifos concentrations ranged from 8.74 to 3,610 μg/kg and 360 to 1,522 μg/kg, respectively.

Other environmental systems utilizing the concept of pesticide–vegetation sorption have reported successful results with chlorpyrifos mitigation. A vegetated treatment system with duckweed (*Lemna* sp.), watercress (*Nasturtium* sp.), and pennywort (*Hydrocotyle ranunculoides* L.f.) was reported to reduce chlorpyrifos by 52% according to Hunt et al. [23]. The system used by these researchers was more pond-like, allowing for more settling and contact time for potential pesticide removal, as compared to the present study. Poletika et al. [24] discovered chlorpyrifos removal efficiency with uniform runoff flow in vegetated filter strips (4.6 m in length) to be 85%. Filter strips comprised 90% smooth brome (*Bromus inermis* Leyss.) and 10% bluegrass (*Poa pratensis* L.). Similar findings by Arora et al. [25] reported 83.1% chlorpyrifos retention in vegetated buffer strips composed of 81% *B. inermis*, 12% *P. pratensis*, 5% *Festuca arundinacea* Schreber, and 2% other with a density of 8.82 M tillers/ha. Schulz and Peall [26] reported essentially 100% chlorpyrifos retention in a South African constructed wetland over a five-month period (inflow concentration was 31 μg/kg on suspended particles; no detection on outflow suspended particles). The constructed wetland (100 m × 36 m) was vegetated with *Typha capensis* (Rohrb.) N.E.Br. (60% coverage), *Juncus kraussii* Hochst. (10% coverage), and *Cyperus dives* Delile (5% coverage). Variable results of pesticide–vegetation sorption can result from differences in hydraulic retention times of vegetated systems, water quality factors such as temperature, pH, and TSS, as well as the type of vegetated system being utilized (constructed wetland versus vegetated filter strip).

When water was present at all sampled sites (inlet, midway, and outlet) within the ditch site, 52% of the measured chlorpyrifos mass was associated with the water column, whereas 16% was associated with sediment. The remaining 32% of measured chlorpyrifos mass was associated with ditch plant material. With regard to plant versus soil sorption, Rogers and Stringfellow [27] reported chlorpyrifos sorption to whole plant stems was 10 times higher than to soil. Their study used batch equilibrium laboratory exposures of either chlorpyrifos plus sediment or chlorpyrifos plus plant stems instead of in situ field collections, as in the present study. In addition, Rogers and Stringfellow [27] had a considerably longer exposure period than the present study. Of the measured masses in the present ditch study, 26% were located at the inlet of the ditch, 34% were in the middle of the ditch, and 40% were from samples near the outlet. After draining, only 1% of measured chlorpyrifos mass was found in the inlet (84% in sediment; 16% in plants). Samples collected in the middle of the ditch held 8% of the measured mass (34% in sediment; 66% in plants). The majority (91%) was located at the outlet of the ditch (96% in sediment; 4% in plants). By examining the estimated mass balance of chlorpyrifos in the ditch, there was a 19% reduction between the inlet (4.45 g) and the outlet (3.63 g).

Tomato field ditch

Mean density of *L. triticoides* in plots were 358 ± 40 plants/m^2, with a 50 ± 22% mean cover. No *C. praegracilis* plugs survived into the irrigation season. By mid-July, the originally bare buffers now had *Echinocloa crus-galli* (L.) Beauv at a mean density of 1 ± 1 plants/m^2, and a 1 ± 1% mean cover.

Ditch pH values were slightly more alkaline than that of the alfalfa field ditch (mean 8.2 ± 0.27) for the 6 h event duration. At acidic and neutral pH ranges, permethrin is relatively stable; however, hydrolysis begins slowly when alkaline conditions are present [28]. Mean ± SE values for temperature, dissolved oxygen, and conductivity were 31.5 ± 3.0°C, 6.5 ± 0.8 mg/L, and 380 ± 35 μS/cm, respectively. Sharom and Solomon [29] reported temperature, pH, and microorganisms can influence permethrin's rate of chemical degradation in aqueous systems. In their laboratory studies, when incubated at 30°C, the initial rate of permethrin degradation was more rapid than at 15°C [29]. In the present study, a 35% reduction in TSS concentration overall from inlet to outlet was observed, with maximum inflow TSS of 566 mg/L and maximum outflow of 146 mg/L. Permethrin, as with many of the synthetic pyrethroids, has an affinity for suspended solids and dissolved organic matter present in runoff water [30,31]. With relatively high TSS concentrations present in the tomato field runoff, it is expected that much of the permethrin would be bound to such material. This would also affect the pesticide's bioavailability to aquatic organisms [31]. The mean flow rate at the top (inlet) of the ditch sampling area was estimated to be 8.9 ± 2.9 L/s, while mean flow rate at the ditch outlet was 9.5 ± 3.1 L/s.

Inflow aqueous permethrin concentrations from irrigation runoff were variable, ranging from 0.23 μg/L (415 min) to a maximum of 0.67 μg/L at the initial (time 0) sample (Fig. 3). Outflow permethrin concentrations at the bottom of the ditch ranged from below detection (1380 min) to a maximum of 0.26 μg/L (60 min). A statistically significant difference ($\alpha = 0.05$) existed between the mean permethrin concentration at the top of the ditch (0.67 μg/L) versus the mean permethrin concentration at the bottom of the ditch (0.26 μg/L). This represented a 62% mean reduction in aqueous concentration from top to bottom. When water was present at all sampled sites (inlet, middle, and outlet) within the ditch, 18% of the measured permethrin mass was associated with the water column, while 67% of the measured permethrin mass was associated with sediment. Based on its chemical profile, permethrin is expected

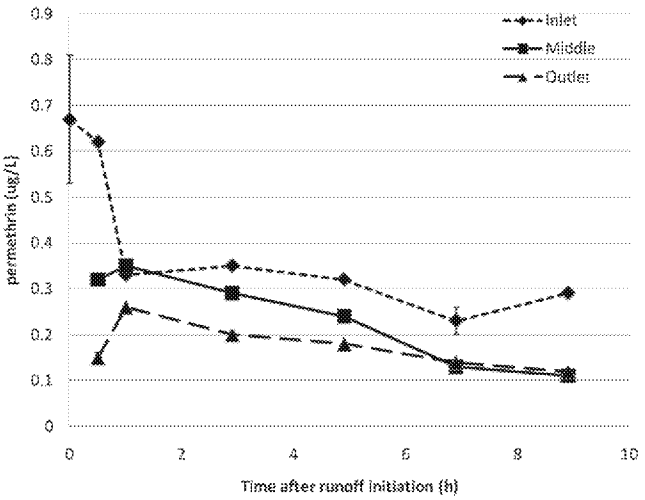


Fig. 3. Mean aqueous permethrin concentration (μg/L) at ditch inlet, middle, and outlet for a 6-h tomato irrigation event.

to prefer sorption to sediment or other forms of organic carbon. The remaining 15% of measured permethrin mass was associated with ditch plant material. As with chlorpyrifos, mitigation of permethrin by use of vegetation has been documented. Schmitt et al. [32] reported that filter strips comprising trees, shrubs, and grasses at widths of 7.5 m and 15 m reduced permethrin-associated contaminants by 27 to 83%. Plant-specific mitigation of *cis*-permethrin in aquatic mesocosms ranged from 67% reduction with *Typha latifolia* L. to 71% reduction with *Leersia oryzoides* (L.) Sw. [33]. Within this same study, however, there was no significant difference in permethrin mass mitigation between different plant species and the unvegetated control [33]. The short hydraulic retention time (4 h) was likely the main factor for this lack of discrepancy. In larger-scale studies from Yolo County, California, Moore et al. [16] reported that permethrin half-distances in vegetated drainage ditches were 21 to 22 m, whereas unvegetated drainage ditches had a half-distance of 50 to 55 m. Inflow sediment and plant permethrin concentrations ranged from below detection to 110.4 μg/kg and 489 to 1232 μg/kg, respectively. Sediment and plant concentrations in the middle sampling location ranged from below detection to 77.2 μg/kg and 58.9 to 463 μg/kg, respectively. Outflow sediment and plant permethrin concentrations ranged from below detection to 12.3 μg/kg and 38.9 to 216 μg/kg, respectively.

Of the measured permethrin masses in the present study, 13% were located at the inlet of the ditch, 33% were in the middle of the ditch, and 54% were from samples at the end of the ditch. After water had drained out of the ditch, only 9% of measured permethrin mass was in the inlet of the ditch (92% in sediment; 8% in plants). Samples collected in the middle of the ditch held 69% of the measured mass (94% sediment; 6% in plants). The 22% remainder was located at the outlet of the ditch (98% in sediment; 2% in plants). The estimated permethrin mass balance indicated a 44% reduction from ditch inlet (0.091 g) to the outlet (0.051 g). With the increased TSS concentrations, mass percentages of measured permethrin indicate a significant portion of the pyrethroid likely sorbed to the TSS and settled out in the sediments as they traveled through the ditch.

CONCLUSION

Results of the present study demonstrate the ability of VADDs to assist in pesticide mitigation of irrigation return flows. Chlorpyrifos concentrations were decreased 20% from inflow to outflow, whereas permethrin concentrations were decreased 63% from inflow to outflow. This translated into a 19% mass reduction of chlorpyrifos and a 44% mass reduction of permethrin from ditch inflow to outflow. Chemical structure, presence of TSS, and microbial activity are just some potential reasons permethrin was decreased by a greater extent than chlorpyrifos. Mean chlorpyrifos concentration in water leaving the alfalfa field ditch was 2.75 μg/L, which is still above the U.S. EPA aquatic life acute criteria of 0.083 μg/L ([34]; http://www.epa.gov/oppefed1/ecorisk_ders/aquatic_life_benchmark.htm). In the tomato field ditch, mean permethrin concentration in outflow water was 0.26 μg/L. This value falls within the acceptable range of U.S. EPA's aquatic life benchmark for fish (acute); however, it is still above the acute aquatic life benchmark for invertebrates [34]. It is imperative to remember that no single management practice is continuously 100% effective. Using suites of in-field (no-till, reduced till, cover crops, etc.) and edge-of-field (vegetated drainage ditches, stiff grass hedges, conservation buffers, etc.) management practices, landowners will have more effective overall control of agricultural runoff. Future research should examine impacts of multiple management practices, while paying special attention to the microbial aspect of pesticide degradation.

The California Department of Water Resources reports that 50% of the state's irrigated crops uses surface irrigation (flood and furrow water application) systems, which results in surface return flows that contain nutrients and chemicals [35]. Although the use of flood and furrow systems is decreasing, the degradation of surface and ground water by irrigation return flows is well documented, and the federal and state governments are implementing programs to reduce future impacts. Implementation of VADDs to reduce nutrients and chemicals in return flows will become an effective tool in these programs.

As a result of this field study, the USDA Natural Resource Conservation Service state office in California made VADDs a cost-share practice under the Environmental Quality Incentives Program (EQIP). Under EQIP, farmers can receive up to 75% cost-sharing for installing conservation practices that improve or benefit natural resource conditions. Evaluation and approval by the state office was obtained in 2008, and the practice is now incorporated in the electronic field office technical guide for California. The practice standard for VADDs was categorized under the current practice standard 607 (surface drain, field ditch).

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Message

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Sent: 4/16/2018 1:22:50 PM
To: Panger, Melissa [Panger.Melissa@epa.gov]; 'Anderson, Brian' [anderson.brian@epa.gov]; Peck, Charles [Peck.Charles@epa.gov]; Rossmeisl, Colleen (Rossmeisl.Colleen@epa.gov) [Rossmeisl.Colleen@epa.gov]; Connolly, Jennifer [Connolly.Jennifer@epa.gov]; Lennartz, Steven [Lennartz.Steven@epa.gov]; Perry, Tracy [Perry.Tracy@epa.gov]; White, Katrina [White.Katrina@epa.gov]; Wendel, Christina [Wendel.Christina@epa.gov]; Blankinship, Amy [Blankinship.Amy@epa.gov]; Donovan, Elizabeth [Donovan.Elizabeth@epa.gov]; Harwood, Douglas [harwood.douglas@epa.gov]
Subject: FW: Usage Workgroup Action Items - NMFS' questions and concerns
Attachments: 4-13-18 NASS Agricultural Chemical Use Surveys- NMFS questions.docx; 4-13-18 EPA usage estimates-NMFS questions and concerns.docx; 4-13-18 EPA surrogacy proposal-NMFS questions and concerns.docx

FYI

From: Tony Hawkes - NOAA Federal [mailto:tony.hawkes@noaa.gov]
Sent: Friday, April 13, 2018 7:05 PM
To: Stilson, Ashley <ashley_stilson@fws.gov>
Cc: Sims, Diann <Sims.Diann@epa.gov>; Nancy Golden <nancy_golden@fws.gov>; Becker, Jonathan <Becker.Jonathan@epa.gov>; David Baldwin <david.baldwin@noaa.gov>; cathy.tortorici@noaa.gov; Echeverria, Marietta <Echeverria.Marietta@epa.gov>; Hill2, Elizabeth - ARS <elizabeth.hill2@ars.usda.gov>; Paisley-Jones, Claire <Paisley-Jones.Claire@epa.gov>; Miller, Wynne <Miller.Wynne@epa.gov>; david epstein <David.Epstein@ARS.USDA.GOV>; henry_schlitzer@fws.gov; Garber, Kristina <Garber.Kristina@epa.gov>; George Noguchi <george_noguchi@fws.gov>; clayton.myers@ars.usda.gov; Omar, Sara <sara_omar@ios.doi.gov>; Shultz, Gina <gina_shultz@fws.gov>; Kunickis, Sheryl <sheryl.kunickis@osec.usda.gov>; Scott Hecht <Scott.Hecht@noaa.gov>; Thomas Hooper - NOAA Federal <thomas.hooper@noaa.gov>; Ryan DeWitt <ryan.dewitt@noaa.gov>
Subject: Usage Workgroup Action Items - NMFS' questions and concerns

Attached please find documents outlining NMFS' questions and concerns regarding A) the NASS chemical use surveys, B) EPA-BEAD's pesticide usage reports, and C) EPA's proposal for using surrogates to fill pesticide usage information gaps. In addition to these specific concerns, NMFS also has the following overarching concerns:

Deliberative Process / Ex. 5

Message

From: Garber, Kristina [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=C4FBFBF6569041F4BD559765E027AA31-KRISTINA GARBER]
Sent: 4/4/2018 8:07:36 PM
To: Echeverria, Marietta [Echeverria.Marietta@epa.gov]; 'Anderson, Brian' [anderson.brian@epa.gov]; Panger, Melissa [Panger.Melissa@epa.gov]
Subject: FW: Weinberg's filing on NMFS BiOp
Attachments: MANA v. NMFS Complaint as Filed.pdf

This filing mentions usage data ("use volumes"):

FACTS GIVING RISE TO PLAINTIFFS' CAUSE OF ACTION:
29. The annual use volume of the products at issue in this case had been substantially reduced by 2017 compared to prior years. Those usage volumes cannot be expected substantially to increase. The 2017 Biological Opinion was required to recognize and consider the implications and impacts of the changes in uses, application methods, and use volumes. Determination of the baseline for analysis of the potential ecological impacts considered in the 2017 Biological Opinion required NMFS to evaluate the implications of the fact that products containing these active ingredients had been used for many previous years in volumes considerably higher than those consistent with current registrations; had been used for a broader range of applications; and had been applied using methods no longer permitted. NMFS was required to evaluate current and potential future impacts against a historic baseline that recognized these facts. (p. 11)

From: Sankula, Sujatha
Sent: Wednesday, April 04, 2018 3:42 PM
To: Garber, Kristina <Garber.Kristina@epa.gov>
Subject: FW: Weinberg's filing on NMFS BiOp

FYI

From: Echeverria, Marietta
Sent: Wednesday, April 04, 2018 3:03 PM
To: OPP EFED Managers <OPP_EFED_Managers@epa.gov>
Subject: FW: Weinberg's filing on NMFS BiOp

From: Knorr, Michele
Sent: Wednesday, April 04, 2018 11:59 AM
To: Baptist, Erik <Baptist.Erik@epa.gov>; Perlis, Robert <Perlis.Robert@epa.gov>; Beck, Nancy <Beck.Nancy@epa.gov>; Keigwin, Richard <Keigwin.Richard@epa.gov>; Echeverria, Marietta <Echeverria.Marietta@epa.gov>; Bertrand, Charlotte <Bertrand.Charlotte@epa.gov>
Cc: Grant, Brian <Grant.Brian@epa.gov>
Subject: Weinberg's filing on NMFS BiOp

Courtesy copy.

Michele L. Knorr
Attorney

Pesticides and Toxics Law Office
Office of General Counsel
202-564-5631

Message

From: Garber, Kristina [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=C4FBFBF6569041F4BD559765E027AA31-KRISTINA GARBER]
Sent: 4/9/2018 3:53:58 PM
To: Peck, Charles [Peck.Charles@epa.gov]; Panger, Melissa [Panger.Melissa@epa.gov]; Eckel, William [Eckel.William@epa.gov]; Blankinship, Amy [Blankinship.Amy@epa.gov]; Connolly, Jennifer [Connolly.Jennifer@epa.gov]; Rossmeisl, Colleen (Rossmeisl.Colleen@epa.gov) [Rossmeisl.Colleen@epa.gov]; Donovan, Elizabeth [Donovan.Elizabeth@epa.gov]; 'Anderson, Brian' [anderson.brian@epa.gov]; Lennartz, Steven [Lennartz.Steven@epa.gov]; White, Katrina [White.Katrina@epa.gov]; Harwood, Douglas [harwood.douglas@epa.gov]; Wendel, Christina [Wendel.Christina@epa.gov]
Subject: FW: Some answers.....
Attachments: Example of Weighting.xlsx

FYI

From: Becker, Jonathan
Sent: Monday, April 09, 2018 11:51 AM
To: Garber, Kristina <Garber.Kristina@epa.gov>; Echeverria, Marietta <Echeverria.Marietta@epa.gov>; Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>; Perry, Tracy <Perry.Tracy@epa.gov>
Cc: Miller, Wynne <Miller.Wynne@epa.gov>; Sims, Diann <Sims.Diann@epa.gov>; Paisley-Jones, Claire <Paisley-Jones.Claire@epa.gov>; Suarez, Mark <Suarez.Mark@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; White, Katrina <White.Katrina@epa.gov>
Subject: Some answers.....

Hi,

I'm still working to revised the draft on surrogate usage and it's taking longer than I thought. Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Jonathan

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Message

From: Garber, Kristina [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=C4FBFBF6569041F4BD559765E027AA31-KRISTINA GARBER]
Sent: 3/8/2018 1:44:04 PM
To: Echeverria, Marietta [Echeverria.Marietta@epa.gov]; 'Anderson, Brian' [anderson.brian@epa.gov]
Subject: FW: NMFS questions
Attachments: NMFS GfK request_OPP_response.docx

FYI: here are questions posed previously by NMFS on usage data (from a year or two ago)

From: Becker, Jonathan
Sent: Wednesday, March 07, 2018 2:58 PM
To: Garber, Kristina <Garber.Kristina@epa.gov>
Subject: NMFS questions

Hi Kris,

I think these are the questions that Tony sent with our responses. I can't swear that this is the final version that we sent out, but it's pretty close.

Jonathan

Message

From: Garber, Kristina [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=C4FBFBF6569041F4BD559765E027AA31-KRISTINA GARBER]
Sent: 2/28/2018 8:58:35 PM
To: White, Katrina [White.Katrina@epa.gov]
Subject: RE: Notes from ESA use workshop attached and final charge

Thank you for the detailed notes!

From: White, Katrina
Sent: Tuesday, February 27, 2018 11:13 AM
To: Anderson, Brian <Anderson.Brian@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>; Connolly, Jennifer <Connolly.Jennifer@epa.gov>; Lennartz, Steven <Lennartz.Steven@epa.gov>; Eckel, William <Eckel.William@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Donovan, Elizabeth <Donovan.Elizabeth@epa.gov>
Cc: Holmes, Jean <Holmes.Jean@epa.gov>; Harwood, Douglas <harwood.douglas@epa.gov>; Mastrota, Nicholas <Mastrota.Nicholas@epa.gov>; Blankinship, Amy <Blankinship.Amy@epa.gov>; Lin, James <lin.james@epa.gov>; Perry, Tracy <Perry.Tracy@epa.gov>
Subject: Notes from ESA use workshop attached and final charge

Attached are notes from the ESA workshop and the final charge below. Please pass this on to anyone I missed, who should receive these. Chuck's shorter version of the notes are at the bottom of the document.

PESTICIDE USAGE WORK GROUP (2/26/18)

Form working group with decision-makers and experts from each agency to find a path forward; decisions will be elevated to the policy team members as needed.
Team members and a team lead will be identified by this Thursday.
Check in on methodologies and next steps in 2 weeks.
EPA will provide the malathion agricultural and non-agricultural usage report by March 31.

CHARGE:
The group will determine where malathion is likely to be applied, consistent with the best available scientific and commercial data, and how those data can be incorporated into the risk assessment method.

- Use the most granular data available.
- Explain the limitations to providing more granular data as needed.
- Include assumptions about the data used to determine areas where usage is likely.
- Step 1: Come up with a timeline and tasks for developing a methodology.

Katrina White
Senior Fate Scientist
Environmental Risk Branch IV
Environmental Fate & Effects Division

703-308-4536
White.katrina@epa.gov

Message

From: Garber, Kristina [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=C4FBFBF6569041F4BD559765E027AA31-KRISTINA GARBER]
Sent: 2/23/2018 10:22:46 PM
To: Garber, Kristina [Garber.Kristina@epa.gov]
Subject: back up of slides
Attachments: butterfly_overlap.xlsx; caracara_overlap.xlsx; Applying_usage_2-23-18_draft2.pptx; caracara_example.pptx

Kris Garber, Senior Science Advisor
Environmental Fate and Effects Division
Office of Pesticide Programs
US Environmental Protection Agency
Mail Code: 7507P
1200 Pennsylvania Ave NW
Washington DC 20460-0001

Phone: 703-347-8940

PESTICIDE USAGE MEETING

How to apply usage data to the risk assessment

EPA OPP EFED
KRIS GARBER

deliberative, for inter-Agency discussion

Outline

- Background and objective
- Description of an approach for incorporating usage data at the species range level
  - Deliberative, idea in progress
- An example to illustrate the approach

ED_002896_00041475-00002

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Message

From: Garber, Kristina [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=C4FBFBF6569041F4BD559765E027AA31-KRISTINA GARBER]
Sent: 2/23/2018 5:17:50 PM
To: Panger, Melissa [Panger.Melissa@epa.gov]; Paisley-Jones, Claire [Paisley-Jones.Claire@epa.gov]
Subject: FW: final drafts of usage meeting slide decks
Attachments: Applying_usage_2-23-18_draft.pptx; Increasing our understanding of geographic specificity_2-23-18_draft.pptx; Pesticide Usage Data Sources Overview_2-23-18_draft.pptx

Melissa and Claire,

When I moved our slides into the EPA template for PPT, the format was modified on most of the slides. I did my best to resize and adjust the contents of each slide. Just want to give you a heads up. You may want to reformat some of the slides based on your preferences.

I hope that this doesn't cause any inconvenience to you.

Kris

From: Garber, Kristina
Sent: Friday, February 23, 2018 12:14 PM
To: Echeverria, Marietta <Echeverria.Marietta@epa.gov>
Cc: Panger, Melissa <Panger.Melissa@epa.gov>; Paisley-Jones, Claire <Paisley-Jones.Claire@epa.gov>; 'Anderson, Brian' <anderson.brian@epa.gov>
Subject: final drafts of usage meeting slide decks

Hi Marietta,

Attached are the final drafts of the presentations that Melissa, Claire and I will be giving at next Monday's meeting with the services. I modified all three slide decks so that they are in the "official" EPA format for PPT. These slides are ready for Nancy Beck's review.

Thanks,
Kris

Kris Garber, Senior Science Advisor
Environmental Fate and Effects Division
Office of Pesticide Programs
US Environmental Protection Agency
Mail Code: 7507P
1200 Pennsylvania Ave NW
Washington DC 20460-0001

Phone: 703-347-8940

Message

From: Garber, Kristina [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=C4FBFBF6569041F4BD559765E027AA31-KRISTINA GARBER]
Sent: 2/22/2018 4:58:20 PM
To: Panger, Melissa [Panger.Melissa@epa.gov]; Connolly, Jennifer [Connolly.Jennifer@epa.gov]
Subject: FW: couple of thoughts
Attachments: Applying_usage_2-22-18draft4.pptx

FYI. **Deliberative Process / Ex. 5**

From: Garber, Kristina
Sent: Thursday, February 22, 2018 11:54 AM
To: Anderson, Brian <Anderson.Brian@epa.gov>
Subject: RE: couple of thoughts

Here's the revised slide deck. Please distribute to Marietta and the other dds.

From: Anderson, Brian
Sent: Thursday, February 22, 2018 11:42 AM
To: Garber, Kristina <Garber.Kristina@epa.gov>
Subject: couple of thoughts

Hi Kris,

I talked with Marietta in the break room a bit after the briefing – a couple of suggestions.

Deliberative Process / Ex. 5

I think she wanted to talk through a couple of questions on the slides a little bit after the 1:00 meeting if we can?

Does that sound ok? If not, please let me know.

Thanks

Brian

Message

From: Garber, Kristina [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=C4FBFBF6569041F4BD559765E027AA31-KRISTINA GARBER]
Sent: 2/22/2018 1:25:44 PM
To: White, Katrina [White.Katrina@epa.gov]; Rossmeisl, Colleen [Rossmeisl.Colleen@epa.gov]
CC: Connolly, Jennifer [Connolly.Jennifer@epa.gov]
Subject: RE: Diazinon aerial use on lettuce

Thank you.

-----Original Message-----
From: White, Katrina
Sent: Thursday, February 22, 2018 8:19 AM
To: Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>
Cc: Connolly, Jennifer <Connolly.Jennifer@epa.gov>
Subject: RE: Diazinon aerial use on lettuce

Wanted to make you aware, although I am sure you already are. Josh and I pulled the CAPUR data and say that usage increased for diazinon in CA between 2014 to 2015. The middle row is pounds diazinon applied.

Grand Total	109247.3723	2015
Grand Total	61126.00208	2014
Grand Total	61224.15479	2013
Grand Total	78524.44429	2012
Grand Total	86646.98686	2011
Grand Total	142060.7126	2010
Grand Total	142060.7126	2009
Grand Total	258533.51	2008
Grand Total	354440.9509	2007
Grand Total	385833.0292	2006
Grand Total	403958.7223	2005

Katrina White
Senior Fate Scientist
Environmental Risk Branch IV
Environmental Fate & Effects Division

703-308-4536
White.katrina@epa.gov

-----Original Message-----
From: Rossmeisl, Colleen
Sent: Thursday, February 22, 2018 8:09 AM
To: Garber, Kristina <Garber.Kristina@epa.gov>; White, Katrina <White.Katrina@epa.gov>
Cc: Connolly, Jennifer <Connolly.Jennifer@epa.gov>
Subject: RE: Diazinon aerial use on lettuce

Deliberative Process / Ex. 5

But maybe there is more info that isn't listed here (but this looks pretty comprehensive).

-----Original Message-----
From: Garber, Kristina
Sent: Thursday, February 22, 2018 7:58 AM
To: White, Katrina <White.Katrina@epa.gov>
Cc: Connolly, Jennifer <Connolly.Jennifer@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>
Subject: Diazinon aerial use on lettuce

Hi Katrina,

Deliberative Process / Ex. 5

Thanks!
Kris

Message

From: Garber, Kristina [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=C4FBFBF6569041F4BD559765E027AA31-KRISTINA GARBER]
Sent: 2/22/2018 1:24:52 PM
To: White, Katrina [White.Katrina@epa.gov]; Rossmeisl, Colleen [Rossmeisl.Colleen@epa.gov]
CC: Connolly, Jennifer [Connolly.Jennifer@epa.gov]
Subject: RE: Diazinon aerial use on lettuce

Thank you. This is helpful.

Deliberative Process / Ex. 5

-----Original Message-----
From: White, Katrina
Sent: Thursday, February 22, 2018 8:17 AM
To: Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>
Cc: Connolly, Jennifer <Connolly.Jennifer@epa.gov>
Subject: RE: Diazinon aerial use on lettuce

Deliberative Process / Ex. 5

Katrina White
Senior Fate Scientist
Environmental Risk Branch IV
Environmental Fate & Effects Division

703-308-4536
White.katrina@epa.gov

-----Original Message-----
From: Rossmeisl, Colleen
Sent: Thursday, February 22, 2018 8:09 AM
To: Garber, Kristina <Garber.Kristina@epa.gov>; White, Katrina <White.Katrina@epa.gov>
Cc: Connolly, Jennifer <Connolly.Jennifer@epa.gov>
Subject: RE: Diazinon aerial use on lettuce

Deliberative Process / Ex. 5

But maybe there is more info that isn't listed here (but this looks pretty comprehensive).

-----Original Message-----
From: Garber, Kristina
Sent: Thursday, February 22, 2018 7:58 AM
To: White, Katrina <White.Katrina@epa.gov>
Cc: Connolly, Jennifer <Connolly.Jennifer@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>
Subject: Diazinon aerial use on lettuce

Hi Katrina,

As you know, aerial applications of diazinon can only be made to lettuce. In the back of my brain, I have a foggy memory of a geographic restriction to the aerial apps (CA?). Is there a geo restriction?

Thanks!
Kris

Message

From: Garber, Kristina [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=C4FBFBF6569041F4BD559765E027AA31-KRISTINA GARBER]
Sent: 2/22/2018 12:31:35 PM
To: Panger, Melissa [Panger.Melissa@epa.gov]
Subject: Re: BEAD Response to FWS questions regarding usage data

Yes!

On Feb 22, 2018, at 7:29 AM, Panger, Melissa <Panger.Melissa@epa.gov> wrote:

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Melissa Panger, Ph.D.
Senior Advisor, ERB2
Environmental Fate and Effects Division
Office of Pesticide Programs
USEPA

703-305-6136
panger.melissa@epa.gov

From: Garber, Kristina
Sent: Thursday, February 22, 2018 7:26 AM
To: Anderson, Brian <Anderson.Brian@epa.gov>
Cc: Panger, Melissa <Panger.Melissa@epa.gov>; Sims, Diann <Sims.Diann@epa.gov>
Subject: Re: BEAD Response to FWS questions regarding usage data

Deliberative Process / Ex. 5

On Feb 22, 2018, at 7:18 AM, Anderson, Brian <Anderson.Brian@epa.gov> wrote:

> Glad you saw that. Diann asked me about that yesterday, I was thinking it was just to address during the call. I didn't think about it in terms of the written response.
>
> So in short- no one right now – but what about the proposed below in yellow?

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

From: Garber, Kristina
Sent: Thursday, February 22, 2018 6:58 AM
To: Anderson, Brian <Anderson.Brian@epa.gov>
Cc: Panger, Melissa <Panger.Melissa@epa.gov>; Sims, Diann <Sims.Diann@epa.gov>
Subject: Fwd: BEAD Response to FWS questions regarding usage data

Brian,

Who in EFED is working on question 4?

Kris

Begin forwarded message:

> **From:** "Sims, Diann" <Sims.Diann@epa.gov>
> **Date:** February 21, 2018 at 4:21:07 PM EST
> **To:** Patrice Ashfield <patrice_ashfield@fws.gov>
> **Cc:** "Paisley-Jones, Claire" <Paisley-Jones.Claire@epa.gov>, "Becker, Jonathan" <Becker.Jonathan@epa.gov>, "Anderson, Brian" <Anderson.Brian@epa.gov>
> **Subject: BEAD Response to FWS questions regarding usage data**
>
> Hello Patrice,
> Thank you for speaking with us earlier today. As agreed, I am sharing our informal response to your data usage questions. We are also providing an update to the example spreadsheet that we provided earlier this morning. I hope that you find the information helpful in preparing for your presentation. If you have additional questions, please do not hesitate to contact me.

Message

From: Garber, Kristina [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=C4FBFBF6569041F4BD559765E027AA31-KRISTINA GARBER]
Sent: 2/21/2018 11:37:51 PM
To: Connolly, Jennifer [Connolly.Jennifer@epa.gov]
Subject: current draft of slide deck for you to comment on
Attachments: Applying_usage_2-21-18draft2.pptx

Kris Garber, Senior Science Advisor
Environmental Fate and Effects Division
Office of Pesticide Programs
US Environmental Protection Agency
Mail Code: 7507P
1200 Pennsylvania Ave NW
Washington DC 20460-0001

Phone: 703-347-8940

Applying agricultural usage data the species range

Pesticide Usage Meeting

Draft

2/21/18

What is our objective?

To determine the total number of acres that are treated on a given year that may fall within a species range

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

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Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Message

From: Garber, Kristina [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=C4FBFBF6569041F4BD559765E027AA31-KRISTINA GARBER]
Sent: 2/20/2018 12:05:01 PM
To: Rossmeisl, Colleen [Rossmeisl.Colleen@epa.gov]
Subject: Re: summary - part two.

Sorry for being unclear. I was just responding to the email chain. The question was for Brian

On Feb 20, 2018, at 7:02 AM, Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov> wrote:

> Was that question for me? Sorry, I wasn't suggesting we need another write up. Or were you asking Brian why we were writing it up again?
>
> **From:** Garber, Kristina
> **Sent:** Tuesday, February 20, 2018 6:54 AM
> **To:** Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>
> **Cc:** Anderson, Brian <Anderson.Brian@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Lennartz, Steven <Lennartz.Steven@epa.gov>; Connolly, Jennifer <Connolly.Jennifer@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Eckel, William <Eckel.William@epa.gov>
> **Subject:** Re: summary - part two.
>
> **Deliberative Process / Ex. 5**
>
> Why do we need another write up?
>
> On Feb 20, 2018, at 6:33 AM, Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov> wrote:
>
> **Deliberative Process / Ex. 5**
>
> Thanks!
> Colleen
>
> **From:** Anderson, Brian
> **Sent:** Friday, February 16, 2018 1:30 PM
> **To:** Panger, Melissa <Panger.Melissa@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>; Lennartz, Steven <Lennartz.Steven@epa.gov>; Connolly, Jennifer <Connolly.Jennifer@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>; Eckel, William <Eckel.William@epa.gov>
> **Subject:** RE: summary - part two.

Just got make sure that was everyone's understanding and that BEAD can get us all the acres from their data sources on the crops within the CDL.

Thanks guys,

Brian

From: Panger, Melissa
Sent: Friday, February 16, 2018 12:45 PM
To: Anderson, Brian <Anderson.Brian@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>; Lennartz, Steven <Lennartz.Steven@epa.gov>; Connolly, Jennifer <Connolly.Jennifer@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>; Eckel, William <Eckel.William@epa.gov>
Subject: RE: summary - part two.

Clap… clap… clap! I think we're getting there. A few minor clarifications (see green text below):

From: Anderson, Brian
Sent: Friday, February 16, 2018 11:56 AM
To: Panger, Melissa <Panger.Melissa@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>; Lennartz, Steven <Lennartz.Steven@epa.gov>; Connolly, Jennifer <Connolly.Jennifer@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>; Eckel, William <Eckel.William@epa.gov>
Subject: summary - part two.

Just had a conversation with Steve and Mel - It seems to jive with what I heard at the Tuesday meeting with Bead – parts of what I was just overhearing. I just want to summarize for the group and make sure it's consistent with our plan. Is it?

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Message

From: Garber, Kristina [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=C4FBFBF6569041F4BD559765E027AA31-KRISTINA GARBER]
Sent: 2/12/2018 8:44:55 PM
To: Connolly, Jennifer [Connolly.Jennifer@epa.gov]
CC: Panger, Melissa [Panger.Melissa@epa.gov]
Subject: revised "map" for usage slide deck
Attachments: Usage_step2_2-12-18.pptx

Jen,

Deliberative Process / Ex. 5

Kris

Kris Garber, Senior Science Advisor
Environmental Fate and Effects Division
Office of Pesticide Programs
US Environmental Protection Agency
Mail Code: 7507P
1200 Pennsylvania Ave NW
Washington DC 20460-0001

Phone: 703-347-8940

Proposed method for incorporating usage data into revised ESA method

2/13/18 DRAFT

Presentation to EFED scientists

Internal deliberative, do not cite or distribute

EFED ESA team

- Amy Blankinship
- Jen Connolly
- Elizabeth Donovan
- Bill Eckel
- Kris Garber
- Ethan Harwood
- Steve Lennartz
- Melissa Panger
- Chuck Peck
- Colleen Rossmeisl
- Christina Wendel
- Katrina White

Deliberative Process / Ex. 5

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Deliberative Process / Ex. 5

Appointment

From: Garber, Kristina [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=C4FBFBF6569041F4BD559765E027AA31-KRISTINA GARBER]
Sent: 2/14/2018 3:36:20 PM
To: Peck, Charles [Peck.Charles@epa.gov]

Subject: FW: use and usage - pre-meet with DDs to go over slides and plan
Attachments: Usage_step2_2-14-18(BEAD scientists).pptx; Why Usage Data Are Important (3).pptx
Location: DCRoomPYS10100/Potomac-Yard-One; DCRoomPYS9100/Potomac-Yard-One

Start: 2/14/2018 4:00:00 PM
End: 2/14/2018 5:00:00 PM
Show Time As: Tentative

-----Original Appointment-----
From: Anderson, Brian
Sent: Wednesday, February 07, 2018 10:02 AM
To: Anderson, Brian; Panger, Melissa; Garber, Kristina; Rossmeisl, Colleen; Connolly, Jennifer; Lennartz, Steven; Eckel, William; Sims, Diann; Becker, Jonathan; Paisley-Jones, Claire; Miller, Wynne; Myers, Clayton; Echeverria, Marietta
Cc: Suarez, Mark; Perry, Tracy; Sherman, Kelly; Schroeder, Carolyn
Subject: use and usage - pre-meet with DDs to go over slides and plan
When: Wednesday, February 14, 2018 11:00 AM-12:00 PM (UTC-05:00) Eastern Time (US & Canada).
Where: DCRoomPYS10100/Potomac-Yard-One; DCRoomPYS9100/Potomac-Yard-One

Please forward to anyone I missed

Appointment

From: Garber, Kristina [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=C4FBFBF6569041F4BD559765E027AA31-KRISTINA GARBER]
Sent: 2/14/2018 2:49:54 PM
To: Rossmeisl, Colleen [Rossmeisl.Colleen@epa.gov]; Panger, Melissa [Panger.Melissa@epa.gov]; Peck, Charles [Peck.Charles@epa.gov]; Lennartz, Steven [Lennartz.Steven@epa.gov]; Connolly, Jennifer [Connolly.Jennifer@epa.gov]; Blankinship, Amy [Blankinship.Amy@epa.gov]; Donovan, Elizabeth [Donovan.Elizabeth@epa.gov]; Eckel, William [Eckel.William@epa.gov]; White, Katrina [White.Katrina@epa.gov]; Paisley-Jones, Claire [Paisley-Jones.Claire@epa.gov]; Atwood, Donald [Atwood.Donald@epa.gov]; Sims, Diann [Sims.Diann@epa.gov]; Corbin, Mark [Corbin.Mark@epa.gov]; Anderson, Brian [anderson.brian@epa.gov]; Becker, Jonathan [Becker.Jonathan@epa.gov]; Myers, Clayton [Myers.Clayton@epa.gov]
CC: Spatz, Dana [Spatz.Dana@epa.gov]; Stebbins, Katherine [Stebbins.Katherine@epa.gov]; Jones, Arnet [Jones.Arnet@epa.gov]; Kiely, Timothy [Kiely.Timothy@epa.gov]; Kaul, Monisha [Kaul.Monisha@epa.gov]; Wyatt, TJ [Wyatt.Tj@epa.gov]; Suarez, Mark [Suarez.Mark@epa.gov]; Chism, William [Chism.Bill@epa.gov]; Berwald, Derek [Berwald.Derek@epa.gov]
BCC: DCRoomPYS7100/Potomac-Yard-One [DCRoomPYS7100@epa.gov]

Subject: discuss draft method for incorporating usage data (PCT) into ESA method
Attachments: Usage_step2_2-14-18(BEAD scientists).pptx; Methomyl SUUM.012918.Final.docx
Location: DCRoomPYS7100/Potomac-Yard-One

Start: 2/15/2018 2:00:00 PM
End: 2/15/2018 3:00:00 PM
Show Time As: Tentative

Hello all,

The ESA team has working on the refined ESA method for steps 1 and 2. The current draft of the method involves incorporating usage data (specifically percent crop treated) into the determination of whether a species is likely or not likely to be adversely affected by a pesticide. The team would like to present the draft approach involving PCT to BEAD scientists and get feedback.

Please let me know if there are other folks that should be added to this invite.

Please consider these slides an internal deliberative, do not cite or distribute.

Thanks,
Kris

Conference phone number: 1 (202) 991-0477
Conference ID: 9623976

Appointment

From: Garber, Kristina [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=C4FBFBF6569041F4BD559765E027AA31-KRISTINA GARBER]
Sent: 2/13/2018 6:47:16 PM
To: Panger, Melissa [Panger.Melissa@epa.gov]; Peck, Charles [Peck.Charles@epa.gov]; Lennartz, Steven [Lennartz.Steven@epa.gov]; Connolly, Jennifer [Connolly.Jennifer@epa.gov]; Blankinship, Amy [Blankinship.Amy@epa.gov]; Donovan, Elizabeth [Donovan.Elizabeth@epa.gov]; Eckel, William [Eckel.William@epa.gov]; White, Katrina [White.Katrina@epa.gov]; Rossmeisl, Colleen (Rossmeisl.Colleen@epa.gov) [Rossmeisl.Colleen@epa.gov]; Thurman, Nelson [Thurman.Nelson@epa.gov]; Barrett, Dena [Barrett.Dena@epa.gov]; Bohaty, Rochelle [Bohaty.Rochelle@epa.gov]; Khan, Faruque [Khan.Faruque@epa.gov]; Corbin, Mark [Corbin.Mark@epa.gov]; Anderson, Brian [anderson.brian@epa.gov]; Odenkirchen, Edward [Odenkirchen.Edward@epa.gov]; Sappington, Keith [sappington.keith@epa.gov]; Steeger, Thomas [steeger.thomas@epa.gov]
BCC: DCRoomPYS10100/Potomac-Yard-One [DCRoomPYS10100@epa.gov]

Subject: discuss draft method for incorporating usage data (PCT) into ESA method
Attachments: Methomyl SUUM.012918.Final.docx; Usage_step2_2-13-18.pptx
Location: DCRoomPYS10100/Potomac-Yard-One

Start: 2/13/2018 8:00:00 PM
End: 2/13/2018 9:00:00 PM
Show Time As: Tentative

Hello all,

The ESA team has working on the refined ESA method for steps 1 and 2. The current draft of the method involves incorporating usage data (specifically percent crop treated) into the determination of whether a species is likely or not likely to be adversely affected by a pesticide. The team would like to present the draft approach involving PCT to EFED scientists and get feedback.

Please let me know if there are other folks that should be added to this invite.

Thanks,
Kris

Conference phone number: 1 (202) 991-0477
Conference ID: 9623976

Message

From: Garber, Kristina [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=C4FBFBF6569041F4BD559765E027AA31-KRISTINA GARBER]
Sent: 1/4/2018 11:22:16 PM
To: Connolly, Jennifer [Connolly.Jennifer@epa.gov]
Subject: RE: ESA overlap and use layer information

Nonresponsive Discussion of Personal Matters / Ex. 6

From: Connolly, Jennifer
Sent: Thursday, January 04, 2018 5:07 PM
To: Lennartz, Steven <Lennartz.Steven@epa.gov>
Cc: Kyle, Lee <Kyle.Lee@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>
Subject: RE: ESA overlap and use layer information

Awesome! I'll try this to see if it helps my network lag. I'm hoping to have the final table mapping out the overlap runs by tomorrow morning, if the computer gods are on my side.

Thanks everyone for being patient with me this week. **Nonresponsive Discussion of Personal Matters / Ex. 6**

Nonresponsive Discussion of Personal Matters / Ex. 6

Nonresponsive Discussion of Personal Matters / Ex. 6 Most of what needs to happen next week for the overlap is computer crunching. I'll need to generate the species composite files, once we finalize the qualitative species list, and do the restructuring of the using layers to help streamline the overlap runs. I should be able to keep this on track without too much hands-on work.

Jen

For those of you with snow envy....even with poor picture quality and a window screen

From: Lennartz, Steven
Sent: Thursday, January 04, 2018 4:24 PM
To: Connolly, Jennifer <Connolly.Jennifer@epa.gov>
Cc: Kyle, Lee <Kyle.Lee@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>; Panger, Melissa

<Panger.Melissa@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>
Subject: RE: ESA overlap and use layer information

Full delivery is up on OneDrive:

Deliberative Process / Ex. 5

-Steve

From: Connolly, Jennifer
Sent: Thursday, January 04, 2018 2:45 PM
To: Lennartz, Steven <Lennartz.Steven@epa.gov>
Cc: Kyle, Lee <Kyle.Lee@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>
Subject: RE: ESA overlap and use layer information

Thanks!

From: Lennartz, Steven
Sent: Thursday, January 04, 2018 2:01 PM
To: Connolly, Jennifer <Connolly.Jennifer@epa.gov>
Cc: Kyle, Lee <Kyle.Lee@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>
Subject: RE: ESA overlap and use layer information

The 30m resampled versions are on the P drive
\Finals\171226\Chemicals\NL48\Resample

From: Lennartz, Steven
Sent: Thursday, January 04, 2018 12:55 PM
To: Connolly, Jennifer <Connolly.Jennifer@epa.gov>
Cc: Kyle, Lee <Kyle.Lee@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>
Subject: RE: ESA overlap and use layer information

I'm rerunning the GU & VI chemical gdb's at 30m, super easy. I'll post after my 1pm meeting.

From: Connolly, Jennifer
Sent: Thursday, January 04, 2018 12:36 PM
To: Lennartz, Steven <Lennartz.Steven@epa.gov>
Cc: Kyle, Lee <Kyle.Lee@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>
Subject: RE: ESA overlap and use layer information

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Jen

From: Lennartz, Steven
Sent: Thursday, January 04, 2018 11:38 AM
To: Connolly, Jennifer <Connolly.Jennifer@epa.gov>
Cc: Kyle, Lee <Kyle.Lee@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>
Subject: RE: ESA overlap and use layer information

Deliberative Process / Ex. 5

-Steve

From: Connolly, Jennifer
Sent: Thursday, January 04, 2018 11:27 AM
To: Lennartz, Steven <Lennartz.Steven@epa.gov>
Cc: Kyle, Lee <Kyle.Lee@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>
Subject: RE: ESA overlap and use layer information

I've attached my tracking sheet for the use layers. This isn't complete yet but there are couple of things I wanted to bring up soon rather than later.

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Jen

From: Lennartz, Steven
Sent: Thursday, January 04, 2018 9:41 AM
To: Connolly, Jennifer <Connolly.Jennifer@epa.gov>
Cc: Kyle, Lee <Kyle.Lee@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>
Subject: RE: ESA overlap and use layer information

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

-Steve

From: Connolly, Jennifer
Sent: Wednesday, January 03, 2018 7:02 PM
To: Lennartz, Steven <Lennartz.Steven@epa.gov>
Cc: Kyle, Lee <Kyle.Lee@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>
Subject: RE: ESA overlap and use layer information

This is great Steve, thanks!

Deliberative Process / Ex. 5

Jen

From: Lennartz, Steven
Sent: Wednesday, January 03, 2018 8:59 AM
To: Connolly, Jennifer <Connolly.Jennifer@epa.gov>
Cc: Kyle, Lee <Kyle.Lee@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>
Subject: RE: ESA overlap and use layer information

The data and documentation are complete, a slim 200GB. I've posted a line by line response to your list on SharePoint. I've copied it below, my comments are in blue.

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Steve Lennartz, *MSc, CMS-RS*
Mapping Scientist, EISB
Environmental Fate and Effects Division, PYS12923
Office of Pesticide Programs
U.S. Environmental Protection Agency
Phone: (703) 347-0145
Email: lennartz.steven@epa.gov

From: Connolly, Jennifer
Sent: Thursday, December 21, 2017 7:26 PM
To: Lennartz, Steven <Lennartz.Steven@epa.gov>
Cc: Kyle, Lee <Kyle.Lee@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>; Panger, Melissa <Panger.Melissa@epa.gov>; Peck, Charles <Peck.Charles@epa.gov>; Rossmeisl, Colleen <Rossmeisl.Colleen@epa.gov>
Subject: ESA overlap and use layer information

Hey Steve,

Here is the final list of layer and tables we've been discussing. If I'm unavailable the first week in January please cc Kris, Melissa, Chuck and Colleen as you send me finalized information. Also, please let them know if you run into any issues.

Deliberative Process / Ex. 5

Happy Holidays! And if I don't see you, good luck with the new little one!

Jen

Deliberative Process / Ex. 5

****

Jennifer Connolly, GIS Analyst
Environmental Information Support Branch

Environmental Fate and Effects Division
Office of Pesticide Programs, U.S. EPA
1200 Pennsylvania Avenue, NW (7507P)
Washington, DC 20460
phone: (703) 347-0405
fax: (703) 305-0619
e-mail: connolly.jennifer@epa.gov
****

Message

From: Garber, Kristina [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=C4FBFBF6569041F4BD559765E027AA31-KRISTINA GARBER]
Sent: 6/7/2019 8:19:10 PM
To: Garber, Kristina [Garber.Kristina@epa.gov]
Subject: RE: backup of ESA notes
Attachments: June 10th Public Meeting Slides_5.31.19(DRAFT)_kg_notes.pptx

---

From: Garber, Kristina
Sent: Friday, June 07, 2019 11:57 AM
To: Garber, Kristina <Garber.Kristina@epa.gov>
Subject: backup of ESA notes

Revised Method for ESA Biological Evaluations

Office of Pesticide Programs,
Environmental Fate and Effects Division
Biological Economic Analysis Division
June 10, 2019
DRAFT internal deliberative, do not cite or distribute

1

Today, senior scientists from EPA will provide a summary of the revised method for conducting national level, pesticide biological evaluations for listed species.

Purpose of Today's Meeting

- EPA has posted a revised method for conducting Biological Evaluations for pesticides
  https://www.epa.gov/endangered-species/draft-revised-method-national-level-endangered-species-risk-assessment-process
- EPA will provide an overview of the approach
  - EPA staff will answer clarifying questions
- The public has the opportunity to provide comments today
  - Written comments may also be submitted via the docket
  - www.regulations.gov in docket EPA-HQ-OPP-2019-0185

During today's meeting, we will discuss the revised method that was posted online at the link provided here.

The presentations from EPA staff that you are about to hear are intended to provide a summary of the revised method.

Additional details can be found in the revised methods document.

After EPA staff discuss the method, there will be time for clarifying questions from the audience.

There will also be time today for the public to provide verbal comments.

Written comments can also be submitted to the docket location on this slide.

That docket is open until July 1.

In addition to general comments on the revised method, There are 4 specific topic areas for which the EPA is soliciting public comments.

These topics are included in the federal register notice for this public comment period

The slides that we use today are already posted to the docket.

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

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Message

From: Garber, Kristina [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=C4FBFBF6569041F4BD559765E027AA31-KRISTINA GARBER]
Sent: 1/18/2018 11:04:07 PM
To: Panger, Melissa [Panger.Melissa@epa.gov]
Subject: RE: Revised slides
Attachments: DRAFT DD Briefing_Revised BE Approach (Jan 2018) (4) 1_18_18kg.pptx

Attached are the revised slides. Do you want to talk through these on Monday and figure out our game plan for how we are going to present?

From: Panger, Melissa
Sent: Thursday, January 18, 2018 5:35 PM
To: Garber, Kristina <Garber.Kristina@epa.gov>
Subject: Re: Revised slides

Great! I was just thinking.. we should add that the current process is not sustainable (resources) on the 'current process limitations' slide... if you don't get to it, I can add it tomorrow.

Thanks!

Sent from my iPhone

On Jan 18, 2018, at 4:04 PM, Garber, Kristina <Garber.Kristina@epa.gov> wrote:

> Thanks. I will work on the slides and send back to you tonight.
>
> ---
>
> **From:** Panger, Melissa
> **Sent:** Thursday, January 18, 2018 3:47 PM
> **To:** Garber, Kristina <Garber.Kristina@epa.gov>
> **Subject:** Revised slides
>
> Here are the revised slides… the only thing that needs to be added is the info on slide 37 (I ran out of time… I have to leave a bit early today). Slide 50 could use some added input, too. Otherwise, I think it's just reviewing the red text.
>
> I'm working tomorrow… so if you could get these back to me before you leave today, I'll be able to get them back to Brian tomorrow.
>
> They are looking good! Thanks!
> Melissa
>
> ---
>
> Melissa Panger, Ph.D.
> Senior Advisor, ERB2
> Environmental Fate and Effects Division
> Office of Pesticide Programs
> USEPA

703-305-6136
panger.melissa@epa.gov

Revised ESA Biological Evaluation Approach

January 23, 2018

Overview

- Background
- Overview of the revised BE process
- Differences among the ESA approaches
- Step 3 case study
- Timeline

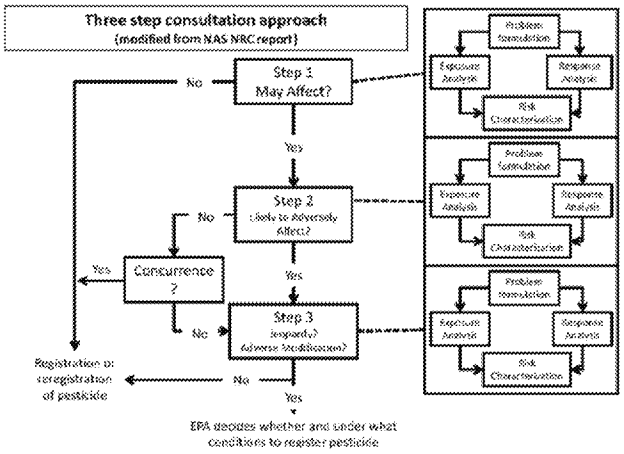
Background

BE Approach

Based on recommendations by the National Research Council (2013)

Three steps process with each step relying upon ecological risk assessment framework

- Steps 1 and 2 = Biological Evaluation (BE)
- Step 3 = Biological Opinion (BO)
- All three steps rely upon the same data sets, with different applications
- Methods used by each step differ in complexity and level of effort, with step 1 being the simplest and fastest and step 3 requiring more species, specific, complex and time intensive analyses



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Message

From: Garber, Kristina [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=C4FBFBF6569041F4BD559765E027AA31-KRISTINA GARBER]
Sent: 6/7/2019 3:57:12 PM
To: Garber, Kristina [Garber.Kristina@epa.gov]
Subject: backup of ESA notes
Attachments: June 10th Public Meeting Slides_5.31.19(DRAFT)_kg_notes.pptx

Revised Method for ESA Biological Evaluations

Office of Pesticide Programs,
Environmental Fate and Effects Division
Biological Economic Analysis Division
June 10, 2019
DRAFT internal deliberative, do not cite or distribute

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Today, senior scientists from EPA will provide a summary of the revised method for conducting national level, pesticide biological evaluations for listed species.

Purpose of Today's Meeting

- EPA has posted a revised method for conducting Biological Evaluations for pesticides
  https://www.epa.gov/endangered-species/draft-revised-method-national-level-endangered-species-risk-assessment-process
- EPA will provide an overview of the approach
  - EPA staff will answer clarifying questions
- The public has the opportunity to provide comments today
  - Written comments may also be submitted via the docket
  - www.regulations.gov in docket EPA-HQ-OPP-2019-0185

During today's meeting, we will discuss the revised method that was posted online at the link provided here.

The presentations from EPA staff that you are about to hear are intended to provide a summary of the revised method.

Additional details can be found in the revised methods document.

After EPA staff discuss the method, there will be time for clarifying questions from the audience.

There will also be time today for the public to provide verbal comments.

Written comments can also be submitted to the docket location on this slide.

That docket is open until July 1.

In addition to general comments on the revised method, There are 4 specific topic areas for which the EPA is soliciting public comments.

These topics are included in the federal register notice for this public comment period

The slides that we use today will also be posted to the docket.

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

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Deliberative Process / Ex. 5

Appointment

From: Anderson, Brian [Anderson.Brian@epa.gov]
Sent: 2/14/2018 3:13:17 PM
To: Anderson, Brian [Anderson.Brian@epa.gov]; Panger, Melissa [Panger.Melissa@epa.gov]; Garber, Kristina [Garber.Kristina@epa.gov]; Rossmeisl, Colleen [Rossmeisl.Colleen@epa.gov]; Connolly, Jennifer [Connolly.Jennifer@epa.gov]; Lennartz, Steven [Lennartz.Steven@epa.gov]; Eckel, William [Eckel.William@epa.gov]; Sims, Diann [Sims.Diann@epa.gov]; Becker, Jonathan [Becker.Jonathan@epa.gov]; Paisley-Jones, Claire [Paisley-Jones.Claire@epa.gov]; Miller, Wynne [Miller.Wynne@epa.gov]; Myers, Clayton [Myers.Clayton@epa.gov]; Echeverria, Marietta [Echeverria.Marietta@epa.gov]; Guilaran, Yu-Ting [Guilaran.Yu-Ting@epa.gov]
CC: Suarez, Mark [Suarez.Mark@epa.gov]; Perry, Tracy [Perry.Tracy@epa.gov]; Sherman, Kelly [Sherman.Kelly@epa.gov]; Schroeder, Carolyn [Schroeder.Carolyn@epa.gov]

Subject: use and usage - pre-meet with DDs to go over slides and plan
Attachments: Usage_step2_2-14-18(BEAD scientists).pptx; Why Usage Data Are Important (3).pptx
Location: DCRoomPYS9100/Potomac-Yard-One

Start: 2/14/2018 4:00:00 PM
End: 2/14/2018 5:00:00 PM
Show Time As: Tentative

Please forward to anyone I missed

Message

From: Smith, Charles [Smith.Charles@epa.gov]
Sent: 10/24/2018 3:22:29 PM
To: Guilaran, Yu-Ting [Guilaran.Yu-Ting@epa.gov]; Friedman, Dana [Friedman.Dana@epa.gov]; Zinn, Nicole [Zinn.Nicole@epa.gov]; Britton, Cathryn [Britton.Cathryn@epa.gov]; Arrington, Linda [Arrington.Linda@epa.gov]; Sherman, Kelly [Sherman.Kelly@epa.gov]
Subject: FW: NEED Responses to Qs for confirmation hearing ASAP
Attachments: Atrazine Qs Only_8-18-17.docx; OP Nomination Questions - 8.17.17.docx; Possible Chlorpyrifos Questions for the AA Nominee _081517.docx; Glyphosate updated Qs only for AA confirmation 8.17.17.docx; Neonic Qs Draft 8-18-17.docx

Importance: High

Last correspondence I have…

Charles "Billy" Smith
Deputy Director
Pesticide Re-Evaluation Division
Office of Pesticide Programs
703-305-0291

From: Anderson, Neil
Sent: Tuesday, August 29, 2017 10:00 AM
To: Jones, Ricardo <Jones.Ricardo@epa.gov>; Zinn, Nicole <Zinn.Nicole@epa.gov>; Sherman, Kelly <Sherman.Kelly@epa.gov>; Costello, Kevin <Costello.Kevin@epa.gov>; Friedman, Dana <Friedman.Dana@epa.gov>; Mannix, Marianne <Mannix.Marianne@epa.gov>; Nguyen, Khue <Nguyen.Khue@epa.gov>; Grable, Melissa <Grable.Melissa@epa.gov>; Keller, Kaitlin <keller.kaitlin@epa.gov>
Cc: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>; Smith, Charles <Smith.Charles@epa.gov>
Subject: NEED Responses to Qs for confirmation hearing ASAP
Importance: High

We started getting word late yesterday afternoon that the IO wants the responses to the Qs sent forward by midday tomorrow, Wednesday 8/30. Some of the Qs have been revised by the IO based on what I saw from one group of Qs sent back (OPs), so pay close attention to what you get back.

Thankfully, I think everyone had mostly prepared responses to all the Qs originally sent for review. You may have been contacted by someone in FEAD late yesterday afternoon asking for this by COB today, but I pushed back and got a few more hours (which is all they would give…).

Let me know if you have heard from your friendly neighborhood FEAD communications rep ☺

Neil

From: Anderson, Neil
Sent: Friday, August 18, 2017 2:29 PM
To: Dinkins, Darlene <Dinkins.Darlene@epa.gov>; Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>
Cc: Smith, Charles <Smith.Charles@epa.gov>; Ricardo Jones <jones.ricardo@epa.gov>; Zinn, Nicole <Zinn.Nicole@epa.gov>; Sherman, Kelly <Sherman.Kelly@epa.gov>; Costello, Kevin <Costello.Kevin@epa.gov>; Friedman, Dana <Friedman.Dana@epa.gov>; Mannix, Marianne <Mannix.Marianne@epa.gov>; Nguyen, Khue <Nguyen.Khue@epa.gov>; Grable, Melissa <Grable.Melissa@epa.gov>; Keller, Kaitlin <keller.kaitlin@epa.gov>
Subject: RE: Examples of confirmation hearing Q&As

Hi Darlene,

Attached are the Qs for the various topics as requested – atrazine, OPs, chlorpyrifos, glyphosate and neonics. Folks from across the relevant OPP divisions have reviewed and contributed to their development. Let us know if you have any comments or questions.

Best,
Neil

Neil Anderson
Chief, Risk Management and Implementation Branch I
Pesticide Re-evaluation Division, Office of Pesticide Programs
US EPA (Mail Code 7508P)
1200 Pennsylvania Avenue, NW
Washington, DC 20460
Phone: 703-308-8187
anderson.neil@epa.gov

Visit us on the Web at: http://www.epa.gov/pesticides/

From: Dinkins, Darlene
Sent: Wednesday, August 16, 2017 11:37 AM
To: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>
Cc: Anderson, Neil <Anderson.Neil@epa.gov>; Smith, Charles <Smith.Charles@epa.gov>
Subject: RE: Examples of confirmation hearing Q&As

Great. Thanks.

Darlene Dinkins
Office of Pesticide Programs
U.S. Environmental Protection Agency
(703) 305-5214

From: Guilaran, Yu-Ting
Sent: Wednesday, August 16, 2017 11:37 AM
To: Dinkins, Darlene <Dinkins.Darlene@epa.gov>
Cc: Anderson, Neil <Anderson.Neil@epa.gov>; Smith, Charles <Smith.Charles@epa.gov>
Subject: RE: Examples of confirmation hearing Q&As

Yes, we (Neil) will provide a set of questions for chlorpyrifos, glyphosate, atrazine, neonics, OPs by Friday. Thanks.

Regards,

Yu-Ting Guilaran, P.E.
Director

Pesticide Re-evaluation Division (PRD)
Office of Pesticide Programs
Office of Chemical Safety and Pollution Prevention

(tel) 703 308 0052
(fax)703 308 8005
Mail code 7508P
Room number PY S9623

From: Dinkins, Darlene
Sent: Wednesday, August 16, 2017 11:33 AM
To: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>
Subject: RE: Examples of confirmation hearing Q&As

Hi Yu-Ting,

I'm following up on the request for Qs&As for the Dourson hearing. Does PRD have any to provide?

Darlene Dinkins
Office of Pesticide Programs
U.S. Environmental Protection Agency
(703) 305-5214

From: Guilaran, Yu-Ting
Sent: Tuesday, August 01, 2017 2:56 PM
To: Dinkins, Darlene <Dinkins.Darlene@epa.gov>
Subject: RE: Examples of confirmation hearing Q&As

Thanks Darlene. I will have my folks develop Q&A for chlorpyrifos, glyphosate, OPs, atrazine (I believe these are the ones that Rick names in staff meeting). Can you check with him to see if he thinks one is needed for the neonicotinoids? Thanks.

Regards,

Yu-Ting Guilaran, P.E.
Director

Pesticide Re-evaluation Division (PRD)
Office of Pesticide Programs
Office of Chemical Safety and Pollution Prevention
(tel) 703 308 0052
(fax)703 308 8005
Mail code 7508P
Room number PY S9623

From: Dinkins, Darlene
Sent: Tuesday, August 01, 2017 2:08 PM
To: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>
Subject: Examples of confirmation hearing Q&As

Hi Yu-Ting,

Sven a little clarification on the "murder board" questions for the Dourson hearing. I will look through press questions and congressional inquiries on some of our hot topics.

Darlene Dinkins
Office of Pesticide Programs
U.S. Environmental Protection Agency
(703) 305-5214

From: Kaiser, Sven-Erik
Sent: Tuesday, August 01, 2017 11:05 AM
To: Dinkins, Darlene <Dinkins.Darlene@epa.gov>
Subject: FW: examples of OECA confirmation hearing Q&A

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Sven

Sven-Erik Kaiser
U.S. EPA
Office of Congressional and Intergovernmental Relations
1200 Pennsylvania Ave., NW (1305A)
Washington, DC 20460
202-566-2753

From: Levine, Carolyn
Sent: Tuesday, August 01, 2017 11:02 AM
To: Kaiser, Sven-Erik <Kaiser.Sven-Erik@epa.gov>
Subject: examples of OECA confirmation hearing Q&A

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Carolyn Levine
Office of Congressional and
Intergovernmental Relations
U.S. EPA
(202) 564-1859
levine.carolyn@epa.gov

Deliberative Process / Ex. 5

Possible Chlorpyrifos Questions for the AA Nominee

Questions Opposing March 2017 Denial

Deliberative Process / Ex. 5

Questions Opposing Use of Currently Available Epidemiology Data in Risk Assessment

Deliberative Process / Ex. 5

Message

From: Perry, Tracy [Perry.Tracy@epa.gov]
Sent: 10/9/2018 9:38:13 PM
To: Guilaran, Yu-Ting [Guilaran.Yu-Ting@epa.gov]
CC: Sherman, Kelly [Sherman.Kelly@epa.gov]
Subject: Review NMFS BiOp slides?
Attachments: IO.DD Briefing.Public Comments on NMFS BiOp.10.9.18.pptx

Yu-Ting,

Would you like to review the attached NMFS BiOp slides for the Thursday, Oct. 11 briefing before I add them to the meeting invite? I've incorporated Kelly's and the team's comments (mainly editorial). I did not receive any comments from Marietta or Wynne.

In addition to adding to the meeting invite, should I send the slides directly to Rick/Sharon J.?

Tracy L. Perry
Senior Regulatory Advisor
Risk Management and Implementation Branch III
Pesticide Re-evaluation Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
(703) 308-0128

Summary of Public Comments on NMFS Biological Opinion (BiOp): chlorpyrifos, malathion, diazinon

IO-DD Briefing

October 11, 2018

126 Public Comments
(63 Substantive)

- Registrants (FMC, Dow, Adama, CropLife America)
- NGOs (CBD, NCAP; Orca Alliance; RISE)
- NASDA and State Departments of Agriculture
- Tribes (Confederated Tribes of the Umatilla Indian Reservation and Columbia River Inter-Tribal Fish Commission)
- USDA/OPMP, CDPR, CASQA, Western IPM Center
- AMCA and mosquito control districts
- Agricultural stakeholder groups (National Cotton Council, Iowa Corn Growers Association, and Georgia Agribusiness Council)
- Academia

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Deliberative Process / Ex. 5

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Deliberative Process / Ex. 5

Message

From: Miller, Wynne [Miller.Wynne@epa.gov]
Sent: 8/13/2018 10:45:50 PM
To: Keller, Kaitlin [keller.kaitlin@epa.gov]
CC: Kiely, Timothy [Kiely.Timothy@epa.gov]; Guilaran, Yu-Ting [Guilaran.Yu-Ting@epa.gov]; Messina, Edward [Messina.Edward@epa.gov]; Dinkins, Darlene [Dinkins.Darlene@epa.gov]; Vogel, Dana [Vogel.Dana@epa.gov]; Dawson, Jeffrey [Dawson.Jeff@epa.gov]; Reaves, Elissa [Reaves.Elissa@epa.gov]; Friedman, Dana [Friedman.Dana@epa.gov]; Anderson, Neil [Anderson.Neil@epa.gov]
Subject: Re: Chlorpyrifos 1-Pager

Hi Kaitlin,

Deliberative Process / Ex. 5

Wynne

Sent from my iPhone

On Aug 13, 2018, at 6:21 PM, Keller, Kaitlin <keller.kaitlin@epa.gov> wrote:

> Thanks Tim!
>
> ---
>
> **From:** Kiely, Timothy
> **Sent:** Monday, August 13, 2018 6:20 PM
> **To:** Keller, Kaitlin <keller.kaitlin@epa.gov>
> **Cc:** Miller, Wynne <Miller.Wynne@epa.gov>; Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>; Messina, Edward <Messina.Edward@epa.gov>; Dinkins, Darlene <Dinkins.Darlene@epa.gov>; Vogel, Dana <Vogel.Dana@epa.gov>; Dawson, Jeffrey <Dawson.Jeff@epa.gov>; Reaves, Elissa <Reaves.Elissa@epa.gov>; Friedman, Dana <Friedman.Dana@epa.gov>; Anderson, Neil <Anderson.Neil@epa.gov>
> **Subject:** Re: Chlorpyrifos 1-Pager
>
> Kaitlin **Deliberative Process / Ex. 5**
>
> **Deliberative Process / Ex. 5**
>
> Tim
> Sent from my iPhone
>
> On Aug 13, 2018, at 5:52 PM, Keller, Kaitlin <keller.kaitlin@epa.gov> wrote:
>
> > Thanks all!! Here's what went forward on the usage. The rest will be back pocket for Charlotte, Nancy, and Erik.
> >
> > **Deliberative Process / Ex. 5**

Thanks again!
Kaitlin

From: Miller, Wynne
Sent: Monday, August 13, 2018 4:02 PM
To: Keller, Kaitlin <keller.kaitlin@epa.gov>; Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>; Messina, Edward <Messina.Edward@epa.gov>
Cc: Dinkins, Darlene <Dinkins.Darlene@epa.gov>; Vogel, Dana <Vogel.Dana@epa.gov>; Dawson, Jeffrey <Dawson.Jeff@epa.gov>; Reaves, Elissa <Reaves.Elissa@epa.gov>; Friedman, Dana <Friedman.Dana@epa.gov>; Kiely, Timothy <Kiely.Timothy@epa.gov>; Anderson, Neil <Anderson.Neil@epa.gov>
Subject: RE: Chlorpyrifos 1-Pager

Hi Kaitlyn,
Here's answers to your questions:

Deliberative Process / Ex. 5

Thx
Wynne

From: Keller, Kaitlin
Sent: Monday, August 13, 2018 3:35 PM
To: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>; Messina, Edward <Messina.Edward@epa.gov>
Cc: Dinkins, Darlene <Dinkins.Darlene@epa.gov>; Vogel, Dana <Vogel.Dana@epa.gov>; Dawson, Jeffrey <Dawson.Jeff@epa.gov>; Reaves, Elissa <Reaves.Elissa@epa.gov>; Miller, Wynne <Miller.Wynne@epa.gov>; Friedman, Dana <Friedman.Dana@epa.gov>
Subject: RE: Chlorpyrifos 1-Pager

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5?

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5?

Deliberative Process / Ex. 5?

From: Keller, Kaitlin
Sent: Monday, August 13, 2018 3:29 PM
To: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>; Messina, Edward <Messina.Edward@epa.gov>
Cc: Dinkins, Darlene <Dinkins.Darlene@epa.gov>; Vogel, Dana <Vogel.Dana@epa.gov>; Dawson, Jeffrey <Dawson.Jeff@epa.gov>; Reaves, Elissa <Reaves.Elissa@epa.gov>; Miller, Wynne <Miller.Wynne@epa.gov>; Friedman, Dana <Friedman.Dana@epa.gov>
Subject: RE: Chlorpyrifos 1-Pager

Deliberative Process / Ex. 5

From: Guilaran, Yu-Ting
Sent: Monday, August 13, 2018 3:26 PM
To: Messina, Edward <Messina.Edward@epa.gov>; Keller, Kaitlin <keller.kaitlin@epa.gov>
Cc: Dinkins, Darlene <Dinkins.Darlene@epa.gov>; Vogel, Dana <Vogel.Dana@epa.gov>; Dawson, Jeffrey <Dawson.Jeff@epa.gov>; Reaves, Elissa <Reaves.Elissa@epa.gov>; Miller, Wynne <Miller.Wynne@epa.gov>; Friedman, Dana <Friedman.Dana@epa.gov>
Subject: RE: Chlorpyrifos 1-Pager

Mine had one minor change…please use mine, thanks.

Regards,

Yu-Ting Guilaran, P.E.
Director

Pesticide Re-evaluation Division (PRD)
Office of Pesticide Programs
Office of Chemical Safety and Pollution Prevention
(tel) 703 308 0052
(fax)703 308 8005
Mail code 7508P
Room number PY S9623

From: Messina, Edward
Sent: Monday, August 13, 2018 3:22 PM
To: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>; Keller, Kaitlin <keller.kaitlin@epa.gov>
Cc: Dinkins, Darlene <Dinkins.Darlene@epa.gov>; Vogel, Dana <Vogel.Dana@epa.gov>; Dawson, Jeffrey <Dawson.Jeff@epa.gov>; Reaves, Elissa <Reaves.Elissa@epa.gov>; Miller, Wynne <Miller.Wynne@epa.gov>; Friedman, Dana <Friedman.Dana@epa.gov>
Subject: RE: Chlorpyrifos 1-Pager

Thanks. Kaitlin it is the same file I sent to you and cc'd Nancy and Charlotte.

_____
Ed Messina
Acting Deputy Office Director (Programs)

Office of Pesticide Programs
U.S. EPA
(703) 347-0209

From: Guilaran, Yu-Ting
Sent: Monday, August 13, 2018 3:21 PM
To: Keller, Kaitlin <keller.kaitlin@epa.gov>
Cc: Messina, Edward <Messina.Edward@epa.gov>; Dinkins, Darlene <Dinkins.Darlene@epa.gov>; Vogel, Dana <Vogel.Dana@epa.gov>; Dawson, Jeffrey <Dawson.Jeff@epa.gov>; Reaves, Elissa <Reaves.Elissa@epa.gov>; Miller, Wynne <Miller.Wynne@epa.gov>; Friedman, Dana <Friedman.Dana@epa.gov>
Subject: RE: Chlorpyrifos 1-Pager

Hello Kaitlin
Please see attached, thanks.

Regards,

Yu-Ting Guilaran, P.E.
Director

Pesticide Re-evaluation Division (PRD)
Office of Pesticide Programs
Office of Chemical Safety and Pollution Prevention
(tel) 703 308 0052
(fax)703 308 8005
Mail code 7508P
Room number PY S9623

From: Dinkins, Darlene
Sent: Monday, August 13, 2018 2:39 PM
To: Vogel, Dana <Vogel.Dana@epa.gov>; Dawson, Jeffrey <Dawson.Jeff@epa.gov>; Reaves, Elissa <Reaves.Elissa@epa.gov>; Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>; Miller, Wynne <Miller.Wynne@epa.gov>
Cc: Messina, Edward <Messina.Edward@epa.gov>
Subject: FW: Chlorpyrifos 1-Pager
Importance: High

Hi everyone,

Just checking on the status of the chlorpyrifos 1-pager. Kaitlin said she has to send something to the DAAs by 3:00 today to allow review time to get it to the Administrator's Office by 4:00 today.

Darlene Dinkins
Office of Pesticide Programs
U.S. Environmental Protection Agency
(703) 305-5214

From: Keller, Kaitlin
Sent: Monday, August 13, 2018 10:28 AM
To: Messina, Edward <Messina.Edward@epa.gov>; Dinkins, Darlene <Dinkins.Darlene@epa.gov>; Keigwin, Richard <Keigwin.Richard@epa.gov>
Subject: Chlorpyrifos 1-Pager
Importance: High

Just learned that a briefing for the Acting Administrator is now scheduled for tomorrow at 4pm, we need to have a briefing paper to the AO by 4 today. Can you send me whatever the team has by 1pm today?

Thanks,
Kaitlin

Kaitlin Keller, Special Assistant
Office of Chemical Safety and Pollution Prevention
U.S. Environmental Protection Agency
(202) 564-7098

<Chlorpyrifos Usage 1-pager 8.13.2018 (1).docx>

Message

From: Messina, Edward [Messina.Edward@epa.gov]
Sent: 8/13/2018 7:21:13 PM
To: Keller, Kaitlin [keller.kaitlin@epa.gov]
CC: Dinkins, Darlene [Dinkins.Darlene@epa.gov]; Beck, Nancy [Beck.Nancy@epa.gov]; Bertrand, Charlotte [Bertrand.Charlotte@epa.gov]; Baptist, Erik [Baptist.Erik@epa.gov]; Miller, Wynne [Miller.Wynne@epa.gov]; Vogel, Dana [Vogel.Dana@epa.gov]; Guilaran, Yu-Ting [Guilaran.Yu-Ting@epa.gov]; Keigwin, Richard [Keigwin.Richard@epa.gov]
Subject: Chlorpyrifos Administrator Information
Attachments: Chlorpyrifos Administrator 1-pager with Attachments_081318.docx

As requested.

Ed Messina
Acting Deputy Office Director (Programs)
Office of Pesticide Programs
U.S. EPA
(703) 347-0209

Message

From: Miller, Wynne [Miller.Wynne@epa.gov]
Sent: 8/13/2018 6:42:59 PM
To: Friedman, Dana [Friedman.Dana@epa.gov]
CC: Kiely, Timothy [Kiely.Timothy@epa.gov]; Anderson, Neil [Anderson.Neil@epa.gov]; Guilaran, Yu-Ting [Guilaran.Yu-Ting@epa.gov]
Subject: BEAD info
Attachments: Chlorpyrifos summary_v2_wfm.docx

Dana,
Please Tim let know if you need anything else. We embedded Charlotte's questions from Messina's email.
Thx

Message

From: Friedman, Dana [Friedman.Dana@epa.gov]
Sent: 9/4/2018 2:19:49 PM
To: Guilaran, Yu-Ting [Guilaran.Yu-Ting@epa.gov]; Smith, Charles [Smith.Charles@epa.gov]
CC: Costello, Kevin [Costello.Kevin@epa.gov]; Zinn, Nicole [Zinn.Nicole@epa.gov]
Subject: most recent cpfos RTCs
Attachments: HED RTC_Master draft 3_7_17_DD.docx; chlorpyrifos_rtc_030617.docx

Here are the most recent versions of the draft RTCs that I have in my files, and were drafted towards a March 2017 rule. Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Dana L. Friedman
Pesticide Re-evaluation Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
703-347-8827

United States Department of Agriculture

January 17, 2017

Jack E. Housenger, Director
Office of Pesticide Programs (7501P)
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue NW
Washington, DC 20460

Dear Mr. Housenger,

USDA appreciates the opportunity to comment on EPA's proposal to revoke chlorpyrifos tolerances, and in particular the new underlying risk assessment that was announced on November 17, 2016 ("Chlorpyrifos; Tolerance Revocations; Notice of Data Availability and Request for Comment," 81 FR 81049, Docket ID EPA-HQ-OPP-2015-0653). As you know, EPA is proposing this action in response to a petition to revoke chlorpyrifos tolerances submitted by the Natural Resources Defense Council and Pesticide Action Network North America in 2007.

USDA has both grave concerns about the EPA process that has led to the Agency publishing three wildly different human health risk assessments for chlorpyrifos within two years, and severe doubts about the validity of the scientific conclusions underpinning EPA's latest chlorpyrifos risk assessment. Even though use of the Columbia Center for Children's Environmental Health (CCCEH) study to derive a point of departure was criticized by the FIFRA Scientific Advisory Panel, EPA continues to rely on this study and has now paired it with an inadequate dose reconstruction approach.

In light of these developments, USDA calls on EPA to deny the NRDC/PANNA petition to revoke chlorpyrifos tolerances. This would allow EPA to ensure the validity of its scientific approach as part of the ongoing registration review process, without the excessive pressure caused by arbitrary, litigation-related deadlines.

Our detailed comments on the latest chlorpyrifos risk assessment follow. We look forward to continuing to work with EPA to ensure that pesticides remain both safe to the public and available to U.S. farmers. Please do not hesitate to contact me if you have any further questions.

Sincerely,

Sheryl H. Kunickis

Sheryl H. Kunickis, Ph.D.
Director

Office of Pest Management Policy
1400 Independence Avenue SW
Washington, D.C. 20250-0314
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January 17, 2017

USDA Comments on the Risk Assessment Underlying the Reopened Proposed Rule "Chlorpyrifos; Tolerance Revocations; Notice of Data Availability and Request for Comment" (Docket ID EPA-HQ-OPP-2015-0653)

Science is the backbone of the EPA's decision-making. The Agency's ability to pursue its mission to protect human health and the environment depends upon the integrity of the science on which it relies. The environmental policies, decisions, guidance, and regulations that impact the lives of all Americans every day must be grounded, at a most fundamental level, in sound, high quality science.

– Excerpt from *Scientific Integrity Policy*, U.S. Environmental Protection Agency

Introduction

The "Revised Human Health Risk Assessment for Registration Review" dated November 3, 2016, is the third human health risk assessment for chlorpyrifos that EPA has released, and that USDA has reviewed and commented on, within the past two years. Typically, three risk assessments for the same hazard published so close together represent successive attempts at improvement and refinement, with the goal of reducing uncertainty and improving the reliability of the results. However, EPA's three risk assessments resemble more of a scattershot approach, with the agency switching between different health outcomes and points of departure, and adopting widely varying dose measurement and reconstruction approaches.

In its latest assessment, EPA has stopped using the dose data from the Columbia Center for Children's Environmental Health (CCCEH) study it had endorsed in its previous risk assessment just eight months earlier, and instead has chosen to rely on a dose-reconstruction approach to identify a point of departure. This dose reconstruction approach supposedly estimates the amount of chlorpyrifos to which women in the CCCEH cohort might have been exposed in their homes around the turn of the century. It is not based on any empirical data, but rather on conversations EPA had with "several" pesticide applicators in 2016 in which they "recalled" what the "predominant" use of chlorpyrifos "in New York City apartment buildings" was 15-20 years earlier. Without any actual data as to use of chlorpyrifos in the cohort members' apartment buildings, let alone their individual apartments, EPA is merely *guessing* that the women in the CCCEH cohort were exposed to one crack-and-crevice application of chlorpyrifos per month.

These exposure guesses are then linked to adverse health outcomes that EPA's own Scientific Advisory Panel (SAP) has questioned as being either statistical artifacts or not caused by chlorpyrifos exposure. In addition, the latest risk assessment is still based on just the single, not replicated, and unconfirmed CCCEH study. Many weaknesses inherent in the study have been identified by the SAP and others, which undermine its suitability for determining a point of departure. These weaknesses remain unaddressed in EPA's latest risk assessment. This cannot be the type of "sound, high quality science" the writers of EPA's Scientific Integrity Policy envisioned as the "backbone of the EPA's decision-making." USDA has grave concerns that ambiguous response data from a single, inconclusive study are being combined with a mere *guess* as to dose levels, and the result is being used to underpin a regulatory decision about a pesticide chemical that is vital to U.S. agriculture, and whose removal from market would have a major economic impact on growers and consumers.

Our more detailed comments follow, and are divided into two sections, substantive and procedural. USDA requests a response that addresses our comments both comprehensively, and on a paragraph-by-paragraph basis.

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Washington, D.C. 20250-0314
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Substantive Concerns

Over the past two years, USDA has observed EPA's chlorpyrifos risk assessments transition from a more traditional, incremental approach based on combining a point of departure from a well-established health outcome (10 % red blood cell acetylcholinesterase inhibition, or 10 % RBC AChEI) with retention of a 10X FQPA safety factor based on some new epidemiological observations, to a completely novel, even radical, approach of basing the entire risk assessment, and through it the regulatory and economic future of this major agricultural chemical, on a single limited and problematic epidemiological study.

Throughout the latest risk assessment and the accompanying notice in the Federal Register, EPA gives the impression that the Agency has addressed concerns voiced by the April 2016 SAP and is following the SAP's recommendations. For example, in the Federal Register Notice published on November 17, 2016, which announced the availability of the latest risk assessment, EPA claimed that it "modif[ied] the methods and risk assessment . . . in accordance with the advice of the SAP" (81 FR 81050). The SAP exists to provide independent scientific advice to the Agency; as such the SAP's findings are particularly important when they disagree with an approach taken by EPA. Even though SAP reports are not legally binding on the Agency, USDA strongly encourages EPA to thoroughly consider the advice received from the SAP. An objective, comprehensive review of the meeting minutes of the SAP's April meeting, published July 20, 2016, simply does not lead to the conclusion that SAP concerns have been addressed. Instead, the latest risk assessment raises additional and more acute concerns about the viability of EPA's risk assessment approach and the reliability of its findings.

EPA's latest risk assessment rests on three central conclusions. USDA disagrees with all three.

1. **EPA concludes that studies show an actual effect on working memory among children in the CCCEH cohort**

In order for a study to be meaningful for deriving a point of departure, it must detect an actual health effect. EPA has chosen a 2 % change in working memory, measured at age 7 and discussed in the Rauh et al. (2011) study of the CCCEH cohort, as the critical effect for its last two risk assessments, the first of which was reviewed by the SAP in April. There was considerable disagreement among SAP members as to whether this 2 % change is even significant or anything more than a statistical artifact:

- "The [SAP] was conflicted with respect to the importance of a 2% change in working memory."
- "Some members considered a 2% change in working memory (less than one standard deviation in the distribution of scores in the general population) to be of questionable biological significance."
- "By definition, the [standard deviation] for an essentially unexposed population is really 15%. A 2% reduction seems to be a particularly low threshold for concluding 'abnormal.'"

Quotes from FIFRA SAP meeting minutes on chlorpyrifos (July 20, 2016)

If the Rauh et al. study failed to detect a true health effect, any further discussion on the use of this study to derive a point of departure would be moot. USDA does not deny that some SAP members did argue that a 2% change in working memory is a significant health effect. Rather than taking a position as to whether the observed 2 % decrement in working memory is "real" or "significant," USDA merely wishes to highlight the considerable disagreement within the EPA SAP as to this very basic question. If the experts convened by EPA cannot even agree that a health effect (let alone an *adverse* health effect) was observed, this severely weakens the study's suitability as the sole quantitative foundation of a major, economically significant risk assessment. Equally concerning is EPA's failure to address in its most recent risk assessment the questions raised by the SAP.

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2. EPA concludes that the 2 % change in working memory was caused by prenatal exposure to chlorpyrifos

Establishing causality between the exposure of interest (in this case, prenatal chlorpyrifos exposure) and the observed health effect (in this case, a 2 % change in working memory measured at age 7) is a crucial prerequisite to using the CCCEH cohort data in quantitative risk assessment. EPA's latest risk assessment does nothing to address the April 2016 SAP's strongly-worded concerns regarding the lack of established causality. If anything, EPA's Federal Register Notice accompanying the risk assessment further obfuscates the SAP's conclusions. It states that "generally, however, the FIFRA SAP agreed with the overall conclusion of the CCCEH study, i.e. the *association* between prenatal chlorpyrifos exposure and neurodevelopmental outcomes in children" (81 FR 81050; emphasis added). Whether or not chlorpyrifos exposure is *associated* with the change in working memory is not the issue here; an association between chlorpyrifos exposure and change in working memory could be the result of a confounding factor or a multiple comparisons problem, and thus be meaningless for risk assessment.

Rather than association, the relevance of the CCCEH study depends on whether chlorpyrifos *caused* the change in working memory. The SAP emphatically commented on question of causality (emphases added):

- "The assumption that the impaired working memory and lower IQ measures observed [in the CCCEH study] are caused primarily by a single insecticide (chlorpyrifos) and predicted by the blood levels at time of delivery <u>is not supported by the scientific weight of evidence</u>."
- "Some members of the [SAP] were also concerned about the lack of knowledge of the sensitive window(s) of exposure during pregnancy that would lead to neurodevelopmental outcomes. Without accurate knowledge that exposure occurred during a sensitive window, <u>it is impossible to derive causation</u>."
- "Without any evidence in the animal literature or elsewhere of a mechanism of action that could explain how pg/g levels in blood could impair IQ and/or working memory, <u>there does not appear to be biological plausibility</u>. This is a significant uncertainty." (Biological plausibility is a crucial element for establishing causality.)
- "The [SAP] is not aware of any scientific evidence where pg/g levels in the blood would lead to deleterious neurotoxicological effects in a mammalian system. This lack of data could indicate a lack of biological plausibility."

The majority of the SAP members drew the correct logical conclusion from the absence of indicated causality, namely that use of the CCCEH study in a highly impactful risk assessment is "premature and possibly inappropriate." This is a necessary conclusion EPA refuses to draw, by continuing to rely on the CCCEH study in its latest risk assessment. Even more worrisome is EPA's willingness to portray its latest risk assessment as responsive to the SAP concerns, when in reality it is extremely difficult to see how any continued use of the CCCEH study as a basis for a point of departure is consistent with the SAP conclusions. The larger passage from the SAP minutes reads as follows (emphases added):

- "The majority of the [SAP] considers the Agency's use of the results from a single longitudinal study to make a decision with immense ramifications based on the use of cord blood measures of chlorpyrifos as a [point of departure] for risk assessment as <u>premature and possibly inappropriate</u>. The basis for this <u>majority view</u> includes: 1) an inability to either know, or confidently make assumptions about, aspects of exposure patterns, labor and delivery, and blood collection . . . [and] 5) <u>lack of biological plausibility</u> for how low cord blood (low parts per trillion) concentrations of chlorpyrifos can alter working memory and produce neurodevelopmental impairment."

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- "Some Panel members stated that the reliance on single cord blood measurements from only one study (i.e. the CCCEH study) as a primary basis for a highly impactful regulatory decision goes against standard practices of science in the field of toxicology and pharmacology."

EPA argues that it addressed SAP concerns about the cord blood data by no longer using them in its latest risk assessment, having replaced them with the dose reconstruction approach. In doing so, EPA misreads the SAP's concerns. While the SAP did criticize EPA for using a single measurement of cord blood, rather than deriving a time-weighted average, the SAP's fundamental disagreement centered on the fact that the risk assessment was based on just a single study with insufficient evidence of causality between exposure and effect. It is not the cord blood data per se that are the problem, and replacing them with a time-weighted average not based on any relevant exposure data does not improve the risk assessment. Rather, it is EPA's "inability to either know, or confidently make assumptions about, aspects of exposure patterns, labor and delivery, and blood collection," as well as the "lack of biological plausibility" that render the CCCEH study unusable. These criticisms are equally valid whether EPA uses the CCCEH cord blood data or a time-weighted average based on the reconstructed dose data.

EPA's reconstructed doses are not based on any additional exposure data collected from the CCCEH cohort, nor do they help overcome the fundamental lack of biological plausibility and thereby causality. In fact, the SAP criticized the use in the risk assessment of not only the dose data (cord blood measurements), but also of the observed outcome data (change in working memory):

- "It was the Panel's conclusion that the Agency provided insufficient justification for using cord blood chlorpyrifos levels and associated neurobehavioral health outcomes to derive a [point of departure]" (emphasis added).

In the end, the fatal flaw of EPA's use of the CCCEH study is that there is insufficient evidence of causality underlying the observed association involving chlorpyrifos. The problems with the chlorpyrifos cord blood data identified by the SAP cannot be isolated from the CCCEH study as a whole. Instead, the entire study, including the cord blood data, is problematic because it fails to indicate a causal relationship between chlorpyrifos and the health effect. Replacing the cord blood data with a different (and arguably inferior) set of reconstructed dose data, as EPA did in its latest risk assessment, does nothing to improve the quality or reliability of the risk assessment, and introduces new and greater uncertainty rather than decreasing it.

The SAP meeting minutes also restated many other concerns about the CCCEH study that have been previously identified by USDA and others, and that continue to be relevant as long as EPA attempts to use the CCCEH study to derive a point of departure. These include the potential presence of numerous confounding factors that further weaken any claim of causality between chlorpyrifos exposure and the change in working memory, the lack of access by EPA or the public to the raw study data, and questions surrounding the analytical methods used to detect the very low (picogram per gram) levels of chlorpyrifos in the cord blood.

- On confounding factors: "In addition to the air sampling study and the cord blood sampling study indicating exposure of the [CCCEH] study cohort to various pesticides, the cohort was additionally exposed to multiple contaminants including PAHs, tobacco smoke, piperonyl butoxide, and phthalates. . . . The fact that the pregnant mothers were exposed to a complex mixture of chemicals, many of which induce deleterious effects on the same neurobehavioral parameters that chlorpyrifos is reported to affect, increases the level of uncertainty for using measurements of chlorpyrifos alone as the basis for the risk assessment . . . . [T]he environment where the exposure occurred contained multiple organophosphate insecticides and multiple carbamate insecticides . . . . Thus, there was the opportunity for the pregnant mothers to be simultaneously exposed to multiple cholinesterase inhibiting chemicals. Following exposure to such a mixture, it would be biologically impossible to separate the independent effects of each

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chemical on a neurochemical or behavioral outcome regardless of the statistical model used" (emphasis added).

- On raw data availability: "Finally, some [SAP] members thought the quality of the CCCEH data is hard to assess when raw analytical data have not been made available, and the study has not been reproduced."
- On the analytical method: "A major source of uncertainty for the [SAP] was the lack of verification and replication of the analytical chemistry results that reported very low levels of chlorpyrifos (pg/g). Imputing quantitative values when the concentration of analyte falls below the level of detection (LOD) was a particular concern, especially given that a large fraction of cord blood samples included in the analyses presented with levels below LOD."

3. EPA uses reconstructed dose estimates that are not based on any empirical data or any actual knowledge of the exposure experienced by members of the CCCEH cohort

EPA used its 2012 Residential SOPs, which are typically used to estimate exposure to a pesticide for the general population, to estimate the doses experienced by the CCCEH cohort. Exposure models, such as the Residential SOPs, are designed to produce conservative exposure estimates that are then compared to experimental dose data derived from animal or human studies. In other words, a study typically supplies actual dose values linked to actual response data, which can then be compared to modeled exposure estimates to determine whether a response is expected in the modeled population.

Dose reconstruction is usually based on an internal dose (biomarker) measurement as a starting point and uses reverse dosimetry to arrive at a corresponding external dose. In this case, EPA has no usable internal dose data, and instead is using exposure models to estimate both the doses received by the individuals in the study, as well as the exposure experienced by the general population. The problem is that there is no cause-and-effect link between the dose estimates provided by the exposure model (Residential SOPs) and the change in working memory observed in the CCCEH study. Any exposure model can produce a huge range of exposure estimates due to both population variability and uncertainty. In this case, the uncertainty around any modeled dose estimate is expected to be massive, since EPA has no way of knowing when, how often, and at what levels chlorpyrifos was applied in the CCCEH cohort members' apartments, nor does EPA know the duration and intensity of exposure experienced by study participants post-application. Did they apply chlorpyrifos themselves and did they do so instead of or in addition to professional applications? How long did they spend in the apartment post-application? Were the windows open or closed? When did they shower? Were they also exposed elsewhere, for example at work?

The wide range of exposure estimates and the vast uncertainty associated with any estimate makes it impossible to identify an actual dose estimate that is linked to the rather small change in working memory observed in the CCCEH study. The fact that EPA's response to the SAP report – which highlighted in a negative way the "inability to either know, or confidently make assumptions about, aspects of exposure patterns, labor and delivery, and blood collection" and the "the lack of knowledge of the sensitive window(s) of exposure during pregnancy" – was to derive dose reconstruction estimates based on absolutely no data related to the cohort members' timing of exposure or sensitive windows, indicates a misunderstanding of the SAP's concerns. The SAP went on to criticize reliance on the CCCEH study, because the data showed "a lack of a clear dose-response relationship and evidence of temporality (i.e., two key concepts in pharmacology and toxicology)." Abandoning the CCCEH cord blood exposure data in favor of EPA's dose reconstruction estimates, which are completely devoid of actual connection to the CCCEH cohort, exacerbates this SAP concern instead of mitigating it.

In addition, EPA is using an inappropriately high level of conservatism in its dose-reconstruction effort given that its stated goal is to derive a lowest observed adverse effect level (LOAEL) dose. In the latest risk assessment, EPA references an earlier dose reconstruction that was part of the 2014 Revised Human

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Health Risk Assessment (2014 HHRA). The goal of the 2014 HHRA exercise was to estimate an "upper limit, bounding level exposure" and as a result it contained very conservative assumptions with regards to exposure duration and bathing frequency. By contrast, EPA states that the purpose of the dose reconstruction in its latest risk assessment is to predict "typical" product usage and behaviors, and therefore the assumptions are essentially realistic or even tend to underestimate exposure (e.g., daily shower taking place immediately after application; exposure duration of only 2 hours/day). However, EPA should be estimating upper limit exposures if its goal is to derive a LOAEL dose. Assuming for a moment that the CCCEH study did observe an actual association between chlorpyrifos exposure and change in working memory, only the most highly exposed cohort members would have experienced this adverse effect. Most cohort members were exposed to comparatively lower levels of chlorpyrifos, which did not cause a change in working memory. Therefore, exposure resulting from "typical" product usage and behaviors should not be expected to cause a response, and if used at all should be considered a no observed adverse effect level (NOAEL) dose, not a LOAEL dose. Instead, upper limit exposures, representing the most highly exposed individuals within the CCCEH cohort, would have been the only doses to be potentially associated with an adverse effect.

Taking a step back, USDA wishes to highlight a logical flaw in EPA's reasoning. In its dose reconstruction, EPA considered the exposure from the monthly residential crack-and-crevice application to be the only contributor to the chlorpyrifos doses experienced by the CCCEH cohort. In other words, EPA is assuming that the crack-and-crevice application, and only the crack-and-crevice application, is causing any adverse effects potentially observed in the CCCEH study cohort, such as a change in working memory. By implication, this indicates that the Agency considers any dietary (food or drinking water) exposure to chlorpyrifos among the CCCEH cohort to be negligible. As EPA points out in its latest risk assessment, all residential uses of chlorpyrifos were cancelled in 2000, meaning that today the only relevant exposures for the general population are food and drinking water exposures. There is no reason to believe that the population today is exposed to significantly higher levels of chlorpyrifos in the diet than the CCCEH cohort was. How then is it possible that food and drinking water exposures were not even considered in the CCCEH cohort dose reconstruction, but EPA now claims that food exposure *alone* causes some individuals to exceed the acceptable level of chlorpyrifos exposure by as much as 140 times?

Conclusion for Substantive Concerns

EPA's latest risk assessment depends on three conclusions related to the existence of a health effect, causality, and the dose-reconstruction approach. For EPA's assessment to be meaningful, all three conclusions would have to be well-supported by the evidence and logically coherent. Instead, they range from questionable to unsupported by the evidence to incorrect. As a result, the latest risk assessment fails to show either a causal or a dose-response relationship between chlorpyrifos exposure and a change in working memory among the CCCEH cohort, even though causality and the existence of a dose-response relationship are two fundamental pillars of regulatory toxicology and risk assessment. USDA concludes by asking whether, before November 2016, EPA has ever derived a point of departure for pesticide risk assessment based on a single study which the Agency has concluded does not contain *any* usable dose data.

Procedural Concerns

USDA strongly urges EPA to abandon use of the CCCEH study to set a point of departure for chlorpyrifos and to return to using AChEI as the critical effect. If EPA chooses to continue to use the CCCEH study, EPA's latest risk assessment should be re-submitted to the SAP for review. USDA finds this to be absolutely crucial for maintaining public confidence in the pesticide regulatory process. Compared to the March 2016 risk assessment that the SAP reviewed, the latest risk assessment is even

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further beyond the "mainstream" of pesticide risk assessment. Mostly, this is due to the dose reconstruction approach that, to USDA's knowledge, has never been externally reviewed. In addition, the fact that the latest risk assessment continues to be based on the CCCEH study clearly weighs in favor of allowing the SAP to review again, in order to determine whether its earlier criticisms of the CCCEH study have been addressed or mitigated.

USDA notes that according to EPA's Peer Review Policy, "external peer review is the approach of choice" for influential scientific information intended to support important decisions. Influential scientific information in turn is characterized, inter alia, by its establishment of a significant precedent, model, or methodology; its material adverse effect on the economy or a sector of the economy; its addressing of significant controversial issues; its significant interagency implications; and its consideration of an "innovative" approach for a previously defined problem (EPA Peer Review Handbook, 4th Ed.). In USDA's opinion, all of these factors are present in EPA's latest chlorpyrifos risk assessment, indicating that an external peer review of the document is warranted. USDA commends EPA for having consulted the SAP three times already on the subject of chlorpyrifos. However, this latest hybrid approach is more than just a refinement or an implementation of previous SAP recommendations. A completely new risk assessment approach is being considered which will have a wide impact on the evaluation chlorpyrifos, as well as other pesticides in the future. USDA strongly urges EPA to present this latest risk assessment to the SAP. Before doing so, EPA should thoughtfully consider and publicly respond to all public comments received on the subject of chlorpyrifos since the 2014 Revised Human Health Risk Assessment was published. This will allow the public to provide an informed opinion at the next SAP meeting, and it will help the SAP in fully understanding the breadth of risk assessment approaches considered by the Agency.

USDA is aware that EPA is under a court-ordered deadline to fully respond to the PANNA/NRDC petition (by issuing a final rule, if necessary) by March 31, 2017. However, USDA strongly feels that EPA should take the necessary time to fully address the SAP concerns and to develop a robust risk assessment. To that end, USDA requests that EPA issue an order denying the petition to revoke chlorpyrifos tolerances and cancel uses. This would allow the Agency to continue its evaluation of chlorpyrifos as part of the ongoing pesticide review process and free from undue litigation-induced pressure, and would not preclude the Agency from taking mitigation action in the future if neurodevelopmental effects related to chlorpyrifos are identified and confirmed.

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Message

From: Miller, Wynne [Miller.Wynne@epa.gov]
Sent: 8/10/2018 7:30:41 PM
To: Guilaran, Yu-Ting [Guilaran.Yu-Ting@epa.gov]
Subject: FW: chlorpyrifos economic analysis
Attachments: Chlorpyrifos Tolerance Revocation EA 2017 02 27_v2.docx

Just FYI … Feb 2017 .. an almost final draft.

From: Kiely, Timothy
Sent: Friday, August 10, 2018 3:20 PM
To: Berwald, Derek <Berwald.Derek@epa.gov>; Wyatt, TJ <Wyatt.Tj@epa.gov>; Kaul, Monisha <Kaul.Monisha@epa.gov>; Miller, Wynne <Miller.Wynne@epa.gov>
Subject: chlorpyrifos economic analysis

Hello. I have attached what I think is the EA for the final rule. Most of the usage data is 2014.

Tim

Message

From: Nguyen, Khue [Nguyen.Khue@epa.gov]
Sent: 7/25/2018 8:14:04 PM
To: Smith, Charles [Smith.Charles@epa.gov]; Guilaran, Yu-Ting [Guilaran.Yu-Ting@epa.gov]
CC: Friedman, Dana [Friedman.Dana@epa.gov]; Jones, Ricardo [Jones.Ricardo@epa.gov]
Subject: FW: Diazinon - EFED update on water monitoring data analysis
Attachments: Diazinon Water Monitoring Analysis HED Briefing 7-10-2018 .pptx

Flag: Follow up

Hi Yu-Ting and Billy,

Deliberative Process / Ex. 5

Next steps: Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Thanks,

Khue Nguyen
Chemical Review Manager
Risk Management and Implementation Branch 1
Pesticide Reevaluation Division (PRD)
Office of Pesticide Programs, EPA
703-347-0248
Nguyen.khue@epa.gov

From: Antoline, Joshua
Sent: Tuesday, July 10, 2018 10:10 AM
To: Blankinship, Amy <Blankinship.Amy@epa.gov>; White, Katrina <White.Katrina@epa.gov>; Holmes, Jean <Holmes.Jean@epa.gov>; Metzger, Michael <Metzger.Michael@epa.gov>; Drew, Danette <Drew.Danette@epa.gov>; Tadayon, Seyed <tadayon.seyed@epa.gov>; Nguyen, Khue <Nguyen.Khue@epa.gov>; Jones, Ricardo <Jones.Ricardo@epa.gov>; Grable, Melissa <Grable.Melissa@epa.gov>
Cc: Miller, David <Miller.DavidJ@epa.gov>; Hrdy, David <Hrdy.David@epa.gov>; Hassan, Ume <Hassan.Ume@epa.gov>; Dobreniecki, Sarah <Dobreniecki.Sarah@epa.gov>; Sutton, Cheryl <Sutton.Cheryl@epa.gov>; Corbin, Mark <Corbin.Mark@epa.gov>
Subject: RE: Diazinon - EFED update on water monitoring data analysis

Hello all,

Attached please find the slide deck for tomorrow's presentation. If you have any questions please send them my way

-----Original Appointment-----
From: Blankinship, Amy
Sent: Monday, June 4, 2018 1:59 PM
To: Blankinship, Amy; Antoline, Joshua; White, Katrina; Holmes, Jean; Metzger, Michael; Drew, Danette; Tadayon, Seyed; Nguyen, Khue; Jones, Ricardo; Grable, Melissa

Cc: Miller, David; Hrdy, David; Hassan, Ume; Dobreniecki, Sarah; Sutton, Cheryl; Corbin, Mark
Subject: Diazinon - EFED update on water monitoring data analysis
When: Wednesday, July 11, 2018 1:00 PM-2:00 PM (UTC-05:00) Eastern Time (US & Canada).
Where: DCRoomPYS7100/Potomac-Yard-One

Hi,

EFED has been working on an analysis of the water monitoring data for diazinon. We are at a point where it would be good to have a meeting with HED and PRD to bring you up-to-date on our analysis. We are working on a write-up of the analysis that we will share with the chemical team prior to the meeting.

Please let me know if there is anyone else who should attend.

Best Regards,
Amy

Conference phone # Conference Line/Code / Ex. 6
Conference ID: Conference Line/Code / Ex. 6

Diazinon Water Monitoring Analysis

Joshua Antoline

ERB IV

7-11-2018

Deliberative Process / Ex. 5

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Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Message

From: Nguyen, Khue [Nguyen.Khue@epa.gov]
Sent: 7/27/2018 4:58:11 PM
To: Smith, Charles [Smith.Charles@epa.gov]; Guilaran, Yu-Ting [Guilaran.Yu-Ting@epa.gov]
CC: Friedman, Dana [Friedman.Dana@epa.gov]; Jones, Ricardo [Jones.Ricardo@epa.gov]
Subject: RE: Diazinon - EFED update on water monitoring data analysis
Attachments: Diazinon state level use info oct2016.pdf

Flag: Follow up

Hi Billy,

Deliberative Process / Ex. 5

Thanks,

Khue Nguyen
Chemical Review Manager
Risk Management and Implementation Branch 1
Pesticide Reevaluation Division (PRD)
Office of Pesticide Programs, EPA
703-347-0248
Nguyen.khue@epa.gov

From: Smith, Charles
Sent: Thursday, July 26, 2018 7:22 AM
To: Nguyen, Khue <Nguyen.Khue@epa.gov>; Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>
Cc: Friedman, Dana <Friedman.Dana@epa.gov>; Jones, Ricardo <Jones.Ricardo@epa.gov>
Subject: RE: Diazinon - EFED update on water monitoring data analysis

Thanks Khue. If there is more discussion to be had here I have some thoughts…

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Charles "Billy" Smith
Deputy Director
Pesticide Re-Evaluation Division
Office of Pesticide Programs
703-305-0291

From: Nguyen, Khue
Sent: Wednesday, July 25, 2018 4:14 PM
To: Smith, Charles <Smith.Charles@epa.gov>; Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>
Cc: Friedman, Dana <Friedman.Dana@epa.gov>; Jones, Ricardo <Jones.Ricardo@epa.gov>
Subject: FW: Diazinon - EFED update on water monitoring data analysis

Hi Yu-Ting and Billy,

Deliberative Process / Ex. 5

Next steps: Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Thanks,

Khue Nguyen
Chemical Review Manager
Risk Management and Implementation Branch 1
Pesticide Reevaluation Division (PRD)
Office of Pesticide Programs, EPA
703-347-0248
Nguyen.khue@epa.gov

From: Antoline, Joshua
Sent: Tuesday, July 10, 2018 10:10 AM
To: Blankinship, Amy <Blankinship.Amy@epa.gov>; White, Katrina <White.Katrina@epa.gov>; Holmes, Jean <Holmes.Jean@epa.gov>; Metzger, Michael <Metzger.Michael@epa.gov>; Drew, Danette <Drew.Danette@epa.gov>; Tadayon, Seyed <tadayon.seyed@epa.gov>; Nguyen, Khue <Nguyen.Khue@epa.gov>; Jones, Ricardo <Jones.Ricardo@epa.gov>; Grable, Melissa <Grable.Melissa@epa.gov>
Cc: Miller, David <Miller.DavidJ@epa.gov>; Hrdy, David <Hrdy.David@epa.gov>; Hassan, Ume <Hassan.Ume@epa.gov>; Dobreniecki, Sarah <Dobreniecki.Sarah@epa.gov>; Sutton, Cheryl <Sutton.Cheryl@epa.gov>; Corbin, Mark <Corbin.Mark@epa.gov>
Subject: RE: Diazinon - EFED update on water monitoring data analysis

Hello all,

Attached please find the slide deck for tomorrow's presentation. If you have any questions please send them my way

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Sent: Monday, June 4, 2018 1:59 PM
To: Blankinship, Amy; Antoline, Joshua; White, Katrina; Holmes, Jean; Metzger, Michael; Drew, Danette; Tadayon, Seyed; Nguyen, Khue; Jones, Ricardo; Grable, Melissa
Cc: Miller, David; Hrdy, David; Hassan, Ume; Dobreniecki, Sarah; Sutton, Cheryl; Corbin, Mark
Subject: Diazinon - EFED update on water monitoring data analysis
When: Wednesday, July 11, 2018 1:00 PM-2:00 PM (UTC-05:00) Eastern Time (US & Canada).
Where: DCRoomPYS7100/Potomac-Yard-One

Hi,

EFED has been working on an analysis of the water monitoring data for diazinon. We are at a point where it would be good to have a meeting with HED and PRD to bring you up-to-date on our analysis. We are working on a write-up of the analysis that we will share with the chemical team prior to the meeting.

Please let me know if there is anyone else who should attend.

Best Regards,
Amy

Conference phone Conference Line/Code / Ex. 6
Conference ID Conference Line/Code / Ex. 6

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OCT 26 2016

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

SUBJECT: Diazinon (57801) National and State Summary Use and Usage Summary

FROM: Claire Paisley-Jones, Biologist
Science Information and Analysis Branch
BEAD (7503-P)

THRU: Diann Sims, Chief
Science Information and Analysis Branch
BEAD (7503-P)

TO: Kristina Garber, Senior Science Advisor
Environmental Fate and Effects Division

Khue Nguyen, Chemical Review Manager
RMIB 1
Pesticide Re-evaluation Division (7508P)

Peer Review Panel date: October 21, 2016

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Diazinon: National and State Use and Usage Summary

Introduction

The Environmental Protection Agency (EPA) is currently working with the United States Fish and Wildlife Service (USFWS) and National Marine Fisheries Service (NMFS) to develop a method for assessing the risks of chlorpyrifos, diazinon, and malathion to populations of endangered and threatened species. Given that many populations of listed species range over large areas, it is necessary to consider use of pesticides on a landscape scale, rather than simply a field or a small watershed. One consideration involves the percent of the crop in a given area (relevant to a listed specie's range) that is treated with a pesticide. There are uncertainties in extrapolating from national level usage data (as provided in the SLUA), to regional and state level ranges of protected species. In particular, national level data does not distinguish if there are areas of a species' range where usage is greater or less than the average national usage. In order to address these concerns, this document contains a listing of all diazinon registered use sites, and all available estimates of pesticide usage data for diazinon, nationally and by state. The intended use of the data presented here are to inform assumptions about where diazinon is used in the United States, including the extent, variability, and rate of that usage. Additionally, the data may inform assumptions about crops and states where diazinon is likely not being used. The state level estimates of pesticide usage presented here (especially percent crop treated) can be used to inform estimates of the proportion of a species range that may be exposed to diazinon.

The pesticide usage data summarized herein was obtained from both public and private (proprietary) sources. The data were averaged and rounded so that the presented information is not proprietary, business confidential, or a trade secret. The most recent five years of available data were used, 2010-2014, in order to represent current and future use patterns for diazinon. Current use patterns will encompass changes in diazinon use directions implemented based on the 2006 Diazinon Registration Eligibility Decision (RED).

Data Sources

- **Market Research Data (MRD)** – proprietary pesticide usage data from 2010 to 2014. These data are collected and sold by a private market research firm. The data collected provides pesticide usage data for about 60 crops by annual surveys of agricultural users in the continental United States. The survey design targets at least 80 percent of US acreage/production of the surveyed commodities. Survey methodology provides statistically valid results, typically at the state level.
- **United States Department of Agriculture's National Agricultural Statistics Service (NASS)** – publicly available pesticide usage data from 2010 to 2014. NASS data are based on surveys that focus on the top-producing states that together account for the majority of U.S. acres or production of the surveyed commodity. NASS survey design targets a minimum of 80 percent of the acreage/production for every fruit, vegetable, and field crop surveyed. Operation level data are combined during summary and, pending compliance with disclosure rules, published at the state and national levels. NASS collects data for various commodities on a rotating schedule.
- **California Department of Pesticide Regulation (CDPR) Pesticide Use Reporting (PUR)** – publicly available pesticide usage data for 2010 to 2014. The PUR database contains detailed records and summaries of agricultural applications of pesticides on crops based on application permits. All agricultural pesticide use are reported monthly and results are published annually.

The presented usage data are averaged over the number of years of available survey data based on sampling frequency (five years for MRD and CDPR, and 1-2 years for NASS), regardless of whether usage is

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observed in each surveyed year. The presented data may thus underestimate the maximum yearly usage. In certain cases, data are unavailable or withheld. These cases are indicated in the tables:

- Some data sources do not provide all data elements. When a data element is not available this is indicated with a "- -"notation in the relevant column.
- In some cases, not enough samples are available to establish a robust average. This is indicated with the notation "Insufficient number of reports to establish an estimate" or "(S)". Generally this indicates that the chemical is only periodically used by a small number of users.
- If a registered use site is surveyed by one of our data sources but no usage is observed, this is indicated with the notation "Surveyed but no usage reported" across the data columns. Lack of reported usage data for the pesticide on a surveyed crop indicates that there is a very low likelihood that the given pesticide is used on that crop.
- If a registered use site is not surveyed nationally by any of our data sources, this is indicated with the notation "Not Surveyed at National Level" across the data columns.

Summary

Nationally, agricultural diazinon usage has been decreasing for over a decade. Total agricultural acres treated with diazinon has decreased by 97%, since its peak in 2005. Annual pounds applied agriculturally has decreased 85% since its peak in 2003 (Figure 1). During the most recent five years of available survey data (2010 and 2014), over 171,000 pounds of diazinon were applied to over 131,000 acres of agricultural crops annually (Table 1), in 19 states (Figure 2). Approximately 60% of agricultural applications of diazinon are made to five crops (apples, lettuce, onions, tomatoes, and cherries), both in terms of total acres treated and pounds a.i. applied annually. The remaining 40% of diazinon applications are spread over nearly 30 other crops. Further information on national usage of diazinon by crop is available in Table 1. While the vast majority of diazinon is only applied to a handful of crops, examination of the percent of individual crops grown by state that are treated with diazinon indicates that it is an important pest control tool for certain crops in certain states. For instance, an average of 88% of spinach, 58% of onions, and 52% of cabbage crops in Texas were treated with diazinon annually. Further information on percent of crops treated with diazinon by state is available in Table 2.

Approximately 30% of operations that grow ornamentals in outdoor nurseries in the six US states surveyed by NASS are treated with diazinon. While the overall percent of surveyed operations using diazinon has remained relatively constant, the percent of operations treated by state differs from year to year. Between 2006 and 2009, the percent of operations treated with diazinon in California increased from 11% to 23%, while those treated in Texas decreased from 14% to only 1%. Further information on percent of ornamental nurseries treated with diazinon by state is available in Table 3.

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Agricultural Usage

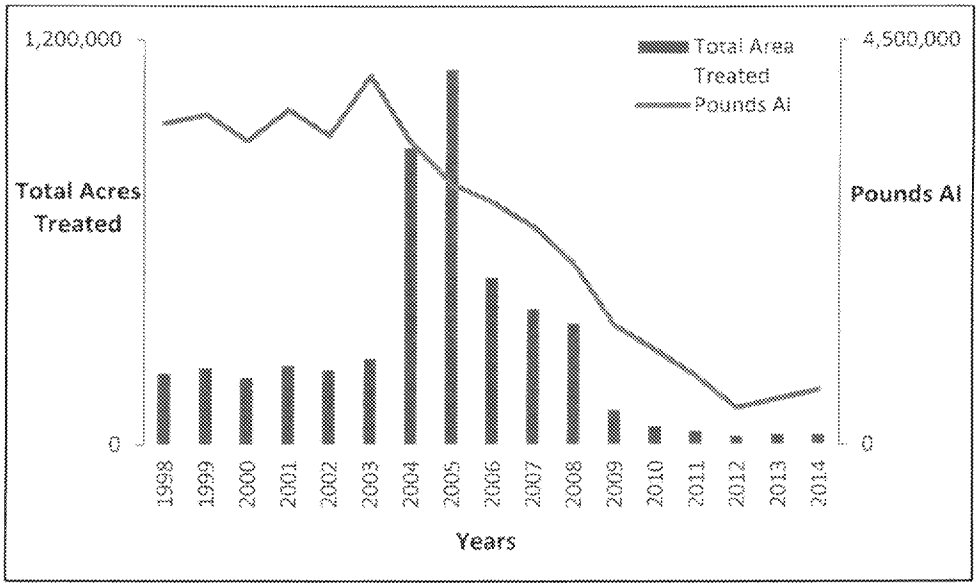

Figure 1. Diazinon Total Acres Treated and Total Pounds A.I. Applied (1998-2014).
Source: *Market Research Data (MRD).* 1998-2014

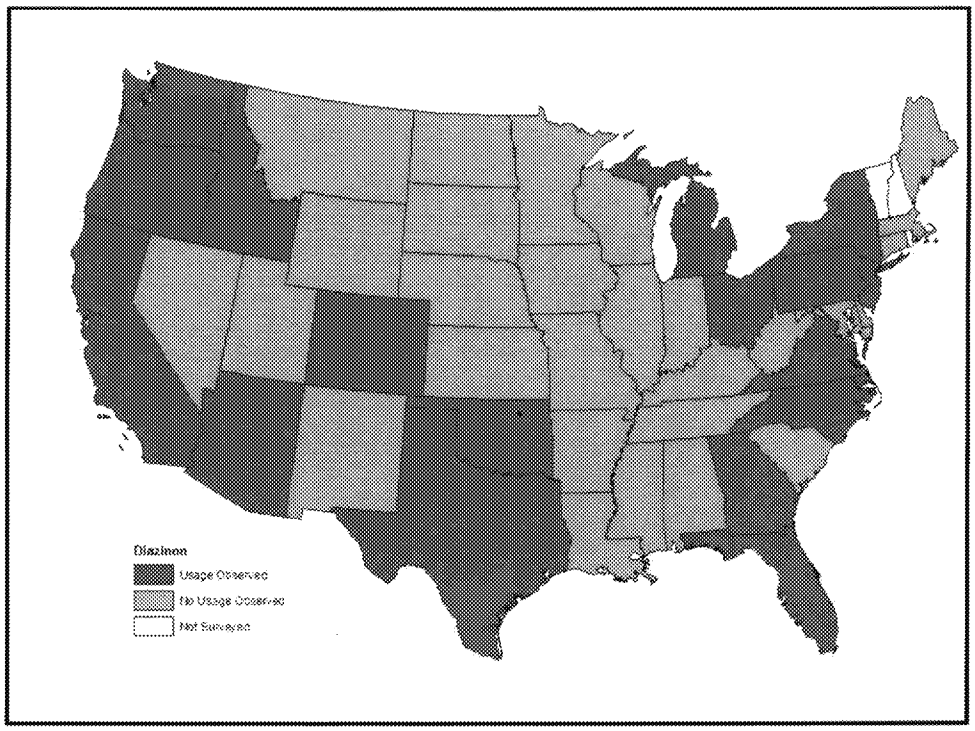

Figure 2. States with observed Diazinon usage (2010-2014).
Source: *Market Research Data (MRD).* 2010-2014
*AK, HI, and US Territories are not surveyed.

Table 1. National Diazinon Agricultural Usage by Crop. Data Averaged Over Reported Years.

Crop	Survey Status	Avg. Annual Pounds AI Applied*	Avg. Annual Total Acres Treated‡	Avg. AI Rate (lb AI/A)	Max Labeled Rate (lb AI/A)
Almonds	MRD (2010-2014)	3,780	2,016	1.87	3.0
Apples	MRD (2010-2014)	26,030	22,900	1.14	2.0
Apricots	MRD (2010-2014)	Insufficient number of reports to establish an estimate.			2.0
Beans (Snap, Bush, Pole, String)	MRD (2010-2014)	Insufficient number of reports to establish an estimate.			4.0
Broccoli	MRD (2010-2014)	4,070	3,660	1.11	4.0
Cabbage	MRD (2010-2014)	8,820	6,320	1.40	4.0
Caneberries	MRD (2010-2014)	7,150	6,650	1.08	2.0
Cantaloupes	MRD (2010-2014)	3,900	5,450	0.72	4.0
Carrots	MRD (2010-2014)	9,970	4,700	2.12	4.0
Cauliflower	MRD (2010-2014)	640	680	0.94	4.0
Cherries	MRD (2010-2014)	13,490	8,500	1.59	2.0
Cucumbers	MRD (2010-2014)	1,590	1,130	1.41	4.0
Dry Beans/Peas	MRD (2010-2014)	Insufficient number of reports to establish an estimate.			4.0
Hazelnut	MRD (2010-2014)	Surveyed but no usage reported			0.5
Lettuce	MRD (2010-2014)	11,120	16,000	0.69	2.0
Lima Beans	MRD (2010-2014)	Insufficient number of reports to establish an estimate.			4.0
Onions	MRD (2010-2014)	25,800	15,360	1.68	4.0
Peaches	MRD (2010-2014)	5,370	3,890	1.38	2.0
Pears	MRD (2010-2014)	1,430	870	1.64	2.0
Peppers	MRD (2010-2014)	1,080	370	2.92	4.0
Plums/Prunes	MRD (2010-2014)	3,470	380	1.46	2.0
Potatoes	MRD (2010-2014)	Insufficient number of reports to establish an estimate.			4.0
Pumpkins	MRD (2010-2014)	610	380	1.60	4.0
Spinach	MRD (2010-2014)	7,850	5,460	1.44	4.0
Squash	MRD (2010-2014)	1,270	1,150	1.10	4.0
Strawberries	MRD (2010-2014)	2,980	4,740	0.63	1.0
Tomatoes	MRD (2010-2014)	23,420	11,750	1.99	4.0
Watermelons	MRD (2010-2014)	4,660	4,530	1.03	4.0
Blueberries	NASS (2011)	7,100	--	0.62	1.0
Honeydew Melon	NASS (2010,2014)	Surveyed but no usage reported			4.0
Brussels Sprouts	CalDPR (2010-2014)	24	32	1.32	4.0

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Crop	Survey Status	Avg. Annual Pounds AI Applied*	Avg. Annual Total Acres Treated‡	Avg. AI Rate (lb AI/A)	Max Labeled Rate (lb AI/A)
Figs	CalDPR (2010-2014)	155	208	1.45	0.5
Garlic	CalDPR (2010-2014)	Insufficient number of reports to establish an estimate.			4.0
Nectarines	CalDPR (2010-2014)	266	140	1.98	2.0
Cranberries	Cranberry Institute (2015)	41,000	--	--	3.0
Beets (Red And Table)	Not Surveyed at National Level				4.0
Casaba Mellon	Not Surveyed at National Level				4.0
Chinese Broccoli	Not Surveyed at National Level				4.0
Chinese Cabbage	Not Surveyed at National Level				4.0
Chinese Mustard	Not Surveyed at National Level				4.0
Chinese Radish Daikon	Not Surveyed at National Level				4.0
Collards	Not Surveyed at National Level				4.0
Crenshaw Melon	Not Surveyed at National Level				4.0
Endive	Not Surveyed at National Level				4.0
Ginseng	Not Surveyed at National Level				0.5
Kale	Not Surveyed at National Level				4.0
Leeks	Not Surveyed at National Level				4.0
Muskmelons	Not Surveyed at National Level				4.0
Mustard Greens	Not Surveyed at National Level				4.0
Parsley	Not Surveyed at National Level				4.0
Parsnips	Not Surveyed at National Level				0.996
Persian Melons	Not Surveyed at National Level				4.0
Pineapple	Not Surveyed at National Level				1.0
Radishes	Not Surveyed at National Level				4.0
Rutabagas	Not Surveyed at National Level				4.0
Shallots	Not Surveyed at National Level				4.0
Sweet Potatoes	Not Surveyed at National Level				4.0
Swiss Chard	Not Surveyed at National Level				4.0
Turnips	Not Surveyed at National Level				4.0

Notes	
MRD (YEAR-YEAR)	Surveyed by MRD Data, and Year(s) of data included
NASS (YEAR)	Surveyed by NASS, and Year(s) of data included
Cal DPR (YEAR)	Surveyed by CalDPR and Year(s) of data included Over than 80% of crop grown in California
*	The pounds AI displayed in this document may differ from those displayed in the SLUA and other BEAD documents, because different calculation methods were used.
‡	Total Acres Treated accounts for multiple applications to a single area. This will overestimate the number of acres treated as some acres are treated more than once.

Table 2. Diazinon Agricultural Usage by Crop and State.

Crop	Survey Status	State	Avg. Annual Crop Acres Grown†	Avg. Annual Total Lbs. AI Applied	Min. Annual PCT	Max. Annual PCT	Avg. Annual PCT
Almonds	MRD (2010-2014)	California	947,400	3776	0	0.5	**0.2**
Apples	MRD (2010-2014)	Virginia	13,200	1,670	0	53	**21**
		California	18,700	4,117	7	31	**16**
		Pennsylvania	23,600	4,612	5	24	**15**
		Washington	164,000	14,627	1	10	**5**
		Oregon	5,400	(S)	0	38	**8**
		New York	49,200	724	0.2	3	**2**
		Ohio	5,000	(S)	0	2	**0.3**
		Michigan	44,000	(S)	0	0.2	**0.05**
		NC, WV	Surveyed but no usage reported				
Apricots	MRD (2010-2014)	California	10,800	(S)	0	11	**3**
Beans (Snap, Bush, Pole, String	MRD (2010-2014)	California	9,500	(S)	0	3	**0.6**
		New York	26,800	(S)	0	0.9	**0.2**
		FL, GA, IL, ID, MI, NC, OR, PA, TN, TX, WI	Surveyed but no usage reported				
Broccoli	MRD (2010-2014)	California	121,500	4067	0.4	8	**3**
Cabbage	MRD (2010-2014)	Texas	7,200	6,253	23	74	**52**
		New York	10,400	1,954	0	33	**20**
		Florida	9,700	(S)	0	12	**2**
		California	13,000	(S)	0	3	**2**
		Michigan	3,100	(S)	0	4	**0.9**
		AZ, CO, GA, NC, WI	Surveyed but no usage reported				
Caneberries	MRD (2010-2014)	Oregon	11,800	1,595	0	15	**8**
		Washington	11,600	5,019	0	10	**3**
		California	6,800	(S)	0	10	**3**
Cantaloupes	MRD (2010-2014)	Texas	2,700	(S)	0	57	**18**
		California	37,300	2,884	4	35	**13**
		Florida	2,800	(S)	0	6	**1**
		AZ, GA, ID, NC	Surveyed but no usage reported				
Carrots	MRD (2010-2014)	Washington	7,500	(S)	0	49	**10**
		California	64,900	8,956	0	22	**6**
		Texas	2,900	(S)	0	33	**7**
		MI, WI	Surveyed but no usage reported				
Cauliflower	MRD (2010-2014)	Arizona	3,400	(S)	0	28	**8**
		California	33,600	(S)	0	4	**1**
Cherries	MRD (2010-2014)	California	35,800	10,814	3	45	**18**
		Oregon	17,600	704	0.3	8	**3**
		Washington	43,000	1,972	1	5	**3**
		MI	Surveyed but no usage reported				

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Crop	Survey Status	State	Avg. Annual Crop Acres Grown†	Avg. Annual Total Lbs. AI Applied	Min. Annual PCT	Max. Annual PCT	Avg. Annual PCT
Cucumbers	MRD (2010-2014)	California	8,300	400	0	11	**7**
		Florida	24,500	(S)	0	5	**2**
		GA, MD, MI, MO, NC, SC, TX, WA, WI	Surveyed but no usage reported				
Dry Beans/Peas	MRD (2010-2014)	California	53,300	(S)	0	6	**1**
		CO, ID, MI, MN, MO, NE, NY, ND, TX, WA, WY	Surveyed but no usage reported				
Hazelnut	MRD (2010-2014)	OR	Surveyed but no usage reported				
Lettuce	MRD (2010-2014)	California	204,400	11,115	0.9	20	**6**
		AZ	Surveyed but no usage reported				
Lima Beans	MRD (2010-2014)	California	8,400	(S)	0	21	**4**
		DE, GA, IL, SC, WA, WI	Surveyed but no usage reported				
Onions	MRD (2010-2014)	Texas	11,900	14,644	31	82	**58**
		California	44,600	5,511	0	27	**11**
		New York	9,500	797	1	12	**7**
		Washington	24,300	4,430	0	10	**5**
		Colorado	6,400	165	0	8	**3**
		Oregon	19,900	(S)	0	1	**0.4**
		GA, ID	Surveyed but no usage reported				
Peaches	MRD (2010-2014)	California	54,200	4,706	1	11	**5**
		Pennsylvania	5,100	(S)	0	19	**8**
		Texas	5,500	(S)	0	19	**4**
		AL, GA, IL, MI, NJ, SC, WA	Surveyed but no usage reported				
Pears	MRD (2010-2014)	California	14,100	1,398	0	16	**6**
		Oregon	17,800	(S)	0	0.4	**0.1**
		WA	Surveyed but no usage reported				
Peppers	MRD (2010-2014)	New Jersey	3,500	(S)	0	21	**6**
		California	29,100	(S)	0	2	**0.4**
		Florida	17,500	(S)	0	1	**0.2**
		AZ, GA, NM, NC, OH, TX	Surveyed but no usage reported				
Plums/Prunes	MRD (2010-2014)	California	88,200	3,472	0.6	4	**2**

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Crop	Survey Status	State	Avg. Annual Crop Acres Grown†	Avg. Annual Total Lbs. AI Applied	Min. Annual PCT	Max. Annual PCT	Avg. Annual PCT
Potatoes	MRD (2010-2014)	Idaho	318,800	(S)	0	0.4	**0.1**
		CA, C0, FL, ME, MI,MN, MT, NE, NY, NC, ND, OR, PA, TX, VA, WA, WI	Surveyed but no usage reported				
Pumpkins	MRD (2010-2014)	New Jersey	2,400	(S)	0	23	**8**
		California	5,900	(S)	0	2	**0.4**
		Michigan	6,900	(S)	0	1	**0.2**
		CT, IL, ID, IN, MD, MA, NM, MO, NM, NY, OH, OR, PA, TN, TX, VA, WA, WI	Surveyed but no usage reported				
Spinach	MRD (2010-2014)	Texas	2,300	2,759	69	100	**88**
		New Jersey	3,200	2,373	0	78	**47**
		California	30,800	2,632	0	10	**3**
		Oklahoma	1,800	(S)	0	34	**12**
		AZ, CO	Surveyed but no usage reported				
Squash	MRD (2010-2014)	California	6,300	385	3	16	**8**
		Florida	9,800	(S)	0	12	**5**
		Pennsylvania	800	(S)	0	9	**2**
		Georgia	3,500	(S)	0	6	**1**
		Texas	1,900	(S)	0	5	**1**
		New Jersey	3,100	(S)	0	3	**0.5**
		New York	4,900	(S)	0	1	**0.3**
		CT,MA, MI, NC, OH, OR, SC,TN, WI	Surveyed but no usage reported				
Strawberries	MRD (2010-2014)	California	39,100	2,024	3	14	**9**
		Florida	9,300	(S)	0	35	**9**
		Washington	1,800	(S)	0	24	**7**
		Pennsylvania	1,000	(S)	0	4	**1**
		MI, NY, OR	Surveyed but no usage reported				
Tomatoes	MRD (2010-2014)	Florida	31,800	713	0	27	**11**
		California	302,000	22,704	2	3	**3**
Watermelons	MRD (2010-2014)	Texas	26,800	3,255	0	33	**13**
		California	10,600	1,378	5	19	**11**
		Florida	25,200	(S)	0	2	**0.3**
		AL, AZ, GA, ID, MS, MO, NC, OK, SC	Surveyed but no usage reported				

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Crop	Survey Status	State	Avg. Annual Crop Acres Grown†	Avg. Annual Total Lbs. AI Applied	Min. Annual PCT	Max. Annual PCT	Avg. Annual PCT
Blueberries NASS	(2011)	Washington	--	2,600	34	47	**41**
		Georgia	--	3,350	23	25	**24**
		Oregon	--	950	16	17	**17**
		New Jersey	--	800	8	19	**14**
		Michigan	--	Insufficient number of reports to establish an estimate.			
		North Carolina	--	Insufficient number of reports to establish an estimate.			
Honeydew Melon	NASS (2010,2014)	AZ, CA, TX	Surveyed but no usage reported				
Beets (Red And Table)	CalDPR (2010-2014)	California (12%)	--	160	0.4	1.6	**0.8**
		Other states (88%)	Crop grown in other states, but not surveyed at national level				
Chinese Cabbage	CalDPR (2010-2014)	California (49%)	--	86	0	1	**0.4**
		Other states (51%)	Crop grown in other states, but not surveyed at national level				
Chinese Radish Daikon	CalDPR (2010-2014)	California (62%)	--	38	0	1.1	**1**
		Other states (38%)	Crop grown in other states, but not surveyed at national level				
Collards	CalDPR (2010-2014)	California (2%)	--	5	0	0.3	**0.1**
		Other states (98%)	Crop grown in other states, but not surveyed at national level				
Endive	CalDPR (2010-2014)	California (54%)	--	18	0	0.1	**0.02**
		Other states (46%)	Crop grown in other states, but not surveyed at national level				
Kale	CalDPR (2010-2014)	California (27%)	--	41	0.03	0.2	**0.1**
		Other states (73%)	Crop grown in other states, but not surveyed at national level				
Mustard Greens	CalDPR (2010-2014)	California	--	(S)	0	0.1	**0.012**
		Other states	Crop grown in other states, but not surveyed at national level				
Radishes	CalDPR (2010-2014)	California (12%)	--	56	0.1	1.6	**0.5**
		Other states (88%)	Crop grown in other states, but not surveyed at national level				
Swiss Chard	CalDPR (2010-2014)	California	--	(S)	0	0.002	**0.0003**
		Other states	Crop grown in other states, but not surveyed at national level				
Brussels Sprouts	CalDPR (2010-2014)	California (93%)	--	24	0	1.5	**0.4**
Figs	CalDPR (2010-2014)	California (96%)	--	208	0	7	**2.7**
Garlic	CalDPR (2010-2014)	California (85%)	--	(S)	0	0.3	**0.1**

Crop	Survey Status	State	Avg. Annual Crop Acres Grown†	Avg. Annual Total Lbs. AI Applied	Min. Annual PCT	Max. Annual PCT	Avg. Annual PCT
Nectarines	CalDPR (2010-2014)	California (89%)	--	140	0.3	0.8	**0.6**
Cranberries	Cranberry Institute (2015)	New Jersey	--	400	--	--	**8**
		Oregon	--	1,300	--	--	**27**
		Washington	--	1,500	--	--	**44**
		Massachusetts	--	12,800	--	--	**57**
		Wisconsin	--	25,100	--	--	**58**

Notes	
MRD (YEAR-YEAR)	Surveyed by MRD Data, and Year(s) of data included
NASS (YEAR)	Surveyed by NASS, and Year(s) of data included
Cal DPR (YEAR)	Surveyed by CalDPR and Year(s) of data included Percent of crop grown in California included under state
(S)	Insufficient number of reports to establish an estimate. This indicates that the chemical is only periodically used by a small number of users.
†	CAG represents the total number of acres that are grown of the crop in each state. It is independent of treatment with any pesticide

Percent of Applications Aerial vs Ground

Lettuce is the only crop registered for aerial application. No aerial applications were recorded between 2010 and 2014 for lettuce.

Non-Agricultural Usage:

Table 3. Diazinon Non-Agricultural Usage by Site. 2009.

Crop	Survey Status	State	Avg. Annual Total Lbs. AI Applied	Percent of Operations 2006	Percent of Operations 2009
Ornamentals grown in outdoor nurseries (trees, bushes, herbs, nonflowering plants, flowers, shrubs, vines)	NASS (2006, 2009)	California	--	11	23
		Florida	--	3	3
		Pennsylvania	--	3	3
		Oregon	--	5	1
		Texas	--	14	1
		Michigan	--	2	Insufficient number of reports to establish an estimate.
Cattle	Not Surveyed				

Notes	
NASS (YEAR)	Surveyed by NASS, and Year(s) of data included

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Message

From: Han, Kaythi [Han.Kaythi@epa.gov]
Sent: 3/23/2018 3:39:54 PM
To: Perry, Tracy [Perry.Tracy@epa.gov]
CC: Sherman, Kelly [Sherman.Kelly@epa.gov]; Guilaran, Yu-Ting [Guilaran.Yu-Ting@epa.gov]; Siedschlag, Gregory [Siedschlag.Gregory@epa.gov]
Subject: RE: 2018-0141

Thanks for the update, Tracy. We've been given a deadline of sending the OPP Update in the next 30 minutes even if the docket is not yet live and ready to receive comments. The OPP Update will read as follows:

Comment Period Open on National Marine Fisheries Service's Biological Opinion for Chlorpyrifos, Diazinon, and Malathion

EPA is seeking comment on the National Marine Fisheries Service's (NMFS's) Biological Opinion for the pesticides chlorpyrifos, diazinon, and malathion. EPA is asking for input from stakeholders on NMFS's jeopardy findings, the Reasonable and Prudent Measures (RPMs) and Reasonable and Prudent Alternatives (RPAs), and to solicit additional use and usage information, including comment on:

1) the scientific approaches and data sources used to support the BiOp and reach determinations for the listed species and critical habitat.
2) the RPAs and RPMs. Can they reasonably be implemented? If not, describe why not. Are there different measures that may provide equivalent protection to the ones in the BiOp but result in less impact on pesticide users?
3) national- and state-level use and usage data and information, in particular, information for non-agricultural use sites (e.g., nurseries, managed forests, pasture, rights-of-way, golf courses, and wide-area mosquito control). If possible, provide sources of data that should be considered.

EPA encourages input from pesticide users, registrants, public interest organizations and other interested parties, as well as from state, tribal, and local governments on the RPMs and RPAs. This request for input is necessary because, as a result of the U.S. District Court Western District of Washington's failure to extend NMFS's court-ordered deadline, NMFS had to issue the final BiOp in December 2017 without having received input from the public and applicants (pesticide registrants). This was at odds with EPA's 2013 public stakeholder process for ESA consultations – an open and transparent process supported by the Services, EPA, and USDA.

This input will be received prior to either reinitiating consultation on the BiOp or implementing the measures of BiOp. EPA will evaluate the comments received in determining how it will proceed with respect to the final BiOp, whether the alternatives or measures can reasonably be implemented, and whether there are different measures that may provide equivalent protection but result in less impact on pesticide users.

The Biological Opinion will soon be available for comment in docket EPA-HQ-OPP-2018-0141 at Regulations.gov. To view the Biological Opinion now go to https://www.fisheries.noaa.gov/resource/document/biological-opinion-pesticides-chlorpyrifos-diazinon-and-malathion. EPA will forward to NMFS for its consideration all public comments we receive on this Biological Opinion.

Submit your comments, identified by the docket identification (ID) number EPA-HQ-OPP-2018-0141 and the pesticides to which the Biological Opinion pertains, by one of the following methods:

- **Federal eRulemaking Portal:** Regulations.gov. Follow the online instructions for submitting comments.
- **Mail:** Office of Pesticide Programs (OPP) Docket, Environmental Protection Agency, Mail Code 28221T, 1200 Pennsylvania Ave. NW., Washington, DC 20460.
- **Delivery:** EPA Docket Center (EPA/DC), EPA West, Room 3334, 1301 Constitution Ave. NW., Washington, DC 20460. Such deliveries are only accepted during the Docket's normal hours of operation, and special arrangements should be made for deliveries of boxed information. Hours of operation are 8:30 a.m. to 4:30 p.m., Monday through Friday, excluding legal holidays. The telephone number is (202) 566-1744.

Kaythi Han
Team Leader, Communications
Office of Pesticide Programs
U.S. Environmental Protection Agency
(703) 305-5642 | han.kaythi@epa.gov

From: Perry, Tracy
Sent: Friday, March 23, 2018 11:32 AM
To: Han, Kaythi <Han.Kaythi@epa.gov>
Cc: Sherman, Kelly <Sherman.Kelly@epa.gov>; Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>
Subject: RE: 2018-0141

Kaythi – I am working with the docket staff now. FDMS is super slow today. As I thought, the problem is that the documents are not live yet and this is a brand new docket – so that is why people can't find it.

From: Han, Kaythi
Sent: Friday, March 23, 2018 10:41 AM
To: Perry, Tracy <Perry.Tracy@epa.gov>
Subject:

https://www.regulations.gov/searchResults?rpp=25&so=DESC&sb=postedDate&po=0&np=0&dct=N%2BFR%2BPR

Kaythi Han
Team Leader, Communications
Office of Pesticide Programs
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(703) 305-5642 | han.kaythi@epa.gov

Message

From: Smith, Charles [Smith.Charles@epa.gov]
Sent: 3/21/2018 4:31:11 PM
To: Guilaran, Yu-Ting [Guilaran.Yu-Ting@epa.gov]; Sherman, Kelly [Sherman.Kelly@epa.gov]
Subject: RE: Action by noon: Mosquito control update for talking pts
Attachments: EPA_AMCA_May2017_NatMtg_smithedits.pptx

There is a lot in here and its old but I think you could use some of this…from last April as a start.

Charles "Billy" Smith
Deputy Director
Pesticide Re-Evaluation Division
Office of Pesticide Programs
703-305-0291

From: Guilaran, Yu-Ting
Sent: Wednesday, March 21, 2018 12:27 PM
To: Sherman, Kelly <Sherman.Kelly@epa.gov>
Cc: Smith, Charles <Smith.Charles@epa.gov>
Subject: RE: Action by noon: Mosquito control update for talking pts

Her request was for mid-afternoon (as stated in the forwarded email) so I built in time for my review. She wanted high-level bullets, and I'm not sure you've delivered that…

Regards,

Yu-Ting Guilaran, P.E.
Director

Pesticide Re-evaluation Division (PRD)
Office of Pesticide Programs
Office of Chemical Safety and Pollution Prevention
(tel) 703 308 0052
(fax)703 308 8005
Mail code 7508P
Room number PY S9623

From: Sherman, Kelly
Sent: Wednesday, March 21, 2018 12:05 PM
To: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>
Cc: Smith, Charles <Smith.Charles@epa.gov>
Subject: RE: Action by noon: Mosquito control update for talking pts

Since it is noon, I will go ahead and forward this info directly to Kaitlin. Thanks.

Kelly Sherman
Chief, Risk Management and Implementation Branch III (RMIB III)
Pesticide Re-Evaluation Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
(703) 305-8401

From: Sherman, Kelly
Sent: Wednesday, March 21, 2018 11:36 AM
To: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>
Cc: Smith, Charles <Smith.Charles@epa.gov>
Subject: RE: Action by noon: Mosquito control update for talking pts

Did you want me to send these directly to Kaitlin, or wait for your approval?

Kelly Sherman
Chief, Risk Management and Implementation Branch III (RMIB III)
Pesticide Re-Evaluation Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
(703) 305-8401

From: Sherman, Kelly
Sent: Wednesday, March 21, 2018 11:07 AM
To: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>
Cc: Smith, Charles <Smith.Charles@epa.gov>
Subject: RE: Action by noon: Mosquito control update for talking pts

Yu-Ting,

There are EPA websites that provide good information on the use of both malathion and naled for wide-area mosquito control. There are no CA specific issues that I am aware of.

Malathion: https://www.epa.gov/mosquitocontrol/malathion

Deliberative Process / Ex. 5

Naled: https://www.epa.gov/mosquitocontrol/naled-mosquito-control##2

Deliberative Process / Ex. 5

Please let me know if you need any more info or if you think that this is sufficient for Kaitlin's needs.

Thanks,
Kelly

Kelly Sherman
Chief, Risk Management and Implementation Branch III (RMIB III)
Pesticide Re-Evaluation Division
Office of Pesticide Programs
U.S. Environmental Protection Agency

(703) 305-8401

From: Guilaran, Yu-Ting
Sent: Wednesday, March 21, 2018 9:31 AM
To: Sherman, Kelly <Sherman.Kelly@epa.gov>
Cc: Smith, Charles <Smith.Charles@epa.gov>
Subject: Action by noon: Mosquito control update for talking pts

Kelly
Please see Kaitlin's request below. I believe you have most of the key mosquitocides. Please, by noon, provide a couple bullets on status of reg review and any relevant issue in CA. Thanks.

Regards,

Yu-Ting Guilaran, P.E.
Director

Pesticide Re-evaluation Division (PRD)
Office of Pesticide Programs
Office of Chemical Safety and Pollution Prevention
(tel) 703 308 0052
(fax)703 308 8005
Mail code 7508P
Room number PY S9623

From: Keller, Kaitlin
Sent: Wednesday, March 21, 2018 9:24 AM
To: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>
Cc: Dinkins, Darlene <Dinkins.Darlene@epa.gov>
Subject: Mosquito control update for talking pts

Morning Yu-Ting,

I am finalizing talking points for the Administrator's trip to California tomorrow and heard that he'll also be talking to mosquito control folks while out there. Are there any upcoming reg review actions for malathion (other than the BiOp), or any other mosquito control actions happening that we should note? We need to send this over by mid-afternoon, so if there's anything to note, a quick bullet or two would be appreciated.

Thanks!
Kaitlin

Kaitlin Keller, Special Assistant
Office of Chemical Safety and Pollution Prevention
U.S. Environmental Protection Agency
(202) 564-7098

Evaluation Of Mosquito Control Pesticides

Jeff Dawson & Charles (Billy) Smith
U.S. EPA/Office of Pesticide Programs

AMCA 19th Annual Conference
Washington D.C.
5/17/17

ED_002896_00063417-00001

Overview

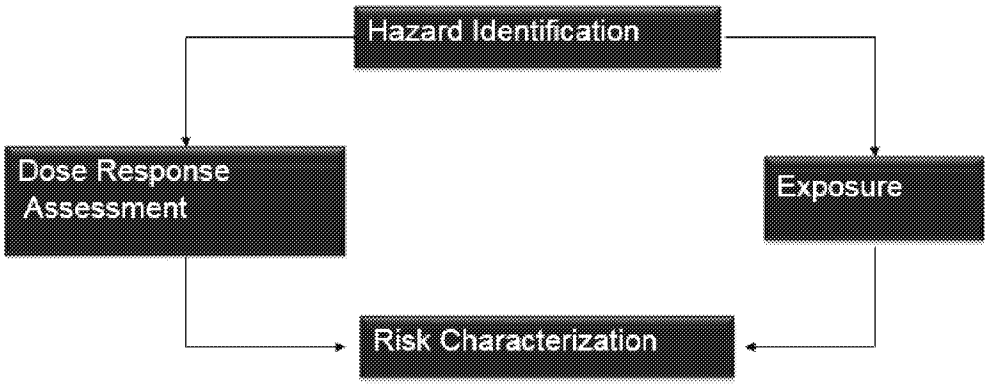
- **Pesticide Risk Assessment** (J Dawson)
  - Malathion As Example Chemical
- **Re-evaluating Mosquito Control Pesticides at EPA** (B Smith)
- **Discussion/Questions**

Risk Assessment Overview

- Pesticide Risk Assessment
  - Malathion As Example Chemical
- Impact of Different Factors On Risk

Basic Approach For Risk Assessment*

Risk is a function of toxicity and exposure and is considered as follows:



*From the National Research Council's *Risk Assessment in the Federal Government: Managing the Process,* 1983.

Hazard Identification

<u>The process quantifies toxicity of a pesticide</u>

- Considers various types of exposure
- Battery of studies required
  - Many types of health effects considered
    - e.g., developmental, reproductive, cancer, neurological, etc.
  - Well established testing guidelines
  - Different species, durations
  - All applicable lifestages
- Outcomes are:
  - Endpoint (the toxic effect of concern)
  - Point of Departure (dose for risk assessment)

Defining a Level of Concern

- Typically at levels where we do not anticipate any effect at 100 times (or more, depending) lower than the POD
- Series of factors (each up to 10) to account for the following:
  - for use of animal data to apply to humans (inter-species)
  - sensitivity among people varies (intra species)
  - to protect children unless evidence supports it is not needed (FQPA)
  - others can be applied for missing data
- Applied in different combinations depending upon scenario

Types of Exposures Considered

- Oral
  - Food,
  - Drinking water,
  - Incidental ingestion (mouthing behavior in children)
- Dermal
  - Occupational (e.g., applying, harvesting)
  - Residential (e.g., homeowner use, children on lawns)
  - Spray Drift (e.g., lawn contact from adulticides)
- Inhalation
  - Applying pesticide
  - Bystander (those around applications)

How Risks Are Calculated

- Risks are generally expressed in several ways but focus here is for adulticide use

- Adulticide risks are expressed as margin of exposure (MOE)

  MOE = POD (e.g., NOAEL)/Exposure

- Compared to the level of concern (LOC)
  - MOEs < LOC raise a potential concern
  - Indicates a need for possible mitigation and/or further evaluation

Malathion Overview

- Malathion is a wide-spectrum OP insecticide
  - It used on a wide variety of agricultural and non-agricultural use sites
  - It is one of most widely used insecticides
  - Products are available to consumers
  - It is also a mosquito adulticide

Risk assessment available at www.regulations.gov
Docket ID EPA-HQ-OPP-2009-0317

Malathion Hazard Assessment

- Endpoint for oral and dermal exposure based on red blood cell AChE inhibition (precursor for neurological effects)
  - LOC = 1000 (inter-, intra- & FQPA)
- Inhalation risk is based on portal of entry effects – nasal lesions
  - LOC = 3,000 are of concern (3X interspecies, 10X intraspecies, 10X LOAEL to NOAEL UF, 10X FQPA)
- Duration of exposure considerations
  - Typically longer exposure leads to more toxicity
  - Toxicity for malathion peaks between 11 to 21 days (known as steady state)
- Steady-state assessments conducted for adulticides

Malathion Endpoint and POD Summary Table

Table 3.2 Summary of Toxicological Doses and Endpoints and Points of Departure for Malathion for Use in Non-occupational Human Health Risk Assessments

Exposure Scenario	Point of Departure	Uncertainty Factors	Level of Concern for Risk Assessment	Study and Toxicological Effects
Incidental Oral Steady State	POD = 10 mg/kg/day	UF_A = 10X UF_H = 10X FQPA SF = 10X	Non-Occupational LOC for MOE = 1000	CCA Studies (MRID 45566201, 46822201, 47373704) Inhibition of RBC AChE in rat pups (PND 11). $BMDL_{10}$ ranged 9-14 mg/kg/day BMD_{10} ranged 13-18 mg/kg/day
Dermal Steady State (21 days and longer) (All populations)	$BMDL_{10}$ = 80 mg/kg/day	UF_A = 10X UF_H = 10X FQPA SF = 10X	Non-Occupational LOC for MOE = 1000	21-day dermal toxicity study in rabbits (MRID 46790501) Inhibition of RBC AChE in adult rabbits. BMD_{10} = 124 mg/kg/day
Inhalation Short-term (21 days and longer) (All populations)	LOAEL = 0.1 mg/L HEC=0.00941 mg/L HED= 1.25mg/kg/day	UF_A = 3X UF_H = 10X UF_L = 10X FQPA SF = 10X	Non-Occupational LOC for MOE = 3000	90-day inhalation toxicity study (MRID 43266601) Histopathological lesions of the nasal cavity and the larynx in respiratory epithelium. Portal-of-Entry LOAEL= 0.1 mg/L (the lowest dose tested) Systemic AChEI NOAEL= 0.1 mg/L; LOAEL= 0.45 mg/L based on RBC AChEI. $BMDL_{10}$= 0.082/0.049 mg/L (M/F); BMD_{10}= 0.167/.0126 mg/L (M/F).
	Residential handler (adults only): HED=1.25 mg/kg/day Residential outdoor post-application (adults and children): HEC = 0.053 mg/L; 52.7 mg/m^3 Residential by-stander post-application (adults and children): HEC= 0.00941 mg/L; 9.41 mg/m^3			
Cancer (oral, dermal, inhalation)	Classification: "Suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential". Quantification of risk using a non-linear approach (i.e. the chronic reference dose) will adequately protect for all chronic toxicity including carcinogenicity.			

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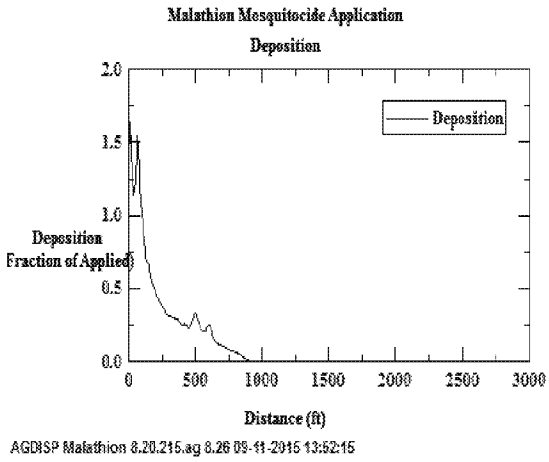
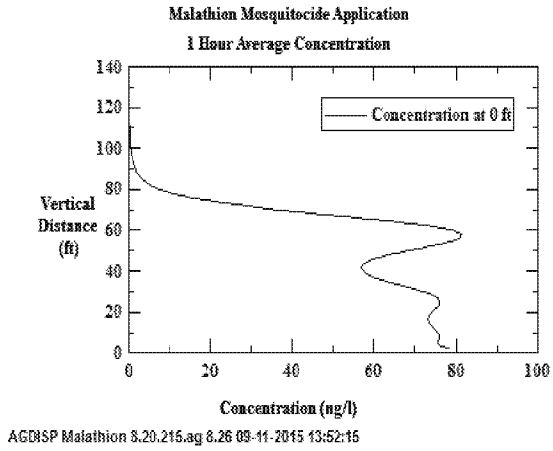
Approach For Adulticide Risks

- Consider exposures for those in treated areas
  - Residents in their yards – children playing outside
- Use peer reviewed methods and monitoring data to:
  - Predict how much pesticide ends up in areas which can result in exposure
    - Aerial – AgDisp model
    - Ground – monitoring data
  - Exposures are then determined for adults and children using standard methods
- Consider applicable application guidance
  - Ground and aerial application methods
  - Label guidance such as spray type (droplets) and aircraft release height

AgDisp Output Examples



Amount deposited



[Air]

Aerial Application Risk Estimates

- Aerial ULV application assessed at current label parameters
  - 100 foot height release
  - 60 µm VMD (Volume Median Diameter of droplets)
  - 1 mph wind speed

- Results indicate that further action may be needed to address exposures for adults and children
  - The lowest estimated MOE (MOE= 93) was calculated for dermal exposure to children 1<2 following an aerial application. Combined with incidental oral, the combined MOE = 80. (LOC =1,000)
  - The lowest MOE (MOE= 930) was calculated for the inhalation exposure to adults and children following ULV application. (LOC = 3,000)

What Do These Represent?

- **Under actual use conditions, there is a range of exposures. The calculated values represent what would be extremely high exposures in this range in order to be protective.**
- **Based On Current Malathion Labels, The Premise Is**:
  - Individuals live in directly treated area
  - Pesticide application evaluated in a way most conducive to highest possible exposure
    - Maximum amount applied per acre
    - Extremely calm weather, little dispersion
    - Droplets are largest allowed, more deposition-bigger objects fall faster
    - Plane height is lowest allowable, less chance to move out of area
    - Nothing to impede drift
  - Focus is children 1-2 years old, they have the highest exposure relative to any age
    - They play rigorously on lawns immediately after treatment, high skin exposure
      - No chance for residue decline but data shows rapid dissipation (<1 day)
    - Children this age put their hands in their mouths which results in oral exposure
      - High rate of contact and transfer each time they put hands in mouth
    - The time they spend in rigorous play is high compared to population
  - Toxicity and the level of concern
    - Basis is biological precursor to more serious effects and the most sensitive
    - Most sensitive children and highest uncertainty for use of animal data used

All conditions must be met simultaneously to experience the calculated risk

Impact of Different Factors On Aerial Application Risks

- Conducted sensitivity analysis to see what parameters had the most impact on exposure and risk
  - Release height
  - Droplet size
- The Agency reached out to CDC and to the mosquito control operators to discuss actual practices and efficacy
  - Increased plane height and lower droplet size would not have a significant impact on efficacy

Release Height and Droplet Size Are Critical

Aerial Mosquitocide Results Including Sensitivity Analysis						
Risk Estimate Scenarios		MOEs				Inhalation Day 0
		Day 0	Day 1	Day 2	Day 3	
Current Label Scenarios						
• 100 foot release • 100% aerial deposition • 60 µm droplet	**Adult dermal**	**340 – 1400 (570)**	**1,400**	**5,800**	**24,000**	**700**
	1< 2 years Dermal + HtM	**170 – 700 (280)**	**700**	**2,900**	**12,000**	
Sensitivity Analysis to Characterize Risks						
• 100 foot release • 33% aerial deposition • 60 µm droplet	Adult dermal	820 – 3,400 (1400)	3,400	14,000	60,000	4,400
	1< 2 years Dermal + HtM	410 – 1,700 (680)	1,700	7,200	30,000	
• 300 foot release • 33% aerial deposition • 60 µm droplet	Adult dermal	1,300 – 5,300 (2,200)	5,400	23,000	94,000	5,300
	1< 2 years Dermal + HtM	650 – 2,700 (1100)	2,700	11,000	47,000	
• 300 foot release • 33% aerial deposition • 45 µm droplet	Adult dermal	1,900 – 7,800 (3200)	7,800	33,000	140,000	6,600
	1< 2 years Dermal + HtM	940 – 3,900 (1,600)	4,000	16,000	68,000	

MOE = Margin of Exposure; Range represents estimated risk based on the highest and lowest turf residues. Values in parenthesis are averages. Dermal/Oral LOC = 1000; Inhalation LOC =3000

Conclusions For Human Health Risk Assessment

- Current label use evaluated for ground foggers indicate no further action needed

- For aerial fogging, results indicate additional action may be needed to address exposures at current label minimum release height (100 feet) and recommended spray droplet criteria (VMD = 60µm)
  - Modifications to application parameters (increased release height to 300 feet and decreased droplet size to 45µm) results in acceptable estimated risk values

Re-Evaluation of Mosquito Pesticides At EPA

- Registration Review
- Mosquito Control Pesticides in Registration Review
- Public Participation in Registration Review
- Registration Review Challenges

EPA's Mission for Pesticides

- Ensure that pesticides are safe and available
- Make best possible regulatory decisions to protect public health, non-target species, and the environment
- Be consistent with core principles
  - Sound science
  - Overwhelming transparency
  - The rule of law

Pesticide Re-evaluation at OPP

- Mandated by law
  - EPA creates a framework for implementing law
- Laws have evolved over time
  - Before 1972, efficacy was only standard for registration, now statute is risk-benefits based
  - Reregistration began in 1988, completed in 2008
  - Registration review began in 2007, completion date of 2022
- Ensure pesticides continue to meet statutory standard of no unreasonable adverse effects
- Function under FIFRA, which is a risk/benefit statute
- Involve sound science, transparency and public involvement
  - Scientific Advisory Panel (SAP)
  - Public involvement and transparency

Registration Review

- Successor program to reregistration
  - FIFRA now requires review of each registered pesticide at least every 15 years to ensure it continues to meet safety standards
- Reviews include national Endangered Species Act (ESA) assessments and endocrine disruption screening program
- Must complete first cycle by 10/1/2022
- Includes over 1,100 active ingredients
- Takes 5-7 years for each pesticide review
- OPP initiates 70 review cases/year

Registration Review: EPA's Role

- Assembles background information, prepares preliminary work plan for case
- Leads focus meeting with registrant(s) to discuss:
  - Areas of uncertainty for risk assessment/decision
  - Label clarity, use/usage information, exposure, other data
- Places preliminary work plan in docket and starts comment period
  - Reviews public comments and any additional information
- Develops final work plan stating if new risk assessments required or additional data needed
  - Issues data call-in (DCI), if needed

Registration Review: Assessment

- If assessment is needed, EPA will
  - Receive and evaluate data, if needed
  - Complete new human health and ecological risk assessment(s), if needed
  - Consult with the Services (Fish and Wildlife Service and National Marine Fisheries Service) if necessary for endangered species concerns
  - Make risk management decisions via Proposed Interim Decision (PID)
  - Publish PID for comment
  - Revise risk management decisions via Interim Decision (ID)
  - Publish ID or final decision document
  - Implement decisions through label amendments

Registration Review: Progress

- All conventional chemicals have had Preliminary Work Plans published
  - Most are have had Final Work Plans published
- Focus thru 2022 will be on:
  - Data evaluation
  - Risk assessment development
  - Risk mitigation via PID and ID development
- 2017 schedule of risk assessments, PIDs, and IDs is available on EPA Registration Review webpage
  - https://www.epa.gov/pesticide-reevaluation/registration-review-schedules

Schedule for Mosquito Control Pesticides

- Pyriproxifen
  - EFED and HED assessments planned for Summer 2017
- Spinosad
  - EFED and HED assessments out in Summer 2016
  - Preliminary Interim Decision planned for Summer 2017
- Malathion
  - EFED and HED assessment out Summer 2016
  - Preliminary Interim Decision planned for 2018
- Naled
  - EFED and HED assessment planned for 2017
- Chlorpyrifos
  - HED Assessment out in December 2017
- Etofenprox
  - EFED assessment out in December 2016, HED assessment planned for Summer 2017
  - Preliminary Interim Decision planned for 2018

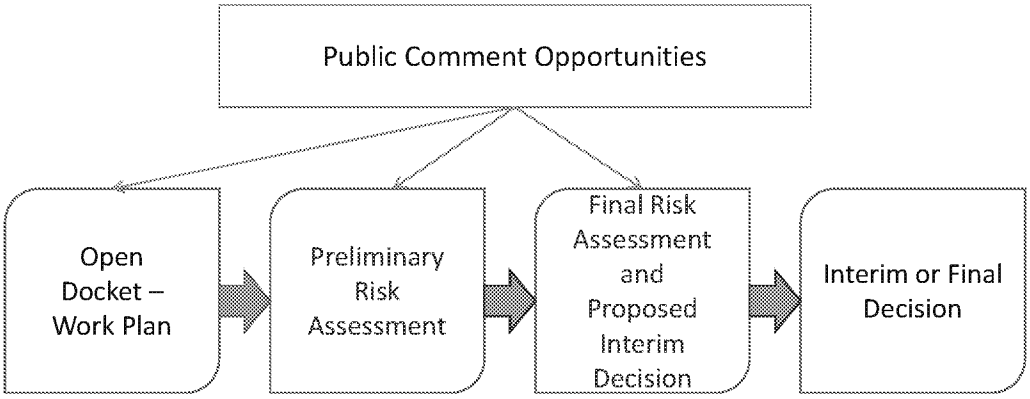
Schedule for Mosquito Control Pesticides

- Phenothrin (Sumithrin)
  - EFED and HED assessments out in December 2016
  - Preliminary Interim Decision planned for 2018
- Prallethrin
  - EFED and HED assessments out in December 2016
  - Preliminary Interim Decision planned for 2018
- Deltamethrin
  - EFED assessment out in December 2016, HED assessment planned for Summer 2017
  - Preliminary Interim Decision planned for 2018
- Pyrethrins
  - EFED assessment out in December 2016, HED assessment planned for Summer 2017
  - Preliminary Interim Decision planned for 2018
- Permethrin
  - EFED assessment out in December 2016, HED assessment planned for Summer 2017
  - Preliminary Interim Decision planned for 2018

Opportunities for Public Participation

- EPA strives to be as transparent as possible and values public input, which:
  - Promotes greater accuracy in decisions
  - Leads to a more robust decision
  - Limits surprises
- Many opportunities for participation and consultation throughout process
  - Especially important for mosquito control products
  - Unique applications that can't be mitigated the same way as typical agricultural products

Registration Review Process and Public Participation



Other Opportunities for Public Participation

- OPP actively collaborates with a variety of stakeholders on science and policy issues
  - FIFRA Scientific Advisory Panel
  - Pesticide Program Dialogue Committee
  - Public health and environmental advocates
  - Pesticide industry
  - Government agencies – all levels
  - International organizations and foreign partners
  - Issue-specific technical experts
  - General public

AMCA Participation: Registration Review

- For registration review, AMCA input will be most valuable in terms of:
  - Label and use patterns will drive risk assessments
    - More detailed use and usage information could refine assessments and ensure more accurate assessments
  - Risk assessment, when geographic locations of use may refine ecological assessments or endangered species assessments
  - Risk mitigation development, letter to AMCA in September on Malathion
  - Risk/Benefits decision, to articulate benefits if EPA needs to make risk/benefits determination

Current Challenges

- Completing first cycle of registration review by 2022
  - Over 200 risk assessments to complete
    - Continuing to evolve science and risk assessment methods
  - Over 250 preliminary interim decisions to complete
    - Develop consistent and useful risk mitigation across similar pesticides
- Implementing the Endocrine Disruptor Screening Program
- Meeting Endangered Species Act obligations
- Finding effective ways to engage the interested parties, particularly on niche products such as those used for mosquito control

Information on EPA's Web Site

- Office of Pesticide Programs

  www.epa.gov/pesticides/
- Registration Review

  www.epa.gov/oppsrrd1/registration_review/
- Focus Meetings

  www.epa.gov/oppsrrd1/registration_review/focus-meetings.html
- Pesticide-specific Information

  www.epa.gov/pesticides/chemicalsearch/

Thank You!

Contact Information	
Jeff Dawson	Charles (Billy) Smith
Deputy Director Health Effects Division	Deputy Director Pesticide Reevaluation Division
Dawson.jeff@epa.gov	Smith.Charles@epa.gov
703-305-7329	703-305-0291

Message

From: Echeverria, Marietta [Echeverria.Marietta@epa.gov]
Sent: 6/12/2018 12:58:59 PM
To: Kunickis, Sheryl - OSEC [Sheryl.Kunickis@osec.usda.gov]; Shultz, Gina [gina_shultz@fws.gov]
CC: Miller, Wynne [Miller.Wynne@epa.gov]; Guilaran, Yu-Ting [Guilaran.Yu-Ting@epa.gov]
Subject: FW: Pesticide usage data
Attachments: Letter from NASS.pdf

FYI

From: Keigwin, Richard
Sent: Tuesday, June 12, 2018 6:13 AM
To: Bertrand, Charlotte <Bertrand.Charlotte@epa.gov>; Beck, Nancy <Beck.Nancy@epa.gov>; Baptist, Erik <Baptist.Erik@epa.gov>; Wise, Louise <Wise.Louise@epa.gov>
Cc: Keller, Kaitlin <keller.kaitlin@epa.gov>; Dinkins, Darlene <Dinkins.Darlene@epa.gov>; Messina, Edward <Messina.Edward@epa.gov>; Echeverria, Marietta <Echeverria.Marietta@epa.gov>; Miller, Wynne <Miller.Wynne@epa.gov>; Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>
Subject: FW: Pesticide usage data

FYI

From: Brett Hartl [mailto:BHartl@biologicaldiversity.org]
Sent: Monday, June 11, 2018 5:35 PM
To: Gary_frazer@fws.gov; Keigwin, Richard <Keigwin.Richard@epa.gov>
Subject: Pesticide usage data

Gary, Rick,

I just wanted to share this letter we received from the USDA-NASS. In essence, it states that NASS data cannot be used or analyzed below that of individual states because the sample size is so small that usage at the county-level would likely reveal the identities of individual pesticide applicants, which is protected by law. USDA also includes a log of "special tabulations" requested by entities other than USDA regarding pesticide use on its website. Neither EPA or USFWS appear to have made any requests to USDA in 2018 regarding the actual use data for any pesticide. Indeed USFWS has never requested any data from USDA in the last six years.

It does make us wonder from where EPA and USFWS intend to augment usage data for the biological opinions. According to EPA's documents, the Biological and Economic Analysis Division only draws its data from NASS, California pesticide usage data and from industry itself. Of course, California requires far greater detail regarding pesticide usage than any other state, so extrapolating based on California may or may not be justified. At the other end of the spectrum, industry hides all of its raw data behind a shield of CBI. One can imagine the conflict of interest and likelihood of deliberate falsification if industry were allowed to provide EPA and USFWS "data" that cannot be verified or subject to independent peer review.

It's all quite interesting, hope you enjoy reading the letter and have a great day,

Brett Hartl
Government Affairs Director
Center *for* Biological Diversity
202-817-8121

MALATHION – Registration Review Status

- Draft Biological Evaluation released for public comment in April 2016
- Draft human health risk assessment released for comment in Sept. 2016
    - National Potato Council did NOT submit comments on HHRA
    - Comments included concerns re: OP 10x SF and use of EPI Data (commenters: USDA, CLA, RISE, IR4, CSCC, Western Growers Association, Cotton Research Protection Council, CA Fresh Fruit Association, National Cotton Council, Minor Crop Framer Alliance, CA Citrus Quality Council, TX TN AKOklahoma Boll Weevil Eradication Foundations, AMCA, several state Depts of Ag, FMC/Cheminova,etc.)
- Final Biological Evaluation released in January 2017
- Proposed Interim Decision anticipated 2019/2020

Malathion ESA Update, 2/27/18

- On December 29, 2017, the National Marine Fisheries Service (NMFS) provided EPA with the final Biological Opinion (BiOp) relative to the potential effects of malathion, chlorpyrifos, and diazinon on federally listed species and their designated critical habitat. NMFS issued the final Biological Opinion in response to a court order although NMFS had sought, but was not provided with, additional time to complete the BiOp. Additional time was needed due to the sheer scope and complexity of technical issues that have arisen during the consultation, as well as the need for more time to assure that the process continues to move forward based on shared and collaborative methodologies.
- EPA is currently reviewing the BiOp and intends to seek public comment on the BiOp, consistent with the 2013 joint Interim Approaches developed by the agencies for assessing risks to listed species from pesticides.
- On January 31, 2018, EPA and the Services signed a Memorandum of Agreement, establishing an Interagency Working Group to involve EPA, FWS, and NMFS. The purpose of the group is to provide recommendations to the three agencies on improving the ESA consultation process for pesticides registration and registration review. USDA, CEQ and OMB will also participate in the Working Group, at the request of the signatory agencies (EPA, DOI, DOC).

[Internal only—not sure if this information is public]

Deliberative Process / Ex. 5

MANCOZEB -- Registration Review Schedule:

- June 2017 – DCI Issued
- June 2019 – Last Study Due Date
- December 2020 – Human Health and Ecological Preliminary Risk Assessments
- September 2021 – Proposed Interim Decision, BEAD Risk/Benefit Analysis
- March 2022 – Interim Decision

NOT ON THE AGENDA, BUT IN CASE IT COMES UP IN THE DISCUSSION…

CHLORPROPHAM – Registration Review Schedule:

- December 2017 – Human Health and Ecological Preliminary Risk Assessment published
- February 13, 2018 – PRA comment period closed
- June 2018 – Proposed Interim Decision, BEAD Risk/Benefit Analysis
- December 2018 – Interim Decision

****The National Potato Council submitted a comment on 2/16/18 on the chlorpropham PRAs.*

- NPC Comment: "Chlorpropham is used on significantly less than 100 per cent of the potato crop …depending on region, **it appears that between 55 percent and 65 per cent of the US potato crop is potentially treated with Chlorpropham**". ***

Deliberative Process / Ex. 5

Phosmet

- Deliberative Process / Ex. 5

Oxamyl

- Deliberative Process / Ex. 5

-
-
-

Deliberative Process / Ex. 5

Diquat Dibromide

-
-
-

Deliberative Process / Ex. 5

-
-

Message

From: Perry, Tracy [Perry.Tracy@epa.gov]
Sent: 6/7/2018 6:46:05 PM
To: Sherman, Kelly [Sherman.Kelly@epa.gov]
CC: Guilaran, Yu-Ting [Guilaran.Yu-Ting@epa.gov]
Subject: response to Kelly Moran re: CA malathion info.

FYI -- see Marietta's response below...in case Kelly contacts you while I am out.

-----Original Message-----
From: Echeverria, Marietta
Sent: Thursday, June 07, 2018 2:35 PM
To: Kelly Moran <kmoran@tdcenvironmental.com>
Cc: TenBrook, Patti <TenBrook.Patti@epa.gov>; Perry, Tracy <Perry.Tracy@epa.gov>
Subject: RE: Quick question - ESA PPDC presentation

Hi Kelly,

Thank you for your note. Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Regards,
Marietta

-----Original Message-----
From: Kelly Moran [mailto:kmoran@tdcenvironmental.com]
Sent: Monday, June 04, 2018 10:02 PM
To: Echeverria, Marietta <Echeverria.Marietta@epa.gov>
Cc: TenBrook, Patti <TenBrook.Patti@epa.gov>
Subject: Quick question - ESA PPDC presentation

Hi Marietta,
Last winter, Patti TenBrook introduced us when you were in the Region 9 office and we had a chat about work that I'm doing with California government agencies to address urban pesticides water pollution (the Urban Pesticides Pollution Prevention Partnership, or UP3 Partnership). I'm writing now to ask a quick question - I saw in your ESA update to the PPDC (slide 7) that EPA is developing use & usage data for the FWS diazinon/chlorpyrifos/malathion BiOP.

Do you know specifically who is doing that?

Deliberative Process / Ex. 5

Thank you!

Kelly Moran
UP3 Partnership

Message

From: Perry, Tracy [Perry.Tracy@epa.gov]
Sent: 2/8/2018 3:37:55 PM
To: Guilaran, Yu-Ting [Guilaran.Yu-Ting@epa.gov]
CC: Smith, Charles [Smith.Charles@epa.gov]; Sherman, Kelly [Sherman.Kelly@epa.gov]
Subject: background on ESA applicant request
Attachments: Request for additional info regarding OP pesticide consultation 1114201....pdf; Response to Request for Additional Info on OP Pesticide Consultation.pdf; Echeverria follow up 12.15.17.pdf; Echeverria follow up 12.17.17.pdf

Yu-Ting,

I'm not sure if you've seen the attached letters between OPP and FWS that led to the request for applicant information (see description of each letter and highlighted information below):

- Deliberative Process / Ex. 5

-Tracy

From: Echeverria, Marietta
Sent: Thursday, January 25, 2018 8:55 AM
To: Perry, Tracy <Perry.Tracy@epa.gov>
Subject: FW: OPP General Follow-up: Timeline for Providing Additional Use and Usage Data to Support ESA Pesticide Consultations

From: Keigwin, Richard
Sent: Wednesday, January 24, 2018 9:18 AM
To: Bertrand, Charlotte <Bertrand.Charlotte@epa.gov>; Beck, Nancy <Beck.Nancy@epa.gov>; Wise, Louise <Wise.Louise@epa.gov>
Cc: Keller, Kaitlin <keller.kaitlin@epa.gov>; Dinkins, Darlene <Dinkins.Darlene@epa.gov>; Echeverria, Marietta <Echeverria.Marietta@epa.gov>; Miller, Wynne <Miller.Wynne@epa.gov>
Subject: FW: OPP General Follow-up: Timeline for Providing Additional Use and Usage Data to Support ESA Pesticide Consultations

A couple of other points:

- Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

From: Keigwin, Richard
Sent: Wednesday, January 24, 2018 7:36 AM
To: Bertrand, Charlotte <Bertrand.Charlotte@epa.gov>; Beck, Nancy <beck.nancy@epa.gov>; Louise Wise (Wise.Louise@epa.gov) <Wise.Louise@epa.gov>
Cc: Keller, Kaitlin <keller.kaitlin@epa.gov>; Darlene Dinkins (Dinkins.Darlene@epa.gov) <Dinkins.Darlene@epa.gov>; Marietta Echeverria (Echeverria.Marietta@epa.gov) <Echeverria.Marietta@epa.gov>; Wynne Miller <Miller.Wynne@epa.gov>
Subject: OPP General Follow-up: Timeline for Providing Additional Use and Usage Data to Support ESA Pesticide Consultations

As a follow-up to yesterday's OPP General, I am attaching the following:

- November 14, 2017, Letter from FWS requesting additional data to support the ongoing consultation for chlorpyrifos, diazinon, and malathion
- November 17, 2017, Letter responding to FWS' request, indicating that EPA would provide the requested use/usage data in approximately 6 months
- December 15, 2017, Letter from FWS acknowledging EPA's November 17, 2017, letter
- December 17, 2017, Letter from correcting an effort in their December 15, 2017, letter

Here is the schedule that we have developed for providing the use/usage data to the Services. We can produce this information on this schedule without significantly impacting BEAD's support for ongoing registration and registration review activities.

- Diazinon: Complete; provided in Fall 2017
- Methomyl: To be completed January 31, 2018 (needed for use in draft BE that will pilot the "strawman" revised methods)
- Carbaryl: To be completed February 28, 2018 ((needed for use in draft BE that will pilot the "strawman" revised methods)
- Chlorpyrifos: To be completed April 30, 2018
- Malathion: To be completed May 31, 2018

Please let me know if you have any questions.
--Rick

Rick Keigwin
Director, Office of Pesticide Programs
US Environmental Protection Agency

Message

From: Friedman, Dana [Friedman.Dana@epa.gov]
Sent: 3/5/2018 11:53:43 AM
To: Guilaran, Yu-Ting [Guilaran.Yu-Ting@epa.gov]
CC: Costello, Kevin [Costello.Kevin@epa.gov]; Zinn, Nicole [Zinn.Nicole@epa.gov]
Subject: FW: Columbia communication issue - follow up
Attachments: LetterDrAndrews_030218 abl_DJM.docx

This is where the conversation on the Columbia email ended last week - David responding to Anna's comments. Did you happen to catch Rick on this before you left on Thursday?

Thanks,
Dana

-----Original Message-----
From: Miller, David
Sent: Friday, March 02, 2018 4:41 PM
To: Friedman, Dana <Friedman.Dana@epa.gov>; Smith, Charles <Smith.Charles@epa.gov>; Dawson, Jeffrey <Dawson.Jeff@epa.gov>; Lowit, Anna <Lowit.Anna@epa.gov>; Vogel, Dana <Vogel.Dana@epa.gov>; Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>; Costello, Kevin <Costello.Kevin@epa.gov>; Zinn, Nicole <Zinn.Nicole@epa.gov>
Subject: RE: Columbia communication issue - follow up

See attached.

-----Original Message-----
From: Friedman, Dana
Sent: Friday, March 02, 2018 11:43 AM
To: Smith, Charles <Smith.Charles@epa.gov>; Dawson, Jeffrey <Dawson.Jeff@epa.gov>; Miller, David <Miller.DavidJ@epa.gov>; Lowit, Anna <Lowit.Anna@epa.gov>; Vogel, Dana <Vogel.Dana@epa.gov>; Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>; Costello, Kevin <Costello.Kevin@epa.gov>; Zinn, Nicole <Zinn.Nicole@epa.gov>
Subject: RE: Columbia communication issue - follow up

I received comments from Anna and Mark, and the attached draft reflects those changes/comments (I have sent the cleanest version for ease of reading, but I can send a redline version if needed). Mark doesn't see any legal issues with the draft or attachment.

David, you will have to weigh in the comments in the attached draft, so please just send the group an updated version of the attachment as soon as possible.

Many thanks,
Dana

-----Original Message-----
From: Friedman, Dana
Sent: Friday, March 02, 2018 9:35 AM
To: Smith, Charles <Smith.Charles@epa.gov>
Cc: Dawson, Jeffrey <Dawson.Jeff@epa.gov>; Miller, David <Miller.DavidJ@epa.gov>; Lowit, Anna <Lowit.Anna@epa.gov>; Vogel, Dana <Vogel.Dana@epa.gov>; Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>
Subject: RE: Columbia communication issue - follow up

Attached are the revisions from Jeff's email (I didn't add the item about additional background, since we indicate that later in the paragraph).

I had sent Mark a note on these documents, so I'll just wait for OGC input before hitting send. I'll keep everyone posted.

Thanks,
Dana

-----Original Message-----
From: Smith, Charles
Sent: Friday, March 02, 2018 9:13 AM
To: Friedman, Dana <Friedman.Dana@epa.gov>
Cc: Dawson, Jeffrey <Dawson.Jeff@epa.gov>; Miller, David <Miller.DavidJ@epa.gov>; Lowit, Anna <Lowit.Anna@epa.gov>; Vogel, Dana <Vogel.Dana@epa.gov>; Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>
Subject: Re: Columbia communication issue - follow up

I'm good with it then. We just wanted everyone to have looked at it and be on the same page. Thanks Jeff.

Sent from my iPhone

> On Mar 2, 2018, at 7:00 AM, Friedman, Dana <Friedman.Dana@epa.gov> wrote:
>
> Adding Billy to the email chain.
>
> -----Original Message-----
> From: Friedman, Dana
> Sent: Friday, March 02, 2018 7:01 AM
> To: Dawson, Jeffrey <Dawson.Jeff@epa.gov>
> Cc: Miller, David <Miller.DavidJ@epa.gov>; Lowit, Anna <Lowit.Anna@epa.gov>; Vogel, Dana
<Vogel.Dana@epa.gov>; Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>
> Subject: RE: Columbia communication issue - follow up
>
> Thanks for taking a look Jeff. I'll hold for others' comments.
>
> -----Original Message-----
> From: Dawson, Jeffrey
> Sent: Thursday, March 01, 2018 5:00 PM
> To: Friedman, Dana <Friedman.Dana@epa.gov>
> Cc: Miller, David <Miller.DavidJ@epa.gov>; Lowit, Anna <Lowit.Anna@epa.gov>; Vogel, Dana
<Vogel.Dana@epa.gov>; Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>
> Subject: Columbia communication issue - follow up
>
> Hi all,
>
> I wanted to follow up from the discussion I had at the end of the day with Yu-Ting and Dana Friedman.

Deliberative Process / Ex. 5

>
> Does this make sense? Just wanted to get something on the table for sake of time. Please comment.
>
> Thanks
>
> Jeff
>
>
> Sent from my iPhone
>
>> On Mar 1, 2018, at 11:04 AM, Friedman, Dana <Friedman.Dana@epa.gov> wrote:
>>
>> Hi all,
>>
>> I've incorporated the feedback I've received on the email, and the attachment has been updated. I'll be sending this along to Columbia at noon.
>>
>> One quick question - the simulated dataset has already been docketed in some capacity, yes? It's my intention to docket both the email and the attachment.
>>
>> Dana
>>
>>
>> Dana L. Friedman
>> Pesticide Re-evaluation Division
>> Office of Pesticide Programs
>> U.S. Environmental Protection Agency
>> 703-347-8827
>>

>>
>> <LetterDrAndrews_022818.ytg.docx>
>> <A synthetic Chlorpyrifos dataset _ Columbia CCCEH_030118.docx>

Message

From: Chun, Melissa [Chun.Melissa@epa.gov]
Sent: 5/1/2018 6:37:30 PM
To: Perry, Tracy [Perry.Tracy@epa.gov]; Guilaran, Yu-Ting [Guilaran.Yu-Ting@epa.gov]
Subject: FW: Title Chlorpyrifos, Diazinon, and Malathion; National Marine Fisheries Service Biological Opinion Issued Under the Endangered Species Act; Extension of Comment Period18P-0099 9977-67
Attachments: NMFSBiOp_EOCP_AIF_2018-05-01.docx

Importance: High

Hi Tracy and Yu-Ting,

Here's a draft action initiation form for your signature package.

I left in the background information from the previous notice, but you can shorten this section if you want to.

-Melissa

Melissa L Chun | US EPA | Regulatory Coordination Staff | Office of Chemical Safety and Pollution Prevention | 1200 Pennsylvania Ave., NW | William Jefferson Clinton Building East Room 3425E, Mail Code 7101M | Washington DC 20460 | phone: (202) 564-1605

From: OCSPP RCS-FRDocuments
Sent: Tuesday, May 01, 2018 9:53 AM
To: Perry, Tracy <Perry.Tracy@epa.gov>
Cc: Hofmann, Angela <Hofmann.Angela@epa.gov>; Chun, Melissa <Chun.Melissa@epa.gov>; Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>; Artis, Sandra-S <artis.sandra-s@epa.gov>
Subject: Title Chlorpyrifos, Diazinon, and Malathion; National Marine Fisheries Service Biological Opinion Issued Under the Endangered Species Act; Extension of Comment Period18P-0099 9977-67
Importance: High

This Email confirms that your FR document has been received by RCS. The initial screening is complete. Your document has been entered into our system, forwarded to the Director to assign an RCS Reviewer to do the typeset review, and it is assigned the following tracking numbers
:

RCS 18P-0099 FRL 9977-67

Once the RCS Director assigns this document, you will receive a follow-up Email from the Director that identifies the RCS Reviewer. That Email will also contain other important information about the processing of your document.

Use the numbers identified above in any subsequent inquiry or communications about this document.

Minnie Lanier
Document Management Specialist
Regulatory Coordination Staff
Office of Chemical Safety and Pollution Prevention
EPA East-West Connecting Wing
Rm. 3385A
RCS Office Telephone: 202-566-1580

From: Perry, Tracy
Sent: Tuesday, May 01, 2018 8:59 AM
To: OCSPP RCS-FRDocuments <OCSPP_RCS-FRDocuments@epa.gov>
Cc: Hofmann, Angela <Hofmann.Angela@epa.gov>; Chun, Melissa <Chun.Melissa@epa.gov>; Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>
Subject: Extension FRN for encoding
Importance: High

Hello,

We believe the publication of the attached FRN extending the comment period on NMFS Biological Opinion is a priority action for Nancy Beck.

Thanks,

Tracy L. Perry
Senior Regulatory Advisor
Risk Management and Implementation Branch III
Pesticide Re-evaluation Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
(703) 308-0128

Action Information Form

SAN: ####; FRL: 9977-67

Action Title: Chlorpyrifos, Diazinon, and Malathion; National Marine Fisheries Service Biological Opinion Issued Under the Endangered Species Act; Extension of Comment Period

Docket No.: EPA-HQ-OPP-2018-0141

Purpose: EPA issued a **Federal Register** notice on March 23, 2018, opening a 60-day comment period for the National Marine Fisheries Service (NMFS) Biological Opinion on chlorpyrifos, diazinon, and malathion. This document extends the comment period for 60 days, from May 22, 2018 to July 23, 2018. EPA is extending the comment period after receipt and consideration of several extension requests citing the length and complexity of the Biological Opinion, and the additional time needed to compile the necessary information requested by EPA.

Background: The Endangered Species Act (ESA) requires Federal agencies to ensure that their actions are not likely to jeopardize the continued existence of species listed as threatened or endangered under the ESA, or destroy or adversely modify the designated critical habitat of such species. The registration of a pesticide under the Federal Insecticide, Fungicide, and Rodenticide Act constitutes an EPA "action" under the ESA. If EPA determines a pesticide may affect a listed species or its designated critical habitat, EPA must initiate consultation with the U.S. Fish and Wildlife Service (FWS) or NMFS (the Service or Services), as appropriate. In response to a Federal agency initiating formal consultation, the Service(s) develops a BiOp in which it provides its opinion on whether the "action" is likely to jeopardize the continued existence of a listed species and/or is likely to destroy or adversely modify designated critical habitat and, if so, describes reasonable and prudent alternatives (RPAs) to avoid the determination. The BiOp will also address whether the action will result in incidental take of listed species and, if so, provide a statement specifying the amount of any permitted incidental take and setting forth reasonable and prudent measures (RPMs) necessary or appropriate to minimize the impact of such take.

Consistent with EPA's responsibility under the ESA, on January 18, 2017, EPA released national-level endangered species Biological Evaluations (BEs) for chlorpyrifos, diazinon, and malathion to assess risks to listed species from registered uses of these pesticides. These BEs were completed in accordance with the joint Interim Approaches developed to implement the recommendations of the April 2013 National Academy of Sciences (NAS) report, *Assessing Risks to Endangered and Threatened Species from Pesticides*. The NAS report outlined recommendations on specific scientific and technical issues related to the development of pesticide risk assessments that EPA and the FWS and NMFS must conduct to meet their obligations under the ESA. In November 2013, the Services, EPA, and the U.S. Department of Agriculture released a white paper containing a summary of their joint Interim Approaches for assessing risks to listed species from pesticides. Details of the joint Interim Approaches are contained in the November 1, 2013 white paper *Interim Approaches for National-Level Pesticide Endangered Species Act Assessments Based on the Recommendations of the National Academy*

of Sciences April 2013 Report. The methods developed as part of the joint Interim Approaches will continue to be vetted before EPA utilizes these methods broadly to meet its ESA obligations.

On December 29, 2017, in response to a court-ordered deadline in the case of *Nw. Coal. for Alternatives to Pesticides, et al. v. NMFS*, Stipulation and Order, Dkt. 50, No. 07-1791-RSL (D. Wash. May 21, 2014), NMFS transmitted to EPA its final BiOp regarding the effects of the registration review under section 3 of FIFRA of these pesticides on listed species. The BiOp addressed the effects of these three pesticides on 77 listed species and 50 designated critical habitats and, in sum, 38 different species would likely be jeopardized with extinction and 37 critical habitat units would be destroyed or adversely modified. NMFS had sought from the court, but was not provided, additional time to complete the BiOp. On January 8, 2018, EPA confirmed receipt of the BiOp and informed NMFS of EPA's intention to reinitiate informal consultation on the BiOp so that the consultation on the pesticides could be informed by (1) input from stakeholders, (2) further interagency discussion and agreement on the jeopardy determination interim methods, and (3) additional data and analysis, including consideration of the best scientific and commercial data available on use and usage information. On February 21, 2018, EPA sent NMFS a letter requesting informal consultation on the same action. EPA will use the information and analysis received and developed in the course of the informal consultation to inform whether formal reinitiation of consultation on the BiOp is appropriate.

EPA issued a **Federal Register** notice on March 23, 2018 which announced the availability of and requested comment on the final BiOp, regarding the potential effects of chlorpyrifos, malathion, and diazinon on federally listed threatened or endangered species (listed species) and their designated critical habitats. The 60-day comment period will close on May 22, 2018. EPA is extending the comment period after receipt and consideration of several extension requests citing the length and complexity of the BiOp and the additional time needed to compile the necessary information requested by EPA. This **Federal Register** notice extends the comment period for 60 days, from May 22, 2018 to July 23, 2018.

Stakeholder Involvement: Stakeholder input will be sought via written comment on the attached notice.

Key Issues/Internal Review: The documents were developed with participation from the program office and the Office of General Counsel.

Deadlines: As soon as possible. This action solicits critical public input on NMFS' BiOp, which should have been completed prior to the finalization of the BiOp in December, 2017.

Timing: Publication in the **Federal Register** should occur before the comment period closes on May 22, 2018.

Contact: Tracy Perry, OPP, (703) 308-0128

Message

From: Perry, Tracy [Perry.Tracy@epa.gov]
Sent: 2/7/2018 6:33:44 PM
To: Guilaran, Yu-Ting [Guilaran.Yu-Ting@epa.gov]
Subject: FW: chlorpyrifos, malathion, diazinon applicants
Attachments: Chlorpyrifos.Malathion.Diazinon.Registrants.docx

See below for more details…

From: Perry, Tracy
Sent: Monday, January 29, 2018 12:03 PM
To: Sherman, Kelly <Sherman.Kelly@epa.gov>; Costello, Kevin <Costello.Kevin@epa.gov>; Anderson, Brian <Anderson.Brian@epa.gov>
Cc: Friedman, Dana <Friedman.Dana@epa.gov>; Javier, Julie <Javier.Julie@epa.gov>; Jakob, Avivah <Jakob.Avivah@epa.gov>; Snyderman, Steven <snyderman.steven@epa.gov>; Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>; Smith, Charles <Smith.Charles@epa.gov>
Subject: FW: chlorpyrifos, malathion, diazinon applicants

Internal***Deliberative***Confidential

Deliberative Process / Ex. 5

Tracy L. Perry
Senior Regulatory Advisor
Risk Management and Implementation Branch III
Pesticide Re-evaluation Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
(703) 308-0128

From: Perry, Tracy
Sent: Monday, January 29, 2018 11:51 AM
To: 'george_noguchi@fws.gov' <george_noguchi@fws.gov>; Patrice Ashfield <patrice_ashfield@fws.gov>; Aubrey, Craig <craig_aubrey@fws.gov>; 'gina_shultz@fws.gov' <gina_shultz@fws.gov>
Cc: Anderson, Brian <Anderson.Brian@epa.gov>; Echeverria, Marietta <Echeverria.Marietta@epa.gov>; Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>
Subject: chlorpyrifos, malathion, diazinon applicants

Hello,

Following up on last Thursday's ESA Pesticide Senior Managers call, I have attached the list of technical registrants for chlorpyrifos, malathion and diazinon. When negotiating pesticide risk reduction measures, EPA routinely works with the technical registrants (i.e., those companies who possess active registrations for products that are solely used to manufacture or formulate other pesticide products, which are referred to as "end-use products"). Working with the technical registrants provides an opportunity for all registrants to be involved in the risk management process.

Please let me know if you have any questions.

Tracy L. Perry
Senior Regulatory Advisor
Risk Management and Implementation Branch III
Pesticide Re-evaluation Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
(703) 308-0128

Message

From: Miller, David [Miller.DavidJ@epa.gov]
Sent: 3/2/2018 5:47:33 PM
To: Friedman, Dana [Friedman.Dana@epa.gov]; Smith, Charles [Smith.Charles@epa.gov]; Dawson, Jeffrey [Dawson.Jeff@epa.gov]; Lowit, Anna [Lowit.Anna@epa.gov]; Vogel, Dana [Vogel.Dana@epa.gov]; Guilaran, Yu-Ting [Guilaran.Yu-Ting@epa.gov]; Costello, Kevin [Costello.Kevin@epa.gov]; Zinn, Nicole [Zinn.Nicole@epa.gov]
Subject: RE: Columbia communication issue - follow up

I have call with FDA at 1, then with USDA/FSIS on Codex at 2:00. Will be able to get to this after that.

David.

-----Original Message-----
From: Friedman, Dana
Sent: Friday, March 02, 2018 11:43 AM
To: Smith, Charles <Smith.Charles@epa.gov>; Dawson, Jeffrey <Dawson.Jeff@epa.gov>; Miller, David <Miller.DavidJ@epa.gov>; Lowit, Anna <Lowit.Anna@epa.gov>; Vogel, Dana <Vogel.Dana@epa.gov>; Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>; Costello, Kevin <Costello.Kevin@epa.gov>; Zinn, Nicole <Zinn.Nicole@epa.gov>
Subject: RE: Columbia communication issue - follow up

I received comments from Anna and Mark, and the attached draft reflects those changes/comments (I have sent the cleanest version for ease of reading, but I can send a redline version if needed). Mark doesn't see any legal issues with the draft or attachment.

David, you will have to weigh in the comments in the attached draft, so please just send the group an updated version of the attachment as soon as possible.

Many thanks,
Dana

-----Original Message-----
From: Friedman, Dana
Sent: Friday, March 02, 2018 9:35 AM
To: Smith, Charles <Smith.Charles@epa.gov>
Cc: Dawson, Jeffrey <Dawson.Jeff@epa.gov>; Miller, David <Miller.DavidJ@epa.gov>; Lowit, Anna <Lowit.Anna@epa.gov>; Vogel, Dana <Vogel.Dana@epa.gov>; Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>
Subject: RE: Columbia communication issue - follow up

Attached are the revisions from Jeff's email (I didn't add the item about additional background, since we indicate that later in the paragraph).

I had sent Mark a note on these documents, so I'll just wait for OGC input before hitting send. I'll keep everyone posted.

Thanks,
Dana

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From: Smith, Charles
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Subject: Re: Columbia communication issue - follow up

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Sent from my iPhone

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Deliberative Process / Ex. 5

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>> Dana
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>>
>> Dana L. Friedman
>> Pesticide Re-evaluation Division
>> Office of Pesticide Programs
>> U.S. Environmental Protection Agency
>> 703-347-8827
>>
>>
>> <LetterDrAndrews_022818.ytg.docx>
>> <A synthetic Chlorpyrifos dataset _ Columbia CCCEH_030118.docx>

Message

From: Friedman, Dana [Friedman.Dana@epa.gov]
Sent: 3/2/2018 4:42:46 PM
To: Smith, Charles [Smith.Charles@epa.gov]; Dawson, Jeffrey [Dawson.Jeff@epa.gov]; Miller, David [Miller.DavidJ@epa.gov]; Lowit, Anna [Lowit.Anna@epa.gov]; Vogel, Dana [Vogel.Dana@epa.gov]; Guilaran, Yu-Ting [Guilaran.Yu-Ting@epa.gov]; Costello, Kevin [Costello.Kevin@epa.gov]; Zinn, Nicole [Zinn.Nicole@epa.gov]
Subject: RE: Columbia communication issue - follow up
Attachments: LetterDrAndrews_030218 abl.docx

I received comments from Anna and Mark, and the attached draft reflects those changes/comments (I have sent the cleanest version for ease of reading, but I can send a redline version if needed). Mark doesn't see any legal issues with the draft or attachment.

David, you will have to weigh in the comments in the attached draft, so please just send the group an updated version of the attachment as soon as possible.

Many thanks,
Dana

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Sent: Friday, March 02, 2018 9:35 AM
To: Smith, Charles <Smith.Charles@epa.gov>
Cc: Dawson, Jeffrey <Dawson.Jeff@epa.gov>; Miller, David <Miller.DavidJ@epa.gov>; Lowit, Anna <Lowit.Anna@epa.gov>; Vogel, Dana <Vogel.Dana@epa.gov>; Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>
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Deliberative Process / Ex. 5

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>> Dana L. Friedman
>> Pesticide Re-evaluation Division
>> Office of Pesticide Programs
>> U.S. Environmental Protection Agency
>> 703-347-8827
>>
>>
>> <LetterDrAndrews_022818.ytg.docx>
>> <A synthetic Chlorpyrifos dataset _ Columbia CCCEH_030118.docx>

Message

From: Lowit, Anna [Lowit.Anna@epa.gov]
Sent: 3/2/2018 3:09:59 PM
To: Friedman, Dana [Friedman.Dana@epa.gov]; Smith, Charles [Smith.Charles@epa.gov]
CC: Dawson, Jeffrey [Dawson.Jeff@epa.gov]; Miller, David [Miller.DavidJ@epa.gov]; Vogel, Dana [Vogel.Dana@epa.gov]; Guilaran, Yu-Ting [Guilaran.Yu-Ting@epa.gov]
Subject: RE: Columbia communication issue - follow up
Attachments: LetterDrAndrews_030218 abl.docx

Edits to the letter to clean it up.

Anna B. Lowit
Senior Science Advisor
Immediate Office
Office of Pesticide Programs
US Environmental Protection Agency
w: +1 703-308-4135
c: +1 703-258-4209

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From: Friedman, Dana
Sent: Friday, March 02, 2018 9:34 AM
To: Smith, Charles <Smith.Charles@epa.gov>
Cc: Dawson, Jeffrey <Dawson.Jeff@epa.gov>; Miller, David <Miller.DavidJ@epa.gov>; Lowit, Anna <Lowit.Anna@epa.gov>; Vogel, Dana <Vogel.Dana@epa.gov>; Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>
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Deliberative Process / Ex. 5

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From: Friedman, Dana [Friedman.Dana@epa.gov]
Sent: 3/2/2018 2:34:07 PM
To: Smith, Charles [Smith.Charles@epa.gov]
CC: Dawson, Jeffrey [Dawson.Jeff@epa.gov]; Miller, David [Miller.DavidJ@epa.gov]; Lowit, Anna [Lowit.Anna@epa.gov]; Vogel, Dana [Vogel.Dana@epa.gov]; Guilaran, Yu-Ting [Guilaran.Yu-Ting@epa.gov]
Subject: RE: Columbia communication issue - follow up
Attachments: LetterDrAndrews_030218.docx; A synthetic Chlorpyrifos dataset _ Columbia CCCEH_ draft.docx

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Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

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>> <LetterDrAndrews_022818.ytg.docx>
>> <A synthetic Chlorpyrifos dataset _ Columbia CCCEH_030118.docx>

Message

From: Nesci, Kimberly [Nesci.Kimberly@epa.gov]
Sent: 3/29/2018 3:39:39 PM
To: Guilaran, Yu-Ting [Guilaran.Yu-Ting@epa.gov]; Miller, Wynne [Miller.Wynne@epa.gov]
Subject: FW: Malathion Usage Data
Attachments: Malathion Extended SUUM.pdf

From: Anderson, Brian
Sent: Thursday, March 29, 2018 11:29 AM
To: Keigwin, Richard <Keigwin.Richard@epa.gov>
Cc: Echeverria, Marietta <Echeverria.Marietta@epa.gov>; Nesci, Kimberly <Nesci.Kimberly@epa.gov>; Corbin, Mark <Corbin.Mark@epa.gov>
Subject: FW: Malathion Usage Data

Hi Rick,

Just wanted to let you know that we sent the malathion usage data that BEAD compiled to FWS today per their request.

Please let me know if you have any questions.

Brian

From: Anderson, Brian
Sent: Thursday, March 29, 2018 9:52 AM
To: Shultz, Gina <gina_shultz@fws.gov>; sheryl.kunickis@osec.usda.gov; Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>; Patrice Ashfield <patrice_ashfield@fws.gov>; cathy.tortorici@noaa.gov; Pease, Anita <Pease.Anita@epa.gov>; Echeverria, Marietta <Echeverria.Marietta@epa.gov>; kayla_miller@fws.gov; Perry, Tracy <Perry.Tracy@epa.gov>; Miller, Wynne (Miller.Wynne@epa.gov) <Miller.Wynne@epa.gov>; Nesci, Kimberly (Nesci.Kimberly@epa.gov) <Nesci.Kimberly@epa.gov>
Subject: Malathion Usage Data

Dear All,

Attached is the usage data on malathion that BEAD put together in response to the FWS request for the information.

Please let us know if you have any questions or need clarification.

Sincerely,

Brian

Message

From: Perry, Tracy [Perry.Tracy@epa.gov]
Sent: 4/17/2018 10:23:45 PM
To: Guilaran, Yu-Ting [Guilaran.Yu-Ting@epa.gov]
Subject: item for ESA senior managers

Yu-Ting,

Deliberative Process / Ex. 5

Tracy L. Perry
Senior Regulatory Advisor
Risk Management and Implementation Branch III
Pesticide Re-evaluation Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
(703) 308-0128

Message

From: Perry, Tracy [Perry.Tracy@epa.gov]
Sent: 2/1/2018 3:44:10 PM
To: Guilaran, Yu-Ting [Guilaran.Yu-Ting@epa.gov]; Smith, Charles [Smith.Charles@epa.gov]
CC: Sherman, Kelly [Sherman.Kelly@epa.gov]; Costello, Kevin [Costello.Kevin@epa.gov]; Anderson, Neil [Anderson.Neil@epa.gov]; Arrington, Linda [Arrington.Linda@epa.gov]; Britton, Cathryn [Britton.Cathryn@epa.gov]
Subject: ESA MOA-Internal***Confidential***Deliberative
Attachments: Executed ESA-FIFRA MOA 1.31.18.pdf

Internal***Confidential***Deliberative

Deliberative Process / Ex. 5

From: Anderson, Brian
Sent: Thursday, February 01, 2018 9:08 AM
To: Perry, Tracy <Perry.Tracy@epa.gov>
Subject:

Message

From: Guilaran, Yu-Ting [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=A698774E93E34A2B9181D4B3032B8A32-YTGUILAR]
Sent: 10/24/2018 11:05:51 AM
To: Keigwin, Richard [Keigwin.Richard@epa.gov]
Subject: RE: Finding time for megasuit prep- settlement talks & information needs

Yes, that would be great! I will double check with Michele and Marietta and put a hold on your calendar.

Regards,

Yu-Ting Guilaran, P.E.
Director

Pesticide Re-evaluation Division (PRD)
Office of Pesticide Programs
Office of Chemical Safety and Pollution Prevention
(tel) 703 308 0052
(fax)703 308 8005
Mail code 7508P
Room number PY S9623

From: Keigwin, Richard
Sent: Wednesday, October 24, 2018 7:05 AM
To: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>
Subject: Re: Finding time for megasuit prep- settlement talks & information needs

It's possible that I could be back by 10:30am tomorrow. Would that work?

Rick Keigwin
Director, Office of Pesticide Programs
U.S. Environmental Protection Agency
Phone: 703-305-7090
Website: www.epa.gov/pesticides
Sent from my iPhone

On Oct 23, 2018, at 4:20 PM, Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov> wrote:

> Hi Rick
> We are trying to find some time Thur afternoon to brainstorm with OGC in preparation for the 11/1 meeting. The only possible slot for you is Thur 2-3 pm during an FEAD all hands meeting. We were wondering if you plan to attend the entire hour or we may use 2:30 to 3 to work with you on this? Thanks.
>
> Regards,
>
> Yu-Ting Guilaran, P.E.
> Director
>
> Pesticide Re-evaluation Division (PRD)
> Office of Pesticide Programs
> Office of Chemical Safety and Pollution Prevention

(tel) 703 308 0052
(fax)703 308 8005
Mail code 7508P
Room number PY S9623

From: Knorr, Michele
Sent: Tuesday, October 23, 2018 3:40 PM
To: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>; Keigwin, Richard <Keigwin.Richard@epa.gov>; Echeverria, Marietta <Echeverria.Marietta@epa.gov>; Perry, Tracy <Perry.Tracy@epa.gov>; Anderson, Brian <Anderson.Brian@epa.gov>
Cc: Perlis, Robert <Perlis.Robert@epa.gov>; Dyner, Mark <dyner.mark@epa.gov>
Subject: RE: megasuit - settlement talks & information needs

So, the Nov. 1st meeting is for EPA to discuss the registration review process and update folks on the ESA consultation process. My hope is that we could talk process and maybe some about how you all are planning your priorities over the next five or so years. The idea here is not to discuss specific dates of actions but to give folks a fuller understanding of the work ahead of you in registration review and ESA consultation generally. Maybe Rick could discuss a little about any meetings under the ESA MOA. I'm happy to have a call with folks this week to discuss this more fully. We are under a court order to have these settlement discussions Attorney Client / Ex. 5

Attorney Client / Ex. 5

Michele L. Knorr, Attorney
Pesticides and Toxic Substances Law Office
Office of General Counsel
202-564-5631

From: Guilaran, Yu-Ting
Sent: Tuesday, October 23, 2018 3:29 PM
To: Knorr, Michele <knorr.michele@epa.gov>; Keigwin, Richard <Keigwin.Richard@epa.gov>; Echeverria, Marietta <Echeverria.Marietta@epa.gov>; Perry, Tracy <Perry.Tracy@epa.gov>; Anderson, Brian <Anderson.Brian@epa.gov>
Cc: Perlis, Robert <Perlis.Robert@epa.gov>; Dyner, Mark <dyner.mark@epa.gov>
Subject: RE: megasuit - settlement talks & information needs

Michele

Can we set up a meeting to discuss DOJ's expectations on the 11/1 face-to-face? If you can give us a few dates/times, we would need Rick's participation as well. Thanks.

Regards,

Yu-Ting Guilaran, P.E.
Director

Pesticide Re-evaluation Division (PRD)
Office of Pesticide Programs

Office of Chemical Safety and Pollution Prevention
(tel) 703 308 0052
(fax)703 308 8005
Mail code 7508P
Room number PY S9623

From: Knorr, Michele
Sent: Tuesday, October 23, 2018 3:19 PM
To: Keigwin, Richard <Keigwin.Richard@epa.gov>; Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>; Echeverria, Marietta <Echeverria.Marietta@epa.gov>; Perry, Tracy <Perry.Tracy@epa.gov>; Anderson, Brian <Anderson.Brian@epa.gov>
Cc: Perlis, Robert <Perlis.Robert@epa.gov>; Dyner, Mark <dyner.mark@epa.gov>
Subject: Fwd: megasuit - settlement talks & information needs

OPP - below is a summary of our initial settlement discussion with CBD today. As we've discussed the next substantive meeting will be on November 1st. DOJ has provided in the summary below the issues that we need to focus on to ensure the next discussions are meaningful. Let's talk later this week about how to start an agenda and times for the November 1 meeting. We plan to bring up the summary of the call at the weekly with OCSPP tomorrow. Thanks

Michele Knorr
OGC
202-564-5631

Begin forwarded message:

> **From:** "McNeil, Bridget (ENRD)" <Bridget.McNeil@usdoj.gov>
> **Date:** October 23, 2018 at 2:55:39 PM EDT
> **To:** "Knorr, Michele" <knorr.michele@epa.gov>
> **Cc:** "Dyner, Mark (dyner.mark@epa.gov)" <dyner.mark@epa.gov>
> **Subject: mega - settlement talks & information needs**
>
> Michele,
>
> Thanks for the conversations earlier this morning on mega, as well as participating with the parties just now in our first settlement call. Please let me know if I missed something in my notes, but I have down that the takeaways and follow-ups/deliverables (along with some editorial comments) are as follows:

Attorney Work Product / Ex. 5

Attorney Work Product / Ex. 5

Thanks!

Bridget Kennedy McNeil, Senior Trial Attorney
Wildlife and Marine Resources Section
Environment and Natural Resources Division
U.S. Department of Justice
999 18th St., Suite 370
Denver, CO 80202
(303) 844-1484
bridget.mcneil@usdoj.gov

Message

From: Guilaran, Yu-Ting [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=A698774E93E34A2B9181D4B3032B8A32-YTGUILAR]
Sent: 8/14/2018 11:35:23 AM
To: Zinn, Nicole [Zinn.Nicole@epa.gov]; Costello, Kevin [Costello.Kevin@epa.gov]
Subject: FYI: Chlorpyrifos 1-Pager
Attachments: Chlorpyrifos Usage 1-pager 8.13.2018 (1).docx

Regards,

Yu-Ting Guilaran, P.E.
Director

Pesticide Re-evaluation Division (PRD)
Office of Pesticide Programs
Office of Chemical Safety and Pollution Prevention
(tel) 703 308 0052
(fax)703 308 8005
Mail code 7508P
Room number PY S9623

From: Keller, Kaitlin
Sent: Monday, August 13, 2018 5:53 PM
To: Miller, Wynne <Miller.Wynne@epa.gov>; Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>; Messina, Edward <Messina.Edward@epa.gov>
Cc: Dinkins, Darlene <Dinkins.Darlene@epa.gov>; Vogel, Dana <Vogel.Dana@epa.gov>; Dawson, Jeffrey <Dawson.Jeff@epa.gov>; Reaves, Elissa <Reaves.Elissa@epa.gov>; Friedman, Dana <Friedman.Dana@epa.gov>; Kiely, Timothy <Kiely.Timothy@epa.gov>; Anderson, Neil <Anderson.Neil@epa.gov>
Subject: RE: Chlorpyrifos 1-Pager

Deliberative Process / Ex. 5

Thanks again!
Kaitlin

From: Miller, Wynne
Sent: Monday, August 13, 2018 4:02 PM
To: Keller, Kaitlin <keller.kaitlin@epa.gov>; Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>; Messina, Edward <Messina.Edward@epa.gov>
Cc: Dinkins, Darlene <Dinkins.Darlene@epa.gov>; Vogel, Dana <Vogel.Dana@epa.gov>; Dawson, Jeffrey <Dawson.Jeff@epa.gov>; Reaves, Elissa <Reaves.Elissa@epa.gov>; Friedman, Dana <Friedman.Dana@epa.gov>; Kiely, Timothy <Kiely.Timothy@epa.gov>; Anderson, Neil <Anderson.Neil@epa.gov>
Subject: RE: Chlorpyrifos 1-Pager

Hi Kaitlyn,

Here's answers to your questions:

Deliberative Process / Ex. 5

Thx
Wynne

From: Keller, Kaitlin
Sent: Monday, August 13, 2018 3:35 PM
To: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>; Messina, Edward <Messina.Edward@epa.gov>
Cc: Dinkins, Darlene <Dinkins.Darlene@epa.gov>; Vogel, Dana <Vogel.Dana@epa.gov>; Dawson, Jeffrey <Dawson.Jeff@epa.gov>; Reaves, Elissa <Reaves.Elissa@epa.gov>; Miller, Wynne <Miller.Wynne@epa.gov>; Friedman, Dana <Friedman.Dana@epa.gov>
Subject: RE: Chlorpyrifos 1-Pager

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5 s?

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5 ?

Deliberative Process / Ex. 5 ?

From: Keller, Kaitlin
Sent: Monday, August 13, 2018 3:29 PM
To: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>; Messina, Edward <Messina.Edward@epa.gov>
Cc: Dinkins, Darlene <Dinkins.Darlene@epa.gov>; Vogel, Dana <Vogel.Dana@epa.gov>; Dawson, Jeffrey <Dawson.Jeff@epa.gov>; Reaves, Elissa <Reaves.Elissa@epa.gov>; Miller, Wynne <Miller.Wynne@epa.gov>; Friedman, Dana <Friedman.Dana@epa.gov>
Subject: RE: Chlorpyrifos 1-Pager

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5? Right now it's a combo a both.

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Sent: Monday, August 13, 2018 3:26 PM
To: Messina, Edward <Messina.Edward@epa.gov>; Keller, Kaitlin <keller.kaitlin@epa.gov>
Cc: Dinkins, Darlene <Dinkins.Darlene@epa.gov>; Vogel, Dana <Vogel.Dana@epa.gov>; Dawson, Jeffrey <Dawson.Jeff@epa.gov>; Reaves, Elissa <Reaves.Elissa@epa.gov>; Miller, Wynne <Miller.Wynne@epa.gov>; Friedman,

Dana <Friedman.Dana@epa.gov>
Subject: RE: Chlorpyrifos 1-Pager

Mine had one minor change…please use mine, thanks.

Regards,

Yu-Ting Guilaran, P.E.
Director

Pesticide Re-evaluation Division (PRD)
Office of Pesticide Programs
Office of Chemical Safety and Pollution Prevention
(tel) 703 308 0052
(fax)703 308 8005
Mail code 7508P
Room number PY S9623

From: Messina, Edward
Sent: Monday, August 13, 2018 3:22 PM
To: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>; Keller, Kaitlin <keller.kaitlin@epa.gov>
Cc: Dinkins, Darlene <Dinkins.Darlene@epa.gov>; Vogel, Dana <Vogel.Dana@epa.gov>; Dawson, Jeffrey <Dawson.Jeff@epa.gov>; Reaves, Elissa <Reaves.Elissa@epa.gov>; Miller, Wynne <Miller.Wynne@epa.gov>; Friedman, Dana <Friedman.Dana@epa.gov>
Subject: RE: Chlorpyrifos 1-Pager

Thanks. Kaitlin it is the same file I sent to you and cc'd Nancy and Charlotte.

_____

Ed Messina
Acting Deputy Office Director (Programs)
Office of Pesticide Programs
U.S. EPA
(703) 347-0209

From: Guilaran, Yu-Ting
Sent: Monday, August 13, 2018 3:21 PM
To: Keller, Kaitlin <keller.kaitlin@epa.gov>
Cc: Messina, Edward <Messina.Edward@epa.gov>; Dinkins, Darlene <Dinkins.Darlene@epa.gov>; Vogel, Dana <Vogel.Dana@epa.gov>; Dawson, Jeffrey <Dawson.Jeff@epa.gov>; Reaves, Elissa <Reaves.Elissa@epa.gov>; Miller, Wynne <Miller.Wynne@epa.gov>; Friedman, Dana <Friedman.Dana@epa.gov>
Subject: RE: Chlorpyrifos 1-Pager

Hello Kaitlin
Please see attached, thanks.

Regards,

Yu-Ting Guilaran, P.E.
Director

Pesticide Re-evaluation Division (PRD)
Office of Pesticide Programs

Office of Chemical Safety and Pollution Prevention
(tel) 703 308 0052
(fax)703 308 8005
Mail code 7508P
Room number PY S9623

From: Dinkins, Darlene
Sent: Monday, August 13, 2018 2:39 PM
To: Vogel, Dana <Vogel.Dana@epa.gov>; Dawson, Jeffrey <Dawson.Jeff@epa.gov>; Reaves, Elissa <Reaves.Elissa@epa.gov>; Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>; Miller, Wynne <Miller.Wynne@epa.gov>
Cc: Messina, Edward <Messina.Edward@epa.gov>
Subject: FW: Chlorpyrifos 1-Pager
Importance: High

Hi everyone,

Just checking on the status of the chlorpyrifos 1-pager. Kaitlin said she has to send something to the DAAs by 3:00 today to allow review time to get it to the Administrator's Office by 4:00 today.

Darlene Dinkins
Office of Pesticide Programs
U.S. Environmental Protection Agency
(703) 305-5214

From: Keller, Kaitlin
Sent: Monday, August 13, 2018 10:28 AM
To: Messina, Edward <Messina.Edward@epa.gov>; Dinkins, Darlene <Dinkins.Darlene@epa.gov>; Keigwin, Richard <Keigwin.Richard@epa.gov>
Subject: Chlorpyrifos 1-Pager
Importance: High

Just learned that a briefing for the Acting Administrator is now scheduled for tomorrow at 4pm, we need to have a briefing paper to the AO by 4 today. Can you send me whatever the team has by 1pm today?

Thanks,
Kaitlin

Kaitlin Keller, Special Assistant
Office of Chemical Safety and Pollution Prevention
U.S. Environmental Protection Agency
(202) 564-7098

Message

From: Guilaran, Yu-Ting [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=A698774E93E34A2B9181D4B3032B8A32-YTGUILAR]
Sent: 8/13/2018 8:05:53 PM
To: Metzger, Michael [Metzger.Michael@epa.gov]
Subject: FW: Chlorpyrifos 1-Pager
Attachments: Chlorpyrifos Administrator 1-pager with Attachments_081318_v2.docx

Regards,

Yu-Ting Guilaran, P.E.
Director

Pesticide Re-evaluation Division (PRD)
Office of Pesticide Programs
Office of Chemical Safety and Pollution Prevention
(tel) 703 308 0052
(fax)703 308 8005
Mail code 7508P
Room number PY S9623

From: Miller, Wynne
Sent: Monday, August 13, 2018 4:02 PM
To: Keller, Kaitlin <keller.kaitlin@epa.gov>; Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>; Messina, Edward <Messina.Edward@epa.gov>
Cc: Dinkins, Darlene <Dinkins.Darlene@epa.gov>; Vogel, Dana <Vogel.Dana@epa.gov>; Dawson, Jeffrey <Dawson.Jeff@epa.gov>; Reaves, Elissa <Reaves.Elissa@epa.gov>; Friedman, Dana <Friedman.Dana@epa.gov>; Kiely, Timothy <Kiely.Timothy@epa.gov>; Anderson, Neil <Anderson.Neil@epa.gov>
Subject: RE: Chlorpyrifos 1-Pager

Hi Kaitlyn,
Here's answers to your questions:

Deliberative Process / Ex. 5

Thx
Wynne

From: Keller, Kaitlin
Sent: Monday, August 13, 2018 3:35 PM
To: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>; Messina, Edward <Messina.Edward@epa.gov>
Cc: Dinkins, Darlene <Dinkins.Darlene@epa.gov>; Vogel, Dana <Vogel.Dana@epa.gov>; Dawson, Jeffrey <Dawson.Jeff@epa.gov>; Reaves, Elissa <Reaves.Elissa@epa.gov>; Miller, Wynne <Miller.Wynne@epa.gov>; Friedman, Dana <Friedman.Dana@epa.gov>
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Deliberative Process / Ex. 5

Deliberative Process / Ex. 5 ?

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Deliberative Process / Ex. 5

Deliberative Process / Ex. 5 ? Right now it's a combo a both.

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Cc: Dinkins, Darlene <Dinkins.Darlene@epa.gov>; Vogel, Dana <Vogel.Dana@epa.gov>; Dawson, Jeffrey <Dawson.Jeff@epa.gov>; Reaves, Elissa <Reaves.Elissa@epa.gov>; Miller, Wynne <Miller.Wynne@epa.gov>; Friedman, Dana <Friedman.Dana@epa.gov>
Subject: RE: Chlorpyrifos 1-Pager

Mine had one minor change…please use mine, thanks.

Regards,

Yu-Ting Guilaran, P.E.
Director

Pesticide Re-evaluation Division (PRD)
Office of Pesticide Programs
Office of Chemical Safety and Pollution Prevention
(tel) 703 308 0052
(fax)703 308 8005
Mail code 7508P
Room number PY S9623

From: Messina, Edward
Sent: Monday, August 13, 2018 3:22 PM
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Cc: Dinkins, Darlene <Dinkins.Darlene@epa.gov>; Vogel, Dana <Vogel.Dana@epa.gov>; Dawson, Jeffrey <Dawson.Jeff@epa.gov>; Reaves, Elissa <Reaves.Elissa@epa.gov>; Miller, Wynne <Miller.Wynne@epa.gov>; Friedman, Dana <Friedman.Dana@epa.gov>
Subject: RE: Chlorpyrifos 1-Pager

Thanks. Kaitlin it is the same file I sent to you and cc'd Nancy and Charlotte.

Ed Messina
Acting Deputy Office Director (Programs)
Office of Pesticide Programs
U.S. EPA
(703) 347-0209

From: Guilaran, Yu-Ting
Sent: Monday, August 13, 2018 3:21 PM
To: Keller, Kaitlin <keller.kaitlin@epa.gov>
Cc: Messina, Edward <Messina.Edward@epa.gov>; Dinkins, Darlene <Dinkins.Darlene@epa.gov>; Vogel, Dana <Vogel.Dana@epa.gov>; Dawson, Jeffrey <Dawson.Jeff@epa.gov>; Reaves, Elissa <Reaves.Elissa@epa.gov>; Miller, Wynne <Miller.Wynne@epa.gov>; Friedman, Dana <Friedman.Dana@epa.gov>
Subject: RE: Chlorpyrifos 1-Pager

Hello Kaitlin
Please see attached, thanks.

Regards,

Yu-Ting Guilaran, P.E.
Director

Pesticide Re-evaluation Division (PRD)
Office of Pesticide Programs
Office of Chemical Safety and Pollution Prevention
(tel) 703 308 0052
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Mail code 7508P
Room number PY S9623

From: Dinkins, Darlene
Sent: Monday, August 13, 2018 2:39 PM
To: Vogel, Dana <Vogel.Dana@epa.gov>; Dawson, Jeffrey <Dawson.Jeff@epa.gov>; Reaves, Elissa <Reaves.Elissa@epa.gov>; Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>; Miller, Wynne <Miller.Wynne@epa.gov>
Cc: Messina, Edward <Messina.Edward@epa.gov>
Subject: FW: Chlorpyrifos 1-Pager
Importance: High

Hi everyone,

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Darlene Dinkins
Office of Pesticide Programs
U.S. Environmental Protection Agency
(703) 305-5214

From: Keller, Kaitlin
Sent: Monday, August 13, 2018 10:28 AM
To: Messina, Edward <Messina.Edward@epa.gov>; Dinkins, Darlene <Dinkins.Darlene@epa.gov>; Keigwin, Richard <Keigwin.Richard@epa.gov>
Subject: Chlorpyrifos 1-Pager
Importance: High

Just learned that a briefing for the Acting Administrator is now scheduled for tomorrow at 4pm, we need to have a briefing paper to the AO by 4 today. Can you send me whatever the team has by 1pm today?

Thanks,
Kaitlin

Kaitlin Keller, Special Assistant
Office of Chemical Safety and Pollution Prevention
U.S. Environmental Protection Agency
(202) 564-7098

Message

From: Guilaran, Yu-Ting [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=A698774E93E34A2B9181D4B3032B8A32-YTGUILAR]
Sent: 10/2/2018 6:01:55 PM
To: Friedman, Dana [Friedman.Dana@epa.gov]
CC: Smith, Charles [Smith.Charles@epa.gov]
Subject: RE: chlorpyrifos

Rick would like to discuss this topic in the next chlorpyrifos OPP meeting, thanks.

Regards,

Yu-Ting Guilaran, P.E.
Director

Pesticide Re-evaluation Division (PRD)
Office of Pesticide Programs
Office of Chemical Safety and Pollution Prevention
(tel) 703 308 0052
(fax)703 308 8005
Mail code 7508P
Room number PY S9623

-----Original Message-----
From: Friedman, Dana
Sent: Tuesday, October 02, 2018 8:26 AM
To: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>
Cc: Smith, Charles <Smith.Charles@epa.gov>
Subject: FW: chlorpyrifos

Yu-Ting,

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5 ?

Many thanks,
Dana

-----Original Message-----
From: Kiely, Timothy
Sent: Tuesday, October 02, 2018 8:23 AM
To: Friedman, Dana <Friedman.Dana@epa.gov>
Cc: Kaul, Monisha <Kaul.Monisha@epa.gov>
Subject: RE: chlorpyrifos

Dana,

Good morning. Please see below for a list of the draft questions for stakeholders. The sooner we go out with these the better.

Tim

USDA OPMP

Deliberative Process / Ex. 5

Sod farms

Deliberative Process / Ex. 5

5. Are there regional differences in the use of chlorpyrifos? Please explain.

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

-----Original Message-----
From: Friedman, Dana
Sent: Monday, October 01, 2018 10:56 AM
To: Kiely, Timothy <Kiely.Timothy@epa.gov>
Subject: chlorpyrifos

Hi Tim,

Nothing much new on chlorpyrifos. I did just want to circle back to see what it will take to scope out the questions that we'd need to get answered by USDA or others regarding the non-ag benefits info, and get that drafted so that once we hear more, we're ready to contact others as needed.

Many thanks,
Dana

Dana L. Friedman
Pesticide Re-evaluation Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
703-347-8827

Message

From: Guilaran, Yu-Ting [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=A698774E93E34A2B9181D4B3032B8A32-YTGUILAR]
Sent: 10/2/2018 2:21:16 PM
To: Friedman, Dana [Friedman.Dana@epa.gov]
CC: Smith, Charles [Smith.Charles@epa.gov]
Subject: RE: chlorpyrifos

Yup, will do, adding it today's general with the IO. Thanks.

Regards,

Yu-Ting Guilaran, P.E.
Director

Pesticide Re-evaluation Division (PRD)
Office of Pesticide Programs
Office of Chemical Safety and Pollution Prevention
(tel) 703 308 0052
(fax)703 308 8005
Mail code 7508P
Room number PY S9623

-----Original Message-----
From: Friedman, Dana
Sent: Tuesday, October 02, 2018 8:26 AM
To: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>
Cc: Smith, Charles <Smith.Charles@epa.gov>
Subject: FW: chlorpyrifos

Yu-Ting,

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5 ?

Many thanks,
Dana

-----Original Message-----
From: Kiely, Timothy
Sent: Tuesday, October 02, 2018 8:23 AM
To: Friedman, Dana <Friedman.Dana@epa.gov>
Cc: Kaul, Monisha <Kaul.Monisha@epa.gov>
Subject: RE: chlorpyrifos

Dana,

Good morning. Please see below for a list of the draft questions for stakeholders. The sooner we go out with these the better.

Tim

USDA OPMP

Deliberative Process / Ex. 5

Sod farms

Deliberative Process / Ex. 5

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Deliberative Process / Ex. 5

-----Original Message-----
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To: Kiely, Timothy <Kiely.Timothy@epa.gov>
Subject: chlorpyrifos

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Many thanks,
Dana

Dana L. Friedman
Pesticide Re-evaluation Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
703-347-8827

Message

From: Guilaran, Yu-Ting [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=A698774E93E34A2B9181D4B3032B8A32-YTGUILAR]
Sent: 8/13/2018 11:24:13 AM
To: Friedman, Dana [Friedman.Dana@epa.gov]
Subject: FYI: chlorpyrifos economic analysis
Attachments: Chlorpyrifos Tolerance Revocation EA 2017 02 27_v2.docx

Regards,

Yu-Ting Guilaran, P.E.
Director

Pesticide Re-evaluation Division (PRD)
Office of Pesticide Programs
Office of Chemical Safety and Pollution Prevention
(tel) 703 308 0052
(fax)703 308 8005
Mail code 7508P
Room number PY S9623

From: Miller, Wynne
Sent: Friday, August 10, 2018 3:31 PM
To: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>
Subject: FW: chlorpyrifos economic analysis

Just FYI … Feb 2017 .. an almost final draft.

From: Kiely, Timothy
Sent: Friday, August 10, 2018 3:20 PM
To: Berwald, Derek <Berwald.Derek@epa.gov>; Wyatt, TJ <Wyatt.Tj@epa.gov>; Kaul, Monisha <Kaul.Monisha@epa.gov>; Miller, Wynne <Miller.Wynne@epa.gov>
Subject: chlorpyrifos economic analysis

Hello. I have attached what I think is the EA for the final rule. Most of the usage data is 2014.

Tim

Message

From: Guilaran, Yu-Ting [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=A698774E93E34A2B9181D4B3032B8A32-YTGUILAR]
Sent: 5/21/2018 5:59:39 PM
To: Perry, Tracy [Perry.Tracy@epa.gov]
Subject: RE: TH call topic?: Malathion--urban use/CA information

Can you come by when you get a chance before 3 so we can chat about this? Thanks.

Regards,

Yu-Ting Guilaran, P.E.
Director

Pesticide Re-evaluation Division (PRD)
Office of Pesticide Programs
Office of Chemical Safety and Pollution Prevention
(tel) 703 308 0052
(fax)703 308 8005
Mail code 7508P
Room number PY S9623

From: Perry, Tracy
Sent: Monday, May 21, 2018 1:59 PM
To: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>
Subject: TH call topic?: Malathion--urban use/CA information

Yu-Ting -- Are you ok with me bringing this topic up at the usage meeting with the Services on Tuesday? Or do you want to run this by Marietta? (see emails below)

From: Perry, Tracy
Sent: Friday, May 18, 2018 3:26 PM
To: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>
Cc: Sherman, Kelly <Sherman.Kelly@epa.gov>
Subject: Malathion--urban use/CA information

Yu-Ting –

I suggested Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

-Tracy

From: Perry, Tracy
Sent: Tuesday, May 15, 2018 11:19 AM

To: Echeverria, Marietta <Echeverria.Marietta@epa.gov>; Garber, Kristina <Garber.Kristina@epa.gov>; Miller, Wynne <Miller.Wynne@epa.gov>; Sims, Diann <Sims.Diann@epa.gov>; Becker, Jonathan <Becker.Jonathan@epa.gov>; Paisley-Jones, Claire <Paisley-Jones.Claire@epa.gov>
Cc: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>; Sherman, Kelly <Sherman.Kelly@epa.gov>; Anderson, Brian <Anderson.Brian@epa.gov>; Jakob, Avivah <Jakob.Avivah@epa.gov>
Subject: Malathion--urban use/CA information

Hi All,

Deliberative Process / Ex. 5

Tracy L. Perry
Senior Regulatory Advisor
Risk Management and Implementation Branch III
Pesticide Re-evaluation Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
(703) 308-0128

Message

From: Guilaran, Yu-Ting [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=A698774E93E34A2B9181D4B3032B8A32-YTGUILAR]
Sent: 3/8/2018 7:36:49 PM
To: Perry, Tracy [Perry.Tracy@epa.gov]
CC: Sherman, Kelly [Sherman.Kelly@epa.gov]
Subject: RE: Notice of public comment for NMFS BiOps
Attachments: final draft.FRN.NMFS.3.08.2018.BiOp.OPs.ytg.docx

One minor edit to change the language back since we are not removing (1) from Section II.C "Comments". After you accepted the change, please forward for encoding. Thank you very much for your thorough and prompt action!

Regards,

Yu-Ting Guilaran, P.E.
Director

Pesticide Re-evaluation Division (PRD)
Office of Pesticide Programs
Office of Chemical Safety and Pollution Prevention
(tel) 703 308 0052
(fax)703 308 8005
Mail code 7508P
Room number PY S9623

From: Perry, Tracy
Sent: Thursday, March 08, 2018 12:48 PM
To: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>
Cc: Sherman, Kelly <Sherman.Kelly@epa.gov>
Subject: RE: Notice of public comment for NMFS BiOps

Yu-Ting,

Here is the revised draft. I believe I've captured the latest from OGC and the IO. Once you've had a final look at this, I'll send it for encoding.

From: Guilaran, Yu-Ting
Sent: Thursday, March 08, 2018 9:21 AM
To: Perry, Tracy <Perry.Tracy@epa.gov>
Cc: Sherman, Kelly <Sherman.Kelly@epa.gov>
Subject: FW: Notice of public comment for NMFS BiOps

Rick is eager to get this going, please work on this as a priority today. Thanks!

Regards,

Yu-Ting Guilaran, P.E.
Director

Pesticide Re-evaluation Division (PRD)
Office of Pesticide Programs

Office of Chemical Safety and Pollution Prevention
(tel) 703 308 0052
(fax)703 308 8005
Mail code 7508P
Room number PY S9623

From: Keigwin, Richard
Sent: Thursday, March 08, 2018 9:00 AM
To: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>; Beck, Nancy <Beck.Nancy@epa.gov>
Subject: RE: Notice of public comment for NMFS BiOps

Nancy mentioned last night that all the comments PRD now has are the only ones to address. Let's move forward with getting the FRN encoded. Thanks!

From: Guilaran, Yu-Ting
Sent: Thursday, March 08, 2018 8:58 AM
To: Beck, Nancy <Beck.Nancy@epa.gov>
Cc: Keigwin, Richard <Keigwin.Richard@epa.gov>
Subject: RE: Notice of public comment for NMFS BiOps

Hi Nancy

Thanks for resolving all the other comments! We've accepted all the comments from USDA and DOI. Just FYI, based on DOI's deletion, we are making one small edit (see blue highlight) to make the section make sense. In today's call with the services, NFMS/commerce metioned that they've also submitted comments through their leadership. I assume you were able to resolve the ones from Commerce? Thanks.

Yu-ting

Deliberative Process / Ex. 5

Regards,

Yu-Ting Guilaran, P.E.
Director

Pesticide Re-evaluation Division (PRD)
Office of Pesticide Programs
Office of Chemical Safety and Pollution Prevention
(tel) 703 308 0052

(fax)703 308 8005
Mail code 7508P
Room number PY S9623

From: Beck, Nancy
Sent: Wednesday, March 07, 2018 12:56 PM
To: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>
Cc: Keigwin, Richard <Keigwin.Richard@epa.gov>
Subject: RE: Notice of public comment for NMFS BiOps

One more comment in the attached. There were a ton more (long story) but we culled them to just this..

Thanks!

Nancy B. Beck, Ph.D., DABT
Deputy Assistant Administrator, OCSPP
P: 202-564-1273
M: 202-731-9910
beck.nancy@epa.gov

From: Guilaran, Yu-Ting
Sent: Monday, March 5, 2018 4:35 PM
To: Beck, Nancy <Beck.Nancy@epa.gov>
Cc: Keigwin, Richard <Keigwin.Richard@epa.gov>
Subject: Re: Notice of public comment for NMFS BiOps

Ok no problem.

Sent from my iPhone

On Mar 5, 2018, at 4:32 PM, Beck, Nancy <Beck.Nancy@epa.gov> wrote:

> Thanks. We gave the group to COB today so we may get a few more comments.
>
> ---
>
> Nancy B. Beck, Ph.D., DABT
> Deputy Assistant Administrator, OCSPP
> P: 202-564-1273
> Personal Phone / Ex. 6
> beck.nancy@epa.gov
>
> ---
>
> **From:** Guilaran, Yu-Ting
> **Sent:** Monday, March 5, 2018 4:31 PM
> **To:** Beck, Nancy <Beck.Nancy@epa.gov>
> **Cc:** Keigwin, Richard <Keigwin.Richard@epa.gov>
> **Subject:** Re: Notice of public comment for NMFS BiOps
>
> Ok. Thanks. We are working on the draft FRN. We will incorporate USDA's comment and will send as soon as it's ready.
>
> Sent from my iPhone

On Mar 5, 2018, at 3:28 PM, Beck, Nancy <Beck.Nancy@epa.gov> wrote:

One suggestion so far.

_____
Nancy B. Beck, Ph.D., DABT
Deputy Assistant Administrator, OCSPP
P: 202-564-1273
Personal Phone / Ex. 6
Beck.Nancy@epa.gov

Begin forwarded message:

From: "Vaden, Stephen - OGC" <Stephen.Vaden@ogc.usda.gov>
Date: March 5, 2018 at 3:25:34 PM EST
To: "Prandoni, Christopher D. EOP/CEQ" EOP / Ex. 6>, "EComstock@doc.gov" <EComstock@doc.gov>, "stu.levenbach@noaa.gov" <stu.levenbach@noaa.gov>, "Moran, John S. EOP/WHO" <John.S.Moran@who.eop.gov>, "todd_willens@ios.doi.gov" <todd_willens@ios.doi.gov>, "dwbernhardt@ios.doi.gov" <dwbernhardt@ios.doi.gov>, "Domesle, Alexander R. EOP/CEQ" EOP / Ex. 6
Cc: "Beck.Nancy@epa.gov" <Beck.Nancy@epa.gov>, "baptist.erik@epa.gov" <baptist.erik@epa.gov>
Subject: RE: Notice of public comment for NMFS BiOps

All,

USDA's comments are attached.

Stephen

From: Prandoni, Christopher D. EOP/CEQ [mailto: EOP / Ex. 6]
Sent: Friday, March 02, 2018 5:21 PM
To: EComstock@doc.gov; stu.levenbach@noaa.gov; Moran, John S. EOP/WHO < EOP / Ex. 6 >; Vaden, Stephen - OGC <Stephen.Vaden@ogc.usda.gov>; todd_willens@ios.doi.gov; dwbernhardt@ios.doi.gov; Domesle, Alexander R. EOP/CEQ < EOP / Ex. 6 >
Cc: Beck.Nancy@epa.gov; baptist.erik@epa.gov
Subject: Notice of public comment for NMFS BiOps

Colleagues - please see Nancy's request below and the attached draft notice of public comment for NMFS's chlorpyrifos, diazinon, and malathion biop. Since this is a short document, let's all get her comments by cob Monday.

Thank you for the quick turnaround.

Chris

Sent from my iPhone

Begin forwarded message:

From: "Beck, Nancy" <Beck.Nancy@epa.gov>
Date: March 2, 2018 at 4:57:45 PM EST
To: "Prandoni, Christopher D. EOP/CEQ
EOP / Ex. 6"
EOP / Ex. 6>
Cc: "Baptist, Erik" <Baptist.Erik@epa.gov>
Subject: Notice of public comment period

Chris,
Attached is the language for our draft FR notice asking for public comments on the BiOps.
Can you please share with the working group—I don't seem to have a good email list of everyone.
Its fine if you want to have them send any comments/suggestions directly to me.
Of course a quick turnaround is appreciated.

Many thanks,
Nancy

****
****
Nancy B. Beck, Ph.D., DABT
Deputy Assistant Administrator
Office of Chemical Safety and Pollution Prevention
P: 202-564-1273
Personal Phone / Ex. 6
beck.nancy@epa.gov

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<Federal Register Notice ESA BiOps with OGC and OPMP edits.docx>

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
CHEMICAL SAFETY AND
POLLUTION PREVENTION

[SEQ CHAPTER \h \r 1]**MEMORANDUM**

Date: December 29, 2016

SUBJECT: Updated Literature Review on Neurodevelopment Effects & FQPA Safety Factor Determination for the Organophosphate Pesticides

PC Code: See Below
Decision No.:
Petition No.: None
Risk Assessment Type: None
TXR No.: 0057252
MRID No.: None

DP Barcode: 437043
Registration No.: None
Regulatory Action: None
Case No.: None
CAS No.: See Below
40 CFR: None

FROM: Ashlee Aldridge
Health Effects Division (7509P)
Office of Pesticide Programs

Anna Lowit, Ph.D., Senior Science Advisory
Immediate Office
Office of Pesticide Programs (7501P)

Virginia C Moser, PhD, DABT, Fellow ATS, Toxicologist
Toxicity Assessment Division
Office of Research and Development

THROUGH: Dana Vogel, Director
Health Effects Division (7509P)

TO: Nicole Zinn, Chemical Review Manager
Neil Anderson, Branch Chief
Risk Management and Implementation Branch 1
Pesticide Re-Evaluation Division (7508P)

This paper supports the use of the 10X FQPA Safety Factor in the individual organophosphate human health risk assessments. This paper updates the September, 2015 paper based on comments from the public and addition of new epidemiology papers from the open literature. No additional studies have been added to the laboratory animal toxicology sections of the literature review.[SEQ CHAPTER \h \r 1][SEQ CHAPTER \h \r 1]

Chemical	PC Code	CAS No.
Dicrotophos	035201	141-66-2
Fosthiazate	129022	98886-44-3
Coumaphos	036501	56-72-4
Terbufos	105001	13071-79-9
Profenofos	111401	41198-08-7
Bensulide	009801	741-58-2
Diazinon	057801	333-41-5
Ethoprop	041101	13194-48-4
Dimethoate	035001	60-51-5
Malathion	057701	121-75-5
Phosmet	059201	732-11-6
Chlorethoxyfos	129006	54593-83-8
Acephate/ Methamidiphos	103301/ 101201	30560-19-1/ 10265-92-6
Pirimiphos-methyl	108102	29232-93-7
TCVP	083701	961-11-5
Tribufos	074801	78-48-8
Phorate	057201	298-02-2
Phostebupirim	129086	96182-53-5
DDVP	084001	62-73-7
Naled	034401	300-76-5
Trichlorfon	057901	52-68-6
Fenamiphos	100601	22224-92-6
AZM	058001	86-50-0
Methidathion	100301	950-37-8
Propetamphos	113601	31218-83-4
ODM	058702	301-12-2
Disulfoton	032501	298-04-4
Methyl parathion	053501	298-00-0
Temephos	059001	3383-96-8
Chlorpyrifos-methyl	059102	5598-13-0

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Updated Literature Review on Neurodevelopment Effects & FQPA Safety Factor Determination for the Organophosphate Pesticides

Office of Pesticide Programs
US Environmental Protection Agency

December 29, 2016

ED_002896_00064133-00003

Table of Contents

[TOC \o "1-3" \h \z \u]

Literature Review on Neurodevelopment Effects & FQPA Safety Factor Determination for the Organophosphate Pesticides

1.0 Introduction and Background

Organophosphate pesticides (OPs), widely used in agricultural and household pesticidal applications, act by inhibiting acetylcholinesterase (AChE) in nerve cells. OPs share the ability to inhibit AChE via phosphorylation of the active site of the enzyme leading to accumulation of acetylcholine and ultimately neurotoxicity, this class of pesticides is subject to assessment of cumulative risk (USEPA, 1999; 2006). Historically the agency has used inhibition of AChE as the point of departure for OP human health risk assessments (HHRAs).

Newer lines of research on OPs in the areas of potential modes of action/adverse outcome pathways (MOAs/AOPs),.[1] *in vivo* animal studies, and notably epidemiological studies in mothers and children, have raised uncertainty about the agency's risk assessment approach with regard to the potential for neurodevelopmental effects in fetuses and children. Many of these studies focus on chlorpyrifos and have been the subject of review by the agency since 2008 (See Appendix 6).

The agency has taken a stepwise, objective and transparent approach in evaluating, interpreting, and characterizing the strengths and uncertainties associated with all of the available lines of scientific information related to the potential for adverse neurodevelopmental effects in infants and children. The stepwise evaluation began with the September 2008 FIFRA Scientific Advisory Panel (SAP) meeting involving a preliminary review of the epidemiology studies on three children's cohorts, with a particular focus on women and children and exposure to chlorpyrifos (USEPA, 2008), followed by the draft "Framework for Incorporating Human Epidemiologic & Incident Data in Health Risk Assessment" for integration of epidemiology with other types of experimental data (USEPA, 2010; FIFRA SAP 2010a,b). In December, 2016, OPP's Framework for Incorporating Human Epidemiologic & Incident Data in Risk Assessments for Pesticides (USEPA, 2016c) was finalized based on input from the 2010 FIFRA SAP, public comment and experience gained over the last few years.

In 2012, the agency convened another meeting of the FIFRA SAP focused on chlorpyrifos which incorporated the newest experimental data related to AChE inhibition and both cholinergic and non-cholinergic adverse outcomes, including neurodevelopmental studies on behavior and cognition effects (FIFRA SAP 2012). Similarly, the agency also performed a more in-depth analysis of the biomonitoring data and of epidemiological studies from three major children's health epidemiology cohort studies in the U.S., as well as plausible hypotheses on MOAs/AOPs

[1] Mode of action (MOA) and adverse outcome pathways (AOPs) describe a set of measureable key events that make up the biological processes leading to an adverse outcome and the causal linkages between such events.

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leading to neurodevelopmental outcomes (USEPA 2012; Appendix 6). Following the 2012 SAP meeting, the agency solicited additional input from federal experts in the areas of Magnetic Resonance Imaging (MRI) and neurobehavioral testing in children to further clarify results obtained by examination of the epidemiological studies.[2] In December, 2014, the agency released "Chlorpyrifos: Revised Human Health Risk Assessment for Registration Review" which included the use of a physiologically-based pharmacokinetic/pharmacodynamic (PBPK-PD) model to derive human PODs, which obviated the need for the animal to human extrapolation factor, and refined intra-species factors for some lifestages (USEPA 2014). The chlorpyrifos 2014 revised HHRA also included retention of the 10X FQPA Safety Factor due to uncertainty regarding the degree of protection the endpoint of AChE inhibition provides for potential neurodevelopmental effects (USEPA, 2014).

A review of the scientific literature on potential MOA/AOP leading to effects on the developing brain was conducted for the 2012 FIFRA SAP meeting (USEPA, 2012) and updated for the 2014 chlorpyrifos revised HHRA (USEPA 2014; Summarized in Appendix 6). In short, multiple biologically plausible hypotheses and pathways are being pursued by researchers including: AChE as a morphogen; cholinergic system; endocannabinoid system; reactive oxygen species; serotonergic system; tubulin, microtubule associated proteins and axonal transport. However, no one pathway has sufficient data to be considered more plausible than the others. Among the available studies, there are effects which are either as sensitive as or more sensitive than AChE inhibition. The fact that there are, however, sparse data to support the *in vitro* to *in vivo* extrapolation, or the extrapolation from biological perturbation to adverse consequence, significantly limits their quantitative use in risk assessment. The SAP concurred with the agency in 2008 and 2012 about the lack of definable key events in a MOA/AOP leading to developmental neurobehavioral effects. Since the 2014 literature review, there have been no substantive changes in the ability to define and quantify steps in an MOA/AOP leading from exposure to effects on the developing brain. The lack of an established MOA/AOP makes quantitative use of the epidemiology study in risk assessment challenging, particularly with respect to dose-response, critical duration of exposure, and window(s) of susceptibility. The agency will continue to monitor the scientific literature for studies on the AOP for neurodevelopmental effects but this document does not include an updated literature review on this line of evidence.

This document (Section 2.0) provides the literature review of *in vivo* laboratory animal studies and epidemiology studies for OPs other than chlorpyrifos to support the single chemical HHRAs. It also provides an integrated weight of evidence (WOE) analysis for all the OPs to support retention of the 10X FQPA Safety Factor (Section 3.0). Section 4.0 states that the 10X FQPA Safety Factor is being retained for all the OPs listed in the table on page 2 above.

[2] [HYPERLINK "http://www.regulations.gov/" \l "!documentDetail;D=EPA-HQ-OPP-2008-0850-0170"]

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2.0 Literature Review

In recent years, the National Academy of Sciences has encouraged the agency to move towards systematic review processes to enhance the transparency of scientific literature reviews that support chemical-specific HHRAs to inform regulatory decision making[3]. The NRC defines systematic review as "a scientific investigation that focuses on a specific question and uses explicit, pre-specified scientific methods to identify, select, assess, and summarize the findings of similar but separate studies"..[4] Consistent with NRC's recommendations, EPA's Office of Chemical Safety and Pollution Prevention (OCSPP) is currently developing systematic review policies and procedures. In short, OCSPP employs fit-for-purpose systematic reviews that rely on standard methods for collecting, evaluating and integrating the scientific data supporting the agency's decisions. The literature review described here uses concepts consistent with systematic review such as detailed tracking of search terms and which literature have been included or excluded.

2.1 Developmental Neurotoxicity (DNT) Research on OPs other than Chlorpyrifos: Laboratory Animal Studies

The literature on neurobehavioral effects of developmental exposure to chlorpyrifos was summarized and discussed at the 2012 FIFRA SAP. More recent studies were added to this summary for the 2014 chlorpyrifos revised HHRA (USEPA, 2014). At that time, the conclusions were that the animal studies clearly showed neurobehavioral outcomes following developmental exposure to chlorpyrifos, but there were inconsistencies in the types of effects reported (neurological domain altered, direction of change, gender specificity). Furthermore, the studies were conducted with doses that most likely produced at least some amount of AChE inhibition at some time during the exposure based on results of guideline studies submitted for registration. The impact of these observations has lead the agency to evaluate whether or not these conclusions extend to other OP pesticides. In this review, the studies of a number of OP pesticides are summarized.

The search aimed to focus on rodent studies involving prenatal/perinatal exposure to OPs in which the offspring were evaluated with *in vivo* neurobehavioral tests. The search methods and analytical scope for this analysis are consistent with the chlorpyrifos analysis from the 2012 FIFRA SAP and 2014 HHRA. Preweaning measurements of behavioral development were noted but not compiled, since these could reflect effects of current exposure to the pesticide rather than long-term neuronal changes. Information on AChE inhibition in either fetuses/pups or dams during this exposure period was evaluated where available. Sections 2.1.1-2.1.3 describe the studies from the open scientific literature. Section 2.1.4 summarizes relevant results from the DNT guidelines studies submitted for pesticide registration (US EPA guideline 870.6300 and/or OECD guideline 426).

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[3] NRC 2011. "Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde" ; NRC 2014. "Review of EPA's Integrated Risk Information System (IRIS) Process"

[4] [HYPERLINK "http://dels.nas.edu/Report/Review-Integrated-Risk/18764"]

2.1.1 Literature Search Strategy & Results

To review and evaluate the developmentally neurotoxic effects of other OPs, a search of the open literature was undertaken. Due to the limited number of studies available, the agency did not limit the search to currently registered OPs. The agency is aware that some OPs listed below are no longer registered for use in the US. In addition, the data evaluation records (DERs) for existing guideline DNT studies were collected from OPP files and summarized. The literature search strategy was developed and conducted by a US EPA reference librarian. Databases searched were PubMed, Web of Science (WoS) and ScienceDirect using key words described below. Duplicates were eliminated after the total database was generated.

1. PubMed (751 results)
(((((organophos* OR Cholinesterase Inhibitors OR acetylcholinesterase inhibitor))) AND ((prenatal OR neonatal OR perinatal OR in utero OR fetal OR foetale OR newborn OR infant* OR postnatal OR gestational OR pregnancy[MeSH Terms]))))) AND ((neurodevelop* OR attention OR birth outcome* OR health outcome* OR cognitive OR cognition OR developmental disability* OR fetal growth OR fetal growth OR foetal development OR fetal development OR sex OR social OR intelligence OR memory OR neurological functioning OR psychomotor OR neurotoxicity OR neurobehavior OR behavior OR learning OR Nervous System[MeSH Terms]) OR Neurotoxicity Syndromes[MeSH Terms]) AND ((((((guinea pigs[MeSH Terms]) OR rabbits[MeSH Terms]) OR mice[MeSH Terms]) OR rats[MeSH Terms]) NOT fishes[MeSH Terms])

2. Web of Science (427 results)
#5 #4 AND #3 AND #2 AND #1
DocType=All document types; Language=All languages;
#4 TS=(guinea pig* OR rabbit* OR mice OR mouse OR rat* OR rodent*) NOT TS=(fish*)
DocType=All document types; Language=All languages;
#3 TS=(neurodevelop* OR attention OR birth outcome* OR health outcome* OR cognitive OR cognition OR developmental disabilit* OR fetal growth OR fetal growth OR foetal development OR fetal development OR sex OR social OR intelligence OR memory OR neurological functioning OR psychomotor OR neurotoxicity OR neurobehavior OR behavior OR learning OR Nervous System OR Neurotoxicity Syndromes)
DocType=All document types; Language=All languages;
#2 TS=(prenatal OR neonatal OR perinatal OR in utero OR fetal OR foetale OR newborn OR infant* OR postnatal OR gestational OR pregnan*)
DocType=All document types; Language=All languages;
#1 TS=(organophos* OR Cholinesterase Inhibitors OR acetylcholinesterase inhibitor OR chlorpyrifos)
DocType=All document types; Language=All languages;

3. Science Direct (19 results)
(ALL(organophos* OR Cholinesterase Inhibitors OR acetylcholinesterase inhibitor OR chlorpyrifos) and ALL(prenatal OR neonatal OR perinatal OR in utero OR fetal OR foetale OR

newborn OR infant* OR postnatal OR gestational OR pregnan*)) AND (neurodevelop* OR attention OR birth outcome* OR health outcome* OR cognitive OR cognition OR developmental disabilit* OR fetal growth OR fetal growth OR foetal development OR fetal development OR sex OR social OR intelligence OR memory OR neurological functioning OR psychomotor OR neurotoxicity OR neurobehavior OR behavior OR learning OR Nervous System OR Neurotoxicity Syndromes) AND (ALL(guinea pig* OR rabbit* OR mice OR mouse OR rat* OR rodent) and not ALL(fish*)).

This broad literature search identified 1012 potential papers, which were reviewed individually. Specific criteria were applied to select suitable studies, as was previously done with chlorpyrifos. Since the literature on chlorpyrifos has been previously reviewed, those papers were excluded here. This resulted in 19 relevant papers with the following specifications:

- Exposure occurred during gestation and/or the postnatal time frame, ending no later than weaning.
- Dosing included maternal and/or pup administration.
- Dosing was via oral or subcutaneous injection. One paper with intracisternal injection was excluded.
- Behavioral testing of the offspring occurred after weaning and/or into adulthood.
- Studies involved only single-chemical exposure, and where two or more chemicals were administered together, only the single-chemical data were included in the summaries.
- Test subjects were rats or mice. Several papers in pigs and rabbits were excluded due to the lack of comparative database for those species.
- The test measures of interest were neurobehavioral endpoints. At least two studies involved only electrophysiological measures, and those were excluded. No neurochemical, genomic, or other molecular endpoints were included.

The OPs examined, and the number of papers for each, are listed below. Of particular interest are studies from one laboratory (Duke University) that included parathion and diazinon, and can be directly compared to studies with chlorpyrifos using similar experimental designs. The majority of studies used rats (13), and exposures periods varied about evenly between gestational and postnatal stages.

- Parathion (5)
- Diazinon (5)
- Methyl parathion (3)
- Methamidophos (2)
- Chlormephos (1)
- Dichlorvos (1)
- Fenitrothion (sumithion) (1)
- Oxydemeton-methyl (demeton-S-methyl, metasystox-R) (1)

These papers dated back to 1968, and there was a wide range in study quality. Shortcomings were noted in almost all papers, including cursory methodological information and presentation of results, inappropriate statistical analyses, contradictory statements, and problematic interpretation of the data. Regardless, the literature is summarized below in terms of the functional domains organized by each neurobehavioral evaluation.

Below are study descriptions and summaries organized by neurological domain. Appendix 1 is an overall summary for each chemical, presenting each endpoint and outcome.

2.1.2 Integration of Literature: Neurobehavioral Domains

Cognition

Fifteen studies measured some aspect of cognition: tests included mazes (radial arm maze, Lashley maze, T-maze spontaneous alternation, M-maze), conditioned response (passive avoidance, conditioned avoidance, operant responding, T-maze), and recognition (novel object). Most of these showed adverse effects of OP exposure, although not always in a consistent or dose-responsive manner.

Rats treated with diazinon (0.5, 2 mg/kg/d, postnatal day (PND) 1-4) showed no differences alternating in a T-maze (Timofeeva *et al.*, 2008a). The same rats were tested several months later in a radial arm maze, and showed increased working memory errors (both males and females), but only at the low dose (0.5 mg/kg/d) with no effect on reference memory performance. Diazinon (1 mg/kg/d) was given to rats on gestational day (GD) 15-18 or PND1-4, and there was no change in the trials to criterion in a passive avoidance test; however, there was clearly decreased step-down latency when tested 24 hr later (Vatanparast *et al.*, 2013). This finding suggests a change in memory but not learning. After *in utero* exposure this effect was only seen in females. In contrast, both genders (greater effect in males) were affected following postnatal exposure. Using a novel object test, male mice (females not tested) exposed postnatally (PND8-11) to diazinon (0.5, 5 mg/kg/d) showed less exploration and discrimination of the new object (Win-Shwe *et al.*, 2013). This was significant at both doses (but no dose-response) when tested at PND49, and only the high dose group showed effects at PND84. Mice exposed to diazinon (0.18, 9 mg/kg/d) throughout gestation were tested in a Lashley III maze, with no changes in the number of errors, suggesting no effect on learning (Spyker and Avery, 1977). Thus, these data on diazinon suggest an effect on learning and/or memory (radial arm maze, passive avoidance, novel object recognition) but no changes in learning a maze task. There was a lack of dose-response across studies.

Parathion exposure in rats (0.1, 0.2 mg/kg/d, PND1-4) produced no changes in T-maze spontaneous alternation (Timofeeva *et al.*, 2008b). These rats showed decreased working memory errors, indicative of improvement, at the low dose only (both males and females), and no changes in reference memory errors when tested at about 3 months of age. However, Levin *et al.* (2008) tested littermates from the Timofeeva study beginning at 14 months, and reported increased working memory errors in male rats treated with the low dose only. Interestingly, there is a difference in direction of change and gender specificity compared to the Timofeeva data. Reference memory errors were also increased at both doses (but no dose-response) in male rats only. When the rats were tested again at 17 months, working memory errors were increased at both doses (but no dose-response), again only in males. There was no effect on

reference errors at 17 months, and no change in either parameter when the rats were again tested at 19 months. In a study by Stamper *et al.* (1988), rats (only males were tested) exposed postnatally to parathion (1.3, 1.9 mg/kg/d, PND5-20) showed decreased spontaneous alternations in a T-maze at both doses (dose-response evident), and increased working memory errors in a radial arm maze at both doses (but no dose-response). Reference memory errors were not altered. Al-Hachim and Fink (1968) administered parathion (3 mg/kg/d) during either the first, second or third week of gestation in mice, and reported no effect on conditioned avoidance (sex not mentioned) following any exposure period. Overall, the radial arm maze showed effects of parathion in several studies, but the direction of change, specificity of errors, and gender selectivity differed. The results with the T-maze were contradictory, with one study out of two reporting effects.

Radial arm maze performance was affected by methyl parathion given directly to pups PND1-21 using an incrementally increasing dose schedule (Johnson *et al.*, 2009). The middle (0.2 to 0.6 mg/kg/d) and high (0.3-0.9 mg/kg/d) dose groups increased both working and reference memory errors. The lowest dose group (0.2 mg/kg/d throughout dosing) also increased reference memory errors; however, on this measure all dose groups had similar averages. Only males were affected on all measures. Rats dosed with methyl parathion (1 mg/kg/d, GD7-15) were trained to go to a specific side in T-maze, and the correct side reversed five times (Crowder *et al.*, 1980). Treated rats had more trials to criterion only on second and fifth switch (note, the text claimed there was an effect on the 4th switch, but the figure does not show it as significant). Effects on only certain reversals is difficult to interpret, and may be a reflection of the multiple t-tests used to analyze the data. Males and females were tested but data were not provided for each sex separately. There were no effects on passive or active avoidance in rats (no mention of gender) exposed to methyl parathion (1, 1.5 mg/kg/d, GD6-20) (Gupta *et al.*, 1985). The low-dose group only showed slower latency to bar press during operant shaping, and more days to asymptote; however, details of operant training and schedule were not provided, the sample size was extremely small (n=4/group), and high variability was mentioned. Thus, the most consistent effect was seen in the radial arm maze (even though there was no dose-response), and other tests were either negative or the data were inconclusive.

Several other OPs were tested in different cognitive tasks, but there is no more than one report for any specific pesticide. After single-trial passive avoidance training, male rats (females not tested) treated with dichlorvos (8 mg/kg/d, GD6-15) showed faster latency to cross when tested 7 days later, suggesting delayed retention (Lazarini *et al.*, 2004). With fenitrothion, male rats (females not tested) exposed gestationally (5, 10, 15 mg/kg/d, GD7-15) were conditioned to climb a pole to escape shock (Lehotzky *et al.*, 1989). The mid and high dose groups (dose-response evident) showed more escapes, were faster, and reached criterion faster than controls. This same pattern seen with reacquisition after a period of extinction, during which time there were no group differences. There was no effect of oxydemeton methyl (0.5, 1.5, 4.5 mg/kg/d, GD6-15) on M-maze learning or memory in rats (Clemens *et al.*, 1990). There was also no effect on retention of single trial passive avoidance in mice exposed to methamidophos (1 mg/kg/d, PND3-9); however, the retention trial occurred at 3 hr instead of the more standard 24 hr or greater (Lima *et al.*, 2013).

Table 2.1.1 Summary of Cognitive Outcomes

	Early gestation ~GD1-7, ~GD8-14, GD6-15 GD7-15	Late gestation ~GD15-21, GD6-20, GD15-18	Gestation GD1-birth	Early postnatal PND1-4, PND3-9	Late postnatal PND8-11, PND5-20	Postnatal PND1-21
Radial arm maze				Diazinon: cognitive deficit – rat, low dose only, M & F[1] Parathion: improved cognition – rat, low dose only, M & F[2] Parathion: cognitive deficit – rat, M not F[3]	Parathion: cognitive deficit – rat, no dose-response, only M tested[4]	Methyl parathion: cognitive deficit – rat, dose-response, M only[5]
T-maze spontaneous alternation				Diazinon: no effect – rat, M & F[1] Parathion: no effect – rat, M & F[2]	Parathion: cognitive deficit – rat, dose-response, only M tested[4]	
T-maze learning	Methyl parathion: cognitive deficit – rat, sex not specified[6]					
Lashley III maze			Diazinon: no effect – mouse, sex not specified[7]			
M-maze	Oxydemeton methyl: no effect – rat, M & F[8]					
Active avoidance	Fenitrothion: improved	Methyl parathion: no				

	cognition – rat, dose-response, only M tested[9] Parathion: no effect – mouse, sex not specified[11]	effect – rat, sex not specified[10] Parathion: no effect – mouse, sex not specified[11]				
Passive avoidance	Dichlorvos: cognitive deficit – rat, only M tested[12]	Diazinon: cognitive deficit – rat, F not M[13] Methyl parathion: no effect – rat, sex not specified[10]		Diazinon: cognitive deficit – rat, M & F[13] Methamidophos: no effect – mouse, sex not specified[14]		
Novel object recognition					Diazinon: cognitive deficit – mouse, no dose-response, only M tested[15]	
Operant responding		Methyl parathion: cognitive deficit – rat, sex not specified[10]				

[1] Timofeeva *et al.*, 2008a
[2] Timofeeva *et al.*, 2008b
[3] Levin *et al.*, 2008
[4] Stamper *et al.*, 1988
[5] Johnson *et al.*, 2009
[6] Crowder *et al.*, 1980
[7] Spyker and Avery, 1977
[8] Clemens *et al.*, 1990
[9] Lehotzky *et al.*, 1989
[10] Gupta *et al.*, 1985
[11] al-Hachim and Fink, 1968
[12] Lazarini *et al.*, 2004
[13] Vatanparast *et al.*, 2013
[14] Lima *et al.*, 2013
[15] Win-Shwe *et al.*, 2013

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Motor Activity

Despite being a commonly tested measure in these studies, only a few of the tested OPs produced changes in motor activity. Rats exposed to dichlorvos (8 mg/kg/d, GD6-15) showed decreased open field locomotion at weaning (males not females), and decreased locomotion and increased immobility as adults (age not specified, only males tested) while rearing was not affected at either age (Lazarini *et al.*, 2004). Male rats exposed to fenitrothion (5, 10, 15 mg/kg/d, GD7-15) showed decreased horizontal activity in the high-dose group only on PND104, with an apparent but not significant effect at PND26, but no effect at PND36 (Lehotsky *et al.*, 1989). Open field testing of rats exposed to methyl parathion (1 mg/kg/d, GD7-15) showed what appeared to be increases only on PND23 and 54, with no differences on PND30, 44, 65 (also not PND18); however, the data are not compelling since statistics are not provided, and the text refers to the data as "a possible change" (Crowder *et al.*, 1980). Methyl parathion-treated rats (1 mg/kg/d, GD6-20) showed a decrease in locomotor activity "accommodation" (apparently the period that is 15-30 min into the activity session) in the low dose group only (Gupta *et al.*, 1985).

Several OPs consistently produced no changes on motor activity, regardless of exposure or test species. No effects were seen with diazinon in rats exposed gestationally (1 mg/kg/d, GD15-18, Vatanparast *et al.*, 2013) or postnatally (0.5, 2 mg/kg/d, PND1-4, Timofeeva *et al.*, 2008a, or 1 mg/kg/d, PND1-4, Vatanparast *et al.*, 2013), or in mice exposed gestationally (0.18, 9 mg/kg/d, GD1-birth, Spyker and Avery, 1977). As with diazinon, there were no motor activity changes following parathion exposure postnatally in rats (0.1, 0.2 mg/kg/d, PND1-4, Timofeeva *et al.*, 2008b, or 1.3, 1.9 mg/kg/d, PND5-20, Stamper *et al.*, 1988) or in mice treated during either the first, second, or third week of gestation (3 mg/kg/d, Al-Hachim and Fink, 1968). There was no effect on measures of activity reported in rats treated with methamidophos (1 mg/kg/d, GD6-15), although high variability of the measures was discussed (deCastro *et al.*, 2000). Methamidophos (1 mg/kg/d, PND3-9) also produced no effect in mice (Lima *et al.*, 2013). Finally, there was no effect of oxydemeton methyl exposure (0.5, 1.5, 4.5 mg/kg/d, GD6-15) on open field activity (Clemens *et al.*, 1990).

In this review, effects on activity are only considered in tests designed specifically for that purpose. During the course of other behavioral tests, e.g., radial arm or T-maze, speed or latency is often measured. These ancillary activity measures were sometimes altered by treatment, but are not included in this domain, since they are not designed to specifically target motor activity.

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Table 2.1.2. Summary of Motor Activity Outcomes

Task/Test Apparatus	Early gestation ~GD1-7, ~GD8-14, GD6-15 GD7-15	Late gestation ~GD15-21, GD6-20, GD15-18	Gestation GD1-birth	Early postnatal PND1-4, PND3-9	Late postnatal PND8-11, PND5-20
Open field	Dichlorvos: decreased activity – rat, only M tested[1] Fenitrothion: decreased activity – rat, dose-response, only M tested[2] Methamidophos: no effect – rat, sex not specified[3] Methyl parathion: increased activity – rat, sex not specified[4] Oxydemeton methyl: no effect – rat, M & F[5] Parathion: no effect – mouse, sex not specified[6]	Diazinon: no effect – rat, M & F[7] Parathion: no effect – mouse, sex not specified[6]	Diazinon: no effect – mouse, sex not specified[8]	Diazinon: no effect – rat, M & F[7] Methamidophos: no effect – mouse, sex not specified[9]	Parathion: no effect – rat, only M tested[10]
Figure-Eight				Diazinon: no effect – rat, M & F[11] Parathion: no effect – rat, M & F[12]	
Donut		Methyl parathion: decreased activity – rat, no dose-response, sex not specified[13]			

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[1] Lazarini *et al*., 2004
[2] Lehotzky *et al*., 1989
[3] deCastro *et al*., 2000
[4] Crowder *et al*., 1980
[5] Clemens *et al*., 1990
[6] al-Hachim and Fink, 1968
[7] Vatanparast *et al*., 2013
[8] Spyker and Avery, 1977
[9] Lima *et al*., 2013
[10] Stamper *et al*., 1988
[11] Timofeeva *et al*., 2008a
[12] Timofeeva *et al*., 2008b
[13] Gupta *et al*., 1985

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Anxiety/Depression

In a series of tests in rats, Roegge *et al*. (2008) showed that early postnatal exposure to diazinon (0.5, 2 mg/kg/d, PND1-4) produced behaviors suggesting higher anxiety at the high dose (decreased open arm time in an elevated plus maze), lower fearfulness (decreased time to start eating in novel environment) in both dose groups, and anhedonia (decreased chocolate milk preference) at the low dose only, but not depression (forced swim test). These effects occurred only in males and did not show a clear dose-response for the novelty eating and chocolate milk preference tests. Using the same tests (same laboratory; Timofeeva *et al*., 2008b), parathion exposure (0.1, 0.2 mg/kg/d, PND1-4) in rats (high dose, both sexes) increased time in the open arm in an elevated plus maze (suggesting decreased anxiety); however, there was also an increase in center crossings, indicating hyperactivity, that may confound overall interpretation. These same rats showed no changes in novelty-suppressed feeding or in chocolate milk preference. Thus, in these two studies the pesticides appear to have different effects on this functional domain.

There are only a few reports of these behaviors with other pesticides. Rats treated with methyl parathion (1, 1.5 mg/kg/d, GD6-20) showed faster emergence from a cage, interpreted by the authors as lowered anxiety, in the low dose group only (Gupta *et al*., 1985). With chlormephos exposure (~0.06, 0.6 mg/kg/d in drinking water, one week premating to weaning), adult mice (both males and females) in the high-dose group showed decreased time spent in the open arms and increased time in the closed arms (no change in latency) of an elevated plus maze, suggesting increased anxiety (Ceh *et al*., 2012). Mice exposed to methamidophos (1 mg/kg/d, PND3-9) showed no differences in time spent in either arm of an elevated plus maze, but spent less time in center (Lima *et al*., 2013). This was interpreted by the authors as effect on choosing arms, which they say is a cognitive effect; however, this measure is often interpreted to reflect only activity levels. The same mice showed increased immobility in forced swim test, suggesting depressive-like behaviors. Overall, these results are varied and did not consistently show a dose-response.

Table 2.1.3. Summary of Anxiety/Depression Outcomes

	Late gestation ~GD15-21, GD6-20, GD15-18	Perinatal premating-weaning	Early postnatal PND1-4, PND3-9
Elevated plus maze		Chlormephos: increased anxiety – mouse, M & F[1]	Diazinon: increased anxiety – rat, dose-response, M not F[2] Methamidophos: no effect –mouse, sex not specified[3] Parathion: decreased anxiety – rat, dose-response, M & F[4]
Chocolate milk preference			Diazinon: increased anhedonia – rat, no dose-response, M not F[2] Parathion: no effect – rat, M & F[4]
Novelty suppressed feeding			Diazinon: decreased fearfulness – rat, dose-response, M not F[2] Parathion: no effect – rat, M & F[4]
Forced swim			Diazinon: no effect – rat, M & F[2] Methamidophos: increased despair – mouse, sex not specified[3]
Open field behaviors	Methyl parathion: decreased anxiety – rat, no dose-response, sex not specified[5]		

[1] Ceh *et al*., 2012
[2] Roegge *et al*., 2008
[3] Lima *et al*., 2013
[4] Timofeeva *et al*., 2008b
[5] Gupta *et al*., 1985

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Social Behavior

Only one study has used tests of social behavior using these OPs. With exposure to fenitrothion (5, 10, 15 mg/kg/d, GD7-15), rats in mid and high dose (dose-response) spent more time actively interacting in conspecific pairs (Lehotsky *et al.*, 1989). The scarcity of data on this measure prevents any conclusions across OPs.

Sensory Function

In a study measuring response to a tactile stimulus, with and without an acoustic prepulse, male (but not female) rats treated with diazinon (0.5, 2 mg/kg/d, PND1-4) showed less prepulse inhibition at both doses; however, no dose-response was evident (Timofeeva *et al.*, 2008a). Using the same paradigm, rats treated with parathion (0.1, 0.2 mg/kg/d, PND1-4) showed a different pattern: lower response to the stimulus alone (high dose, both sexes), but no change in the inhibition produced by the prepulse (Timofeeva *et al.*, 2008b). Mice treated with diazinon through gestation showed no change in response to noise (auditory startle) or smell (olfactory orientation), but did show change in visual cliff behavior (more steps off a "cliff") which occurred only in females in the low dose group (Spyker and Avery, 1977). Additional studies are needed for general conclusions regarding the effects of these pesticides on sensory function.

Table 2.1.4. Summary of Sensory Outcomes

	Gestation GD1-birth	Early postnatal PND1-4, PND3-9
Auditory	Diazinon: no effect – mouse, sex not specified[1]	
Tactile with or without prepulse		Diazinon: decreased sensory gating – rat, no dose-response, M not F[2] Parathion: decreased startle response – rat, dose-response, M & F[3]
Visual	Diazinon: decreased function – mouse, no dose-response, F not M[1]	
Olfactory	Diazinon: no effect – mouse, sex not specified[1]	

[1] Spyker and Avery, 1977
[2] Timofeeva *et al.*, 2008a
[3] Timofeeva *et al.*, 2008b

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Neuromotor Function

One study assessed neuromotor function in mice exposed to diazinon (0.18, 9 mg/kg/d, GD1-birth), and reported some changes in motor abilities measured at about 2 months of age; however, these were not consistent in dose or direction of change (Spyker and Avery, 1977). Increased ability was suggested by a longer time to cling to a rod (both doses, no dose-response). In contrast, there was less ability to stay on an increasingly inclined plane (both doses, no dose-response) or perhaps on a rotarod (group means not significant due to large variability, suggestive of effect at both doses).

Rats in the high-dose group exposed to fenitrothion (5, 10, 15 mg/kg/d, GD7-15) fell off a rotarod faster on PND26 and PND104, but not PND36. Only males were tested (Lehotsky *et al.*, 1989).

There were no neuromotor changes in terms of rotarod performance in rats following exposure to parathion (1.3, 1.9 mg/kg/d, PND5-20) (Stamper *et al.*, 1988) or methyl parathion (1, 1.5 mg/kg/d, GD6-20) (Gupta *et al.*, 1985). Overall, there is little support for conclusions of neuromotor outcomes following these pesticides, but more studies are needed.

Table 2.1.5. Summary of Neuromotor Outcomes

	Early gestation ~GD1-7, ~GD8-14, GD6-15 GD7-15	Late gestation ~GD15-21, GD6-20, GD15-18	Gestation GD1-birth	Late postnatal PND8-11, PND5-20
Rotarod	Fenitrothion: decreased performance – rat, dose-response, only M tested[1]	Methyl parathion: no effect – rat, sex not specified[2]	Diazinon: no effect – mouse, sex not specified[3]	Parathion: no effect – rat, only M tested[4]
Inclined plane			Diazinon: decreased performance – mouse, dose-response, sex not specified[3] (Spyker)	
Rod cling			Diazinon: increased performance – mouse, no dose-response, sex not specified[3]	

[1] Lehotzky *et al.*, 1989
[2] Gupta *et al.*, 1985
[3] Spyker and Avery, 1977
[4] Stamper *et al.*, 1988

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2.1.3 Integration with AChE Inhibition

There are some data with which to compare effective doses in these DNT studies with doses producing AChE inhibition. A number of studies in this literature review included AChE inhibition (brain and/or blood) in their measurements. Information on diazinon and parathion can be taken from a separate (non-behavioral) study (Slotkin *et al.*, 2006) conducted in the same laboratory with the same dosing paradigm used in several studies (Levin *et al.*, 2008; Roegge *et al.*, 2008, Timofeeva *et al.*, 2008a, 2008b).

Neurobehavioral effects of diazinon were reported in rats at doses of 0.5-2 mg/kg/d (Roegge *et al.*, 2008; Timofeeva *et al.*, 2008a). Slotkin *et al.* (2006) reported that a dose of 0.5 mg/kg/d produced some (<10%, statistically significant) brain AChE inhibition when measured the day after the last dose. At 2 hr after a higher dose (2 mg/kg/d; lower doses were not tested at 2 hr), there was greater brain inhibition (25-30%) compared to 24 hr (10-20%). Thus it is probable that for diazinon, inhibition during and shortly after the dosing period (i.e., within hours) was greater at lower doses. While there is no direct AChE data following diazinon exposure at 1 mg/kg/d (Vataparast *et al.*, 2013), it can be assumed from the Slotkin data that this dose also inhibited brain AChE at some time during/after dosing. No AChE activity was measured in mice by Spyker and Avery (1977), but the high dose of 9 mg/kg/d resulted in depressed weight gain, a sign of maternal toxicity. There is no mention of toxicity at the lower doses (0.5, 5 mg/kg/d) used in mice by Win-Shwe *et al.* (2013).

The parathion studies showing effects at 0.1 and 0.2 mg/kg/d (Timofeeva *et al.*, 2008b; Levin *et al.*, 2008) are also informed by the AChE inhibition presented in Slotkin *et al.* (2006) in which a dose of 0.1 mg/kg/d inhibited brain AChE 5-15% on the day after the last dose. There are no AChE data available for a higher dose, 0.2 mg/kg/d, but Timofeeva noted 5% mortality in that dose group. Much higher doses (1.3, 1.9 mg/kg/d in rats) were reported to produced 35, 68% brain inhibition on the day after the last dose (PND5-20), with 26, 36% inhibition persisting a week later (Stamper *et al.*, 1988).

The methyl parathion postnatal incrementing dose paradigm in rats used by Johnson *et al.* (2009) produced brain AChE inhibition in all dose groups at the end of dosing, persisting for 10 to 20 days later; recovery was evident 30-40 days later. The low dose (0.2 mg/kg/d throughout) produced 13-15% inhibition. During gestational exposure to higher doses of methyl parathion at 1, 1.5 mg/kg/d, the dams had 20, 60% brain inhibition at the end of dosing (GD6-20) (Gupta *et al.*, 1985). At the high dose, cholinergic signs and increased resorptions were noted. Furthermore, the offspring (fostered to control dams) showed brain inhibition as high as 50% in both dose groups when measured at birth and also PND7, 14, 21, and 28. The low dose showed recovery at PND28, but not the high dose.

In the study of rats treated with fenitrothion, there was postnatal mortality of 16-17.5% at all doses (5, 10, 15 mg/kg/d, GD7-15), compared to 5% in controls (Lehotsky *et al.*, 1989). In separate study of rats, 5, 25 mg/kg on GD19 produced 40, 80% brain AChE inhibition in dams, and fetal brains showed about 90% inhibition (no dose response) (Sochaski *et al.*, 2007).

Assuming similar responses, the postnatal mortality observed in the Lehotsky study could be at least partly due to this high degree of AChE inhibition in both dams and fetuses.

In rats, nonpregnant females dosed methamidophos 1 mg/kg/d for 10 days showed 16% plasma AChE inhibition, but brain AChE was apparently not measured. This suggests that dams treated at the same dose in a study by deCastro *et al*. (2000) most likely experienced some plasma inhibition. Mouse pups dosed with methamidophos 1 mg/kg/d PND3-9 showed ~36, 46% brain inhibition 1, 4 hr after first dose, 53, 61% inhibition at 1, 4 hr after last dose, and ~19% brain inhibition the day after last dose (Lima *et al*., 2013). The mice therefore experienced considerable brain AChE inhibition throughout dosing.

In the oxydemeton methyl study, dams showed 22-68% brain AChE inhibition on the day after the last dose (0.5-4.5 mg/kg/d, dose response), and 5 days later (GD20) there was 20-54% brain AChE inhibition. Fetal brains taken the day after dosing showed no inhibition (Clemens *et al*., 1990); however, there were no fetal AChE tissues collected during or shortly after the dosing period when AChE inhibition would be greatest.

Overall, in the studies for which there are direct or comparable data, it is clear that the dosing paradigms produced AChE inhibition and in some cases maternal toxicity. Indeed, there are no studies reporting or even suggesting a lack of AChE inhibition in the dam and/or fetus/pup at any time during dosing. Thus, it is not known whether exposure paradigms that do not inhibit AChE would produce any neurobehavioral effects.

2.1.4 Summary of Findings from the Developmental Neurotoxicity (DNT) Guideline Studies

DNT studies have been submitted for 20 OPs, summarized in Appendix 2. These studies follow the US EPA guideline 870.6300 and/or OECD guideline 426 which require testing of motor activity, acoustic startle response, learning and memory, and brain morphometrics in the offspring around weaning and also in adulthood. In general, these studies provide exposure during development either via diet or oral gavage dosing, including direct dosing of the pup preweaning. As with the literature studies, these submitted studies have shortcomings such as inappropriate statistical analyses, limited methodological information and presentation of results. Many measures tend to show high variability, which reduces their interpretability and utility.

In order to compare the submitted guideline and published studies under the scope identified under Section 2.1 and to be consistent with the chlorpyrifos 2012/2014 review, only changes that occurred after dosing had ended (i.e., shortly after weaning or as adults) were considered here. Across the seven submitted studies that reported effects, there are mostly changes in acoustic startle reactivity, cognitive function, and to a lesser extent, motor activity. Some OPs altered multiple domains, others only one. There are both submitted guideline studies and literature studies for only four OPs: diazinon, methyl parathion, methamidophos, and

dichlorvos. Diazinon produced cognitive changes but no effects on motor activity or acoustic startle in the DNT: these results are generally in agreement with published studies. Male offspring in the high dose group (~33 mg/kg/d in the dam diet) showed increased errors and longer latency in Biel maze performance at both PND24 and PND62, and similar effects were seen in females but only in the middle dose group (~3.4 mg/kg/d via diet) at PND24. Following methamidophos exposure, female rats in the middle and high dose groups (~1.7, 5.2 mg/kg/d in the dam diet) showed decreased peak amplitudes of the startle response, which was statistically significant at PND38 and apparent but not significant at PND60. In contrast to the literature reports of cognitive and motor effects of methyl parathion, there were no reported changes in the submitted guideline study. The submitted study of dichlorvos was uninterpretable due to high pup mortality in all groups, including control.

In these studies, AChE activity was assessed as part of the DNT itself, or by means of a separate study comparing the response in pups and adults (comparative cholinesterase, or CCA, studies). Thus, in almost all guideline DNT studies there are adequate data describing AChE inhibition in the pups at some time during development. It is clear from these DNT studies that the doses used did produce AChE inhibition in the offspring, sometimes at all doses tested. It was noted that in these studies, most of the reported effects occurred before weaning, which is the period during which there was likely to be ongoing AChE inhibition.

Thus, as with the literature studies, there are scant data that could inform potential neurodevelopmental changes occurring at doses lower than those needed to inhibit AChE. Furthermore, there is little consistency in patterns of effects across studies or chemicals. Thus, there is uncertainty as to whether lower, non-inhibiting exposures are developmentally neurotoxic; this uncertainty was described in the chlorpyrifos reviews and remains applicable for the available data for other OPs as well.

2.1.5 Conclusions on *In Vivo* Laboratory Animal Studies

For chlorpyrifos, there are >30 papers on developmental neurotoxicity; for the remaining OPs, the literature is sparse with very few studies for each OP (including DNT guideline studies). The studies span over decades, and many of the lower quality studies were the earlier ones; however, some very recent papers also have significant deficits. Methodological detail is lacking, inappropriate statistical analyses are applied, results are cursorily described and/or inaccurately presented, and interpretation of some behavioral changes is faulty. Overall, most studies have significant shortcomings and/or are of low quality.

The most commonly tested behaviors considered aspects of cognition. In the majority of studies, some sort of cognitive deficit was detected, especially with working memory performance (radial arm maze) and conditioned response retention (passive avoidance). However, in many cases there was no dose-response, there was some gender specificity which did not replicate in multiple studies, and cognitive improvement instead of deficit was noted in a few papers. Changes in motor activity in offspring were generally not reported, and the direction of change differed in the papers reporting such effects. There is generally not enough

information to make definitive statements about OP effects on other types of neurological disorders.

Few published papers included AChE measurements of the dams and/or offspring, but where measured, all doses used inhibited AChE to some degree. Some papers even reported overt maternal and fetal toxicity. This was also the case in the guideline studies, most of which included concurrent or supplemental data on AChE inhibition. Since there are no studies with low doses that definitively do not inhibit AChE, there is no information in the animal literature that shows whether or not there would be developmentally neurotoxic outcomes at those lower exposures.

2.2 Epidemiology Research on OPs other than Chlorpyrifos

2.2.1 Overview of Literature Reviews: 2012/2014, 2015, and updated in 2016

In April 2012, EPA presented to the FIFRA Scientific Advisory Panel (SAP) its review and assessment of several epidemiological investigations of the potential adverse neurodevelopmental outcomes of *in utero* and early life exposure to chlorpyrifos. In this effort, EPA limited its review to studies conducted within three major US based prospective birth cohort studies: 1) Mother's and Newborn Study of North Manhattan and South Bronx conducted by Columbia University, referred to in this document as CCCEH 2) Mount Sinai Inner-City Toxicants, Child Growth and Development Study, or the "Mount Sinai Study/Cohort;" and 3) Center for Health Assessment of Mothers and Children of Salinas Valley (CHAMACOS) conducted by the University of California Berkeley, or "CHAMACOS Study/ Cohort." The conclusion of EPA's evaluation, supported by the FIFRA SAP (2008, 2012), was that "chlorpyrifos likely played a role in the neurodevelopmental outcomes observed in these studies."

In 2015, the agency expanded its consideration of the epidemiological data to include studies of any OP pesticide; several different types of development and neurological, neurodevelopmental, and neurobehavioral health outcomes; studies performed in non-U.S. countries as well as US based studies; and non-cohort studies. This 2016 updated literature review includes edits made in response to public comments on the 2015 literature review and addition of new epidemiology papers: Yolton et al. (2013), Cartier et al. (2015), Rauh et al (2015), Ranaan et al, (2015), Engel et al. (2015), Fiedler et al. (2015), Harley et al (2016), Stein et al (2016), Rauh et al (2015), Ranaan et al, 2015; Engel et al. (2015), and Donauer et al (2016).The agency has developed individual study reviews for each study in the 2015 and 2016 updated literature review; these study reviews contain details about study design, results, strengths, uncertainties, and interpretation. The study reviews can be found in USEPA, 2015b, USEPA, 2016, and USEPA,2016b. This literature review document summarizes key information, strengths and uncertainties and provides a concise, coherent weight of evidence analysis.

The 2012/2014 literature review was limited to studies from CCCEH, Mt. Sinai, and CHAMACOS; all studies from these cohorts are considered high quality. In the CCCEH, Mt. Sinai, and

CHAMACOS studies, mother-infant pairs were recruited for the purpose of studying the potential health effects of environmental exposures during pregnancy on subsequent child development. Importantly, each of these cohorts evaluated the association between prenatal chlorpyrifos or OP exposure with adverse neurodevelopmental outcomes in children through age 7 years. For those studies in 2012/2014 literature review detailed summaries and evaluation, associated strengths and limitations, and accompanying detailed evidence table for the CCCEH, CHAMACOS, and Mt. Sinai cohorts can be found in the white paper for the 2012 SAP review, and the 2014 chlorpyrifos revised HHRA. Limited summary information is provided here for consideration with the studies identified in the 2015 and updated 2016 literature reviews.

Publication bias is a type of bias associated with the kinds of academic research likely to be published in the open literature; generally, this bias tends towards lack of publishing of null findings. However, in the case of the neurodevelopmental epidemiology studies on OPs, as noted by commenter, there are already significant amount of null findings reported in this body of evidence. Moreover, these studies are derived from existing cohorts of mothers and children which are well-known to the science community; these kinds of cohorts are expensive and resource intensive to develop and maintain making the number of studies from other groups unlikely. Given the amount of null findings already reported in the literature, the agency believes that publication bias with regard to lack of publishing of null findings does not impact the interpretation of the existing studies on neurodevelopmental outcomes associated with OPs.

The findings from the birth outcome studies are summarized in 2.2.7. The major findings of CCCEH, Mt. Sinai, and CHAMACOS on neurodevelopmental outcomes are briefly summarized in Section 2.2.8.1. Results from other studies on neurodevelopmental outcomes are summarized in 2.2.8.2.

2.2.2 Literature Search Methodology

To identify the epidemiological investigations of the association between OP exposure and adverse neurological, neurodevelopmental or neurobehavioral effects, EPA scientists queried PubMed/Medline and Web of Science directly on January 21 and January 23, 2015. In this literature search, emphasis was placed upon identification of all possible epidemiological studies available, and the ability to use the identical search string in both PubMed/Medline and Web of Science. The following search string was utilized:

> ((Chlorpyrifos OR Organophosphates) AND (prenatal OR neonatal OR perinatal OR in utero OR fetal OR foetal OR newborn OR infant* OR infancy OR preschool OR child* OR maternal OR mother* OR pregnan*) AND (neurodevelop* OR attention OR birth outcome* OR health outcome* OR birth height OR birth weight OR birth length OR cephalometry OR head circumference OR child development OR cognitive OR cognition OR developmental disability* OR fetal growth OR foetal growth OR foetal development

OR fetal development OR intelligence OR memory OR neurological functioning OR psychomotor) AND (human)) English language only((Chlorpyrifos OR Organophosphates) AND (prenatal OR neonatal OR perinatal OR in utero OR fetal OR foetal OR newborn OR infant* OR infancy OR preschool OR child* OR maternal OR mother* OR pregnan*) AND (neurodevelop* OR attention OR birth outcome* OR health outcome* OR birth height OR birth weight OR birth length OR cephalometry OR head circumference OR child development OR cognitive OR cognition OR developmental disability* OR fetal growth OR foetal growth OR foetal development OR fetal development OR intelligence OR memory OR neurological functioning OR psychomotor) AND (human)) English language only((Chlorpyrifos OR Organophosphates) AND (prenatal OR neonatal OR perinatal OR in utero OR fetal OR foetal OR newborn OR infant* OR infancy OR preschool OR child* OR maternal OR mother* OR pregnan*) AND (neurodevelop* OR attention OR birth outcome* OR health outcome* OR birth height OR birth weight OR birth length OR cephalometry OR head circumference OR child development OR cognitive OR cognition OR developmental disability* OR fetal growth OR foetal growth OR foetal development OR fetal development OR intelligence OR memory OR neurological functioning OR psychomotor) AND (human)) (Filter: English language only)

With the aid of an EPA reference librarian, EPA also searched the following databases: PsycInfo, Agricola, Biosis, Embase, Enviroline, Gale Health and Wellness, Global Health, Pascal and Pollution Abstracts. EPA used similar, but modified search terms as listed above. Upon identification of the final set of relevant articles (n=38), limited hand-searching of the reference lists and citation mapping *(Science citation index)* of articles deemed to be most relevant to the review question was performed.

In 2015, EPA identified 299 articles across these several biomedical search engines. The determination of relevance to the study question was made by two EPA epidemiologists who agreed by consensus as to article disposition in the 2015 literature search. Removing duplicates (56), there were 243 articles, and 79 were determined to be epidemiological investigations of potential relevance. The 164 studies excluded from the analysis comprised 57 exposure only studies; 51 review articles; 33 reports of acute OP intoxication; 20 studies in non-human systems; and 3 were otherwise not relevant (See Appendix 4). Among the 79 potentially relevant epidemiologic studies, 41 were excluded; 17 articles were previously reviewed in 2012; 16 were epidemiological methods papers including exposure validation studies without an original epidemiological risk estimate; and 8 were otherwise not relevant for various reasons. Among the 40 remaining studies, 2 were additionally excluded (one was a duplicate study published a second time; the other did not make a measure of an OP pesticide. Therefore, 38 articles are included in the 2015 narrative literature review (referred to herein as "2015 Literature Review/Studies"). (See Appendix 5).

No additional formal literature search was conducted prior to the development of this 2016 update. However, the agency maintains active inquiry of the open scientific literature on issues related to OPs and the agency believes the added studies represent the available, relevant information. Of the 9 new epidemiology studies, two are meta-analyses.

The following sections provide the results of this literature review. Section 2.2.3 describes the breadth and depth of the 2015/2016 literature review, with Section 2.2.4 summarizing the approach for assigning a quality ranking and Section 2.2.5 providing the results of this quality ranking. In Section 2.2.6, these studies were further analyzed with focus on identification of the most appropriate exposure assessment and relevant outcomes for this assessment. Studies focusing solely on birth outcomes are discussed in Section 2.2.7. However, the emphasis in this assessment is on those studies focusing on neurodevelopmental outcomes, which are discussed in detail in Section 2.2.8 and summarized in Section 2.2.9.

2.2.3 Breadth and Depth of the 2015/2016 Literature Review

Key features of each of the 47 articles in the 2015/2016 literature review are summarized in Table 2.2.5.1-1, 2.2.5.1-2, and 2.2.5.2-1 as well as Appendix 3. These articles cover wide range of study designs, study locations and time periods, and exposure and outcome measurement approaches and are listed in Table 2.2-a. The majority of the studies utilized a cross-sectional or a prospective birth cohort study design.

- Mothers and Newborn Study of North Manhattan and South Bronx conducted by Columbia University, New York, NY (See tables 2.2.5.1-1 & 2.2.5.1-2)
- Mount Sinai Inner-City Toxicants, Child Growth and Development Study, New York, NY (See tables 2.2.5.1-1 & 2.2.5.1-2)
- Center for Health Assessment of Mothers and Children of Salinas Valley (CHAMACOS) conducted by the University of California Berkeley (See tables 2.2.5.1-1 & 2.2.5.1-2)
- Denmark, Birth Cohort (Andersen *et al.* 2015)
- Saint Peter's University Hospital, New Brunswick, New Jersey, Birth Cohort (Barr *et al.* 2010)
- Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT), Mexico City, Mexico, Birth Cohort (Fortenberry *et al.* 2014, 2014a)
- EcoSalud Project, Cayambe-Tabacundo region, Ecuador, Infant and Young Child Cohort (Handal *et al.* 2007, 2007b, 2008)
- University Hospital of Heraklion, Crete, Greece, Birth Cohort (Koutroulakis *et al.* 2014)
- Children's Pesticide Survey (CPS), Yuma County, Arizona (Lizardi *et al.* 2008)

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- Infancio y Medio Ambiente (INMA) (Environment and Childhood), Spain, Birth Cohort (Llop *et al*. 2013)

- Perturbateurs endocriniens: Étude Longitudinale sur les Anomalies de la Grossesse, l'Infertilité et l'Enfance (PELAGIE), Brittany, France, Birth Cohort (Petit *et al*. 2010; Cartier et al, 2015)

- Health Outcomes and Measure of the Environment (HOME), Cincinnati, OH, Birth Cohort (Rauch *et al*. 2012; Yolton et al, 2013; Donauer et al, 2016; Harley et al, 2016; Engel et al, 2015)

- Embilipitiya Base Hospital, Southern Sri Lanka, Birth Cohort (Samarawickrema *et al*. 2008)

- Ontario Farm Family Health Study, Ontario, Canada, Birth Cohort (Savitz *et al*. 1997)

- Childhood Autism Risks from Genetics and the Environment (CHARGE), California, Child Cohort (Shelton *et al*. 2014)

- Secondary Exposure to Pesticides Among Infants, Children and Adolescents (ESPINA), Pedro Moncayo County, Pichincha, Ecuador, Child Cohort (Suarez-Lopez *et al*. 2012, 2013, 2013a)

- Shenyang, China, Birth Cohort (Zhang *et al*. 2014)

- Rice and aquaculture farming regions outside of Bangkok, Thailand (Fiedler et al, 2015)

Exposures were assessed via environmental samples, biomarkers, and/or proxy methods.

- Only one study used environmental samples as an exposure measure - methyl parathion in household wipe samples (Ruckart *et al*., 2004).

- Among the studies, 11 biomarkers were used to assess exposure. One study utilized a direct measure of OP pesticide – chlorpyrifos (CPF) in maternal and cord serum (Barr *et al*., 2010). Several studies looked at specific OP parent metabolites (IMPY, 3,5,6-trichloro-2-pyridinol [TCPy], PNP, MDA). The majority of studies used non-specific OP biomarkers: dialkyl phosphates (DAPs), diethyl phosphates (DEPs), and dimethyl phosphates (DMPs). Two effect biomarkers, AChE and butyl cholinesterase (BuChE), were used as the exposure metric.

- Proxy exposure methods included questionnaire and non-questionnaire approaches. For example, questionnaire-based exposures included maternal and or paternal self-report of living with an exposed worker, occupational exposure/

employment, and home pesticide use and child outdoor play exposure. Non-questionnaire-based exposures included Community/area of residence, distance to treated area/farm, percent of area treated with pesticide, level of urbanization, pounds OP pesticide used/year, and pesticide spray season.

Likewise, there were numerous outcome measures examined across the studies, falling into six broad categories: birth characteristics, autonomic nervous system (ANS) effects, Attention Deficit Hyperactivity Disorder (ADHD)/attention problems, autism, general neurodevelopment (cognitive, behavioral, IQ), and physiological effects. The most common outcome measures were birth characteristics (with birth weight, birth length, head circumference and gestational age being the most frequent) and neurodevelopment tests and test batteries. Table 2.2-b lists the many specific neurodevelopment tests employed in the studies. Most studies utilized more than one test, and few tests were utilized in more than one study.

Other features that varied widely among the studies include:

- Study periods ranged from 1991 to 2012 (reports were published from 1997 to 2016).
- Study sizes varied from 25 to 3,159 participants.
- Children's ages ranged from newborns to age 15 years.

Pre-natal exposures were assessed in 23 reports, post-natal exposures in 14 reports, and both pre- and post-natal exposures in 7 reports.

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Table 2.2-a Study Designs, Exposure Measurement Methods, and Outcome Measurement Methods Used across the 2015/2016

Study design (# studies used)	Exposure measurement (# studies used*)	Outcome measurement (# studies used*)
Prospective Cohort (16) Retrospective Cohort (3) Case-control (2) Cross-sectional (16) Ecological (1)	**Biomarkers** **AChE** - acetyl cholinesterase (maternal 1, child 2) **BuChE** - butyl cholinesterase (child 1) **CPF** -chlorpyrifos parent (maternal 1, cord serum 1) **DAP** – dialkyl phosphate (maternal 9, child 10, amniotic fluid 1) **DCCA** - dimethylvinylcyclopropane carboxylic acid (child 1) **DEP** – diethyl phosphate (maternal 3, child 8, amniotic fluid 1) **DETP** – diethylthiophosphate (maternal 2) **DEDTP** – diethyldithiophosphate (maternal 2) **DMP** – dimethyl phosphate (maternal 8, child 8, amniotic fluid 1) **DMTP** – dimethylthiophosphate (maternal 1) **DMDTP** – dimethyldithiophosphate (maternal 2) **DZN** – diazinon parent (1) **IMPY** – diazinon metabolite (child 1) **MAL** - malathion parent (0) **MDA** - malathion metabolite (maternal 1) **OP** - organophosphate (cord blood 1) **PNP** - para-nitrophenol (child 2) **TCPy** - chlorpyrifos metabolite (maternal 1, child 2) **Environmental**	**Birth characteristics** (10) • Birth Weight/LBW/FGR (records 7, NP 1, report 2) • Birth Length (records 3) • Head circumference (records 3, NP 1) • Abdominal circumference (records 1) • Gestational age (GA)/preterm (report 2, records 3) • Ponderal index (records 1) • Placental maturity index (1) • Spontaneous abortion/miscarriage (report 1) • Altered sex ratio (report 1) **Autonomic Nervous System** (4) **ADHD/attention problems** (2) • Diagnosis DISC-IV (1) • Use of medication (1) • Screening (CPRS-R, CPT, BASC-PRS) (1) **Autism** (2) • CA Department of Developmental Services (CDDS) reports • US Individuals with Disabilities Education Act (IDEA) reports • Autism spectrum disorders (ASD) • Autism Diagnostic Observation Schedule (ADOS) combined with ADI-R. **Neurodevelopmental (ND) test/battery** (19) – see Table 2.2-2b for details **IQ** (57) - see Table 2.2-2b for details

Study design (# studies used)	Exposure measurement (# studies used*)	Outcome measurement (# studies used*)
	Methyl parathion - Household wipe (1) **Proxy (questionnaire)** • Maternal occupational exposure/ employment (6) • Paternal occupational exposure/ employment (2) • Living with exposed worker (1) • Home and outdoor play exposure (1) **Proxy (non-questionnaire)** • Community/area of residence (3) • Distance to treated area/farm (2) • Percent of area treated with pesticide (1) • Level of urbanization (1) • Pounds OP used/year (1) • Pesticide spray season (1)	**Physiological** (3) • AChE activity, child • BuChE activity, maternal • Antioxidant status: superoxide dismutase (SOD) activity • Fetal oxidative stress: malondialdehyde (MDA) concentrations) • Fetal DNA fragmentation: electrophoresis • Respiratory symptoms, child

*A single study may include biomarkers at multiple endpoints and / or multiple outcome measurements; thus the total number of studies used may exceed the total number of studies in this manuscript

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Table 2.2-b Detailed Neurological Outcome Measurement Methods Used across the 2015/2016 Review Studies

Outcome measurement

Neurodevelopmental (ND) test/battery (USA-based studies)
• Pediatric Environmental Neurobehavioral Test Battery (PENTB) : cognitive, motor, sensory, and affect domains
  ○ Developmental Test of Visual-Motor Integration (VMI)
  ○ Kaufman Brief Intelligence test (K-BIT)
  ○ Purdue Pegboard
  ○ Story Memory and Story Memory-Delay from Wide Range Assessment of Memory and Learning
  ○ Trail-Making test, Part A and Part B
  ○ Verbal Cancellation test
• Children's Memory Scale (CMS)
• Bayley Scales of Infant Development, Second Edition (Bayley-II)
• Mental Developmental Index (MDI)
• Psychomotor Developmental Index
• Clinical Evaluation of Language Fundamentals-Preschool, Second Edition
• Archimedes spirals
• NICU Network Neurobehavioral Scale (NNNS)
• Behavioral measures:
  ○ The Child Behavior Checklist/4-18
  ○ The Teacher Report Form
• Developmental delay (DD):
  ○ Mullen Scales of Early Learning (MSEL)
  ○ Vineland Adaptive Behavioral Scale (VABS)
  ○ Reciprocal social interaction: Social Responsiveness Scale

ND test/battery (Non-USA-based studies)
• Bayley Scales of Infant Development - mental and psychomotor development
• Behavioral Assessment and Research System (BARS): Memory and attention, response speed and coordination, visual memory, attention, divided attention, recall and recognition memory, dexterity, hand-eye coordination
• Figure drawing task: child's perception and dexterity
• Long-term memory test
• Ages and Stages Questionnaire (ASQ) – communication, fine motor, gross motor, problem solving, and personal–social skills
• Strengths and Difficulties Questionnaire, parent version (SDQ): behavioral problems
• NEPSY-II test (trained examiners): general assessment battery:
  ○ attention and executive functioning
  ○ language
  ○ memory and learning
  ○ sensorimotor (visuomotor precision),
  ○ visuospatial processing
  ○ Statue and Knock Tap
• Neonatal Behavioral Neurological Assessment (NBNA)
  ○ Behavior
  ○ Passive Tone
  ○ Active Tone
  ○ Primary Reflexes
  ○ General Assessment
• Reach-and-grasp, bi-manual coordination: Prehension abilities
• UC Berkeley Preferential Looking Test Cards: Visual acuity skills
• Visual Motor Integration (VMI), Beery-Buktenica, 4th Ed.
• Finger Tapping test: Manual motor speed
• Catsys equipment: Simple reaction time
• Conners' Continuous Performance Test II (CPT II, v5): Attention
• Woodcock-Johnson III Tests of Cognitive Abilities (WJ-III) Verbal Comprehension test: Long-term memory and language function
• Visuospatial performance and memory functions:
  ○ Raven's Colored Progressive Matrices

ED_002896_00064133-00032

- Stanford–Binet Copying Test, 4th ed
- Physical examination (social response, spontaneous motility, involuntary movements, Romberg's sign, walking straight line, standing on one leg, number hops, biceps and patellar reflexes, finger opposition, diadochokinesis, finger–nose coordination, hearing, vision)
- Gesell Development Schedule (GDS): motor, adaptive, language, and social
- Santa Ana Form Board: Motor coordination
- Child's developmental delay - Parent interview

IQ

- Wechsler Intelligence Scale for Children-Revised (WISC-R) Digit Span Test, Card sorting Test
- Wechsler Intelligence Scale for Children, 4th edition (WISC-IV)
- Wechsler Preschool and Primary Scale of Intelligence, Third Edition
- Stanford-Binet Memory for Sentences and Digit String test
- Recall and recognition test

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2.2.4 Study Designs & Considerations for Study Quality Evaluation

2.2.4.1 Study Designs

Besides the two meta-analyses, four basic study designs were used in the literature reviewed for this document: cohort study, case-control study, cross-sectional study, and ecologic study. The first of these two constitute the two basic types of observational (i.e. non-interventional) studies used to evaluate relative incidence of health and disease outcomes by exposure status. The latter two are generally considered descriptive or hypothesis generating study designs, though they too can be used to test hypotheses regarding relative prevalence of health outcomes and, under certain conditions, incidence as well.

Cohort Study

A commonly used design in this literature was the cohort study (See Tables 2.2.5.1-1, 2.2.5.1-2, and Appendix 3 for examples). In a typical cohort study, individuals are classified according to exposure status (i.e., presence, absence, or magnitude of exposure), and then followed over time to quantify and compare the development (i.e., incidence) of the health outcome of interest by exposure group. Conceptually, the non-exposed comparison group in a cohort study provides an estimate of the incidence of the outcome among the exposed, had they, counter-to-fact, not been exposed. Apart from chance variations, a valid cohort study comparing exposed individuals to non-exposed individuals provides an estimate of the relative risk (or rate) of the disease associated with exposure. Ideally, the exposed and non-exposed groups are exchangeable, in the sense that switching the exposed to non-exposed, and non-exposed to exposed would yield the same measure of association (e.g., relative risk). If this were the case then, apart from chance, a cohort study would yield a measure of association equivalent to that produced in a corresponding (intervention) study where exposure status was randomly assigned.

The chief advantage of the cohort study design is that it affords the investigator the opportunity to avoid and/or adjust for potential biases (i.e., selection bias, information bias, and confounding). Cohort studies also allow for discernment of the chronological relationship between exposure and outcome, and can be particularly efficient for studying uncommon exposures. The primary disadvantage of the cohort study design is logistical inefficiency with respect to the necessary time, expense, and other resources needed to conduct them. Cohort studies are particularly inefficient for evaluating associations with rare outcomes and diseases with long induction or latency periods. Though prospective studies are often logistically less efficient relative to other study designs (e.g., the case-control study), these logistical concerns can be minimized in cohort studies of short duration, such as those used to evaluate prenatal OP pesticide exposure effects on birth outcomes or other outcomes of neonatal development assessed shortly after birth.

Two sub-categories of cohort studies – prospective and retrospective - are often applied to distinguish between studies in which the health outcome has occurred (retrospective study), or

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has not occurred (prospective study) at the time the investigators initiate the study. This distinction is important primarily as it pertains to the potential differences in the quality (e.g., completeness, accuracy, and precision) of information that can be ascertained by the investigators, and also as it relates to potential sources of bias. Although not always true, the prospective study design is considered the preferable of the two, as investigators can potentially have more choices in determining how exposure, outcome, and covariate information is collected. In a retrospective study conducted to evaluate the same hypothesis, by contrast, the investigators would have to rely on exposure information such as maternal self-report. Such reporting is subject to (human) errors in recall. Moreover, the outcome status of the child (i.e., whether a child has developmental delays that are known to the mother) may influence the recall of prenatal OP pesticide exposure by the mother.

Case-control Study

In a typical case-control study (see, for example, Dawbrowski *et al.*, 2003 in Appendix 3 and Shelton *et al.* 2014 in Table 2.2.5.2-1), individuals are classified according to their outcome status (i.e., cases who have developed the outcome of interest, and controls who represent the population from which the cases arise). The relative odds of exposure are then compared between cases and controls. The primary advantage of case-control studies is that they are logistically efficient relative to cohort studies. In fact, properly conducted case-control study can be conceptualized as a cohort study with efficient sampling of exposure among the cohort, yet they can often be conducted at a fraction of the cost, in a fraction of the time as a corresponding cohort study. Case-control studies can be used to examine associations between multiple exposures and a given health outcome. They are particularly efficient for evaluating rare outcomes but are inefficient for studying uncommon exposures. The primary weakness of the case-control study is the potential for selection bias, which arises if the exposure distribution among the control subjects is not representative of the exposure distribution among the population that gave rise to the cases. Case-control studies that rely on self-reported exposure measures are also susceptible to information bias.

Cross-sectional study

Cross-sectional studies (see, for example, Suarez-Lopez *et al.* in Table 2.2.5.2-1and Grandjean *et al.* 2006 in Appendix 3) are used to evaluate associations between exposure and outcome prevalence in a population at a single point in (or period of) time. The primary advantage of a cross-sectional study is logistical efficiency; they are relatively quick and inexpensive to conduct, as a long period of follow-up is not required, and exposure and outcome assessments occur simultaneously. Cross sectional studies have three primary *potential* disadvantages: 1) potential difficulty in discerning the temporal relationships (i.e., whether the exposure precedes the outcome); 2) estimating outcome prevalence rather than incidence of the outcome; and 3) the possible overrepresentation of cases of the outcome with long duration relative to the average in the population, and often with a better prognosis.

Ecological study

Ecological studies are used to evaluate associations between exposures and outcomes using population-level rather than individual-level data. For example, Nevison (2014, Appendix 3)

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uses annual estimates of pesticides applied to crops and population level autism prevalence to assess the association between OP pesticide exposure and autism. The primary advantages of ecological studies are related to logistical efficiency, as they often rely on pre-existing data sources and require no individual-level exposure, outcome, or covariate assessments. The primary weakness of the ecologic study is the potential for confounding and resultant inappropriate extrapolation of associations observed on the aggregate-level to associations on an individual level. The mistaken belief that associations observed at the population level exist at the individual level is referred to as the ecological fallacy.

In judging an individual study's contribution to the strength of evidence in the epidemiologic literature base, the following hierarchy of observational study designs was considered (from most to least preferred): prospective cohort study, retrospective cohort study, case-control study, cross-sectional study, ecological study. It is important to note, however, that this hierarchy of study designs reflects the *potential* for the collection of high quality information (related to exposure, outcome, confounders, and effect modifiers) and *potential* for efficient and valid estimation of the true association. Thus, in deliberating on quality, care has been taken to consider the circumstances and particulars of each individual study. For example, a well-conducted case-control study of a rare outcome can provide much higher quality evidence vis-à-vis the association of interest than a poorly conducted prospective cohort study of the same relationship. For this report, the placement of the study design in the aforementioned hierarchy of observational study designs was but one facet of the judgment of study design quality. Additional consideration was given to whether the study was *well conducted*, independent of study design type. The particulars of a study's design, specifically the design elements employed to minimize and adjust for biases, were also considered. Finally, the relevance of each study with respect to the association of primary interest in this initiative, namely the relationship between prenatal (and early life) OP pesticide exposures and fetal and child neurodevelopment was considered.

2.2.4.2 Considerations for Study Quality

This section summarizes how specific study characteristics factored in overall quality category. [Note: these study quality considerations are specific to issue of relevance to this document, namely potential for neurodevelopmental effects of OPs. These considerations are considered 'fit for purpose' under this context and could differ in another regulatory or scientific context.]

The literature base evaluated is heterogeneous, as noted in Section 2.2.3. Pesticide exposure assessments variously relied on, for example, exposure biomarkers, maternal self-reports, and other proxy indicators of OP pesticide exposures. Outcome assessments were similarly varied, relying, for example, on biomarkers of biological effects, birth records, maternal self-reports, and clinical instruments designed to evaluate neurocognitive and neurobehavioral development. These design elements have potential impacts on study quality and relevance to this document. Each study was therefore judged to be of high, moderate, or low quality in five

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domains effecting study quality: exposure assessment, outcome assessment, confounder control, statistical analysis, susceptibility to bias (See Table 2.2.4-1 for general considerations under each domain).

Table 2.2.4-1 Study Quality Considerations

Parameter	High	Moderate	Low
Exposure assessment	Exposure assessment includes information on specific OP a.i.'s (e.g., CPF, MAL), or urinary metabolite (TCPy, IMPy,), or high quality questionnaire based chemical specific exposure assessment during relevant exposure window (pre-natal, early life)	Non-specific biomarker of exposure (DAP), or effect (AChE/BuChE), or questionnaire based individual level information on the OP class, or sub-class	Low quality questionnaire based exposure assessment, or ecologic exposure assessment, with or without validation
Outcome Assessment	Standardized tool, validated in study population; or, medical record review with trained staff (birth characteristics)	Standardized tool, not validated in population, or screening tool; or, medical record review, methods unstated (birth characteristics)	Selected sections of test, or maternal report, other; or, maternal/paternal self-report (birth characteristics)
Confounder control	Good control for important confounders relevant to OP-ND question, and standard confounders	Moderately good control confounders, standard variables, not all variables for OP-ND question	Multi-variable analysis not performed, no adjustments
Statistical Analysis	Appropriate to study question and design, supported by adequate sample size, maximizing use of data, reported well (not selective)	Acceptable methods, questionable study power (especially sub-analyses), analytic choices that lose information, not reported clearly	Minimal attention to statistical analyses, comparisons not performed or described clearly
Risk of (other) bias (selection, differential misclassification, effect size magnification, other)	Major sources of other potential biases not likely present, present but analyzed, unlikely to influence magnitude and direction of the risk estimate	Other sources of bias present, acknowledged but not addressed in study, may influence magnitude but not direction of estimate	Major study biases present, unacknowledged or unaddressed in study, cannot exclude other explanations for study finding

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(Adapted from Munoz-Quezada *et al.* 2013)

2.2.4.2.1 Exposure Measures

There were three major categories of exposure assessment employed in this literature: exposure biomarkers, participant-reported proxy exposure via questionnaire, and objectively obtained proxy indicators of exposure. Although one study included environmental wipe sampling results in the exposure assessment, urinary biomarker measures were also included and no differentiation of the two approaches was presented, so this exposure assessment category has not been included. The merits and the disadvantages of the three primary exposure assessment strategies are discussed below. For this evaluation, studies employing exposure assessments that quantified biomarkers of specific OP pesticides (e.g., chlorpyrifos, malathion, diazinon), or urinary metabolites of these pesticides (e.g., TCPy, malathion dicarboxylic acid, 2-isopropyl-4-methyl-6-hydroxypyrimidine), or high-quality chemical-specific exposure quantitation during relevant exposure-time windows (i.e. pre-natal, early life) were given the highest weight. Studies that quantified levels of non-specific biomarker of OP pesticide exposure (e.g., DAPs), or exposure effects (e.g., AChE, BuChE,) were given a moderate weight. Studies relying on high-quality survey-based individual-level information on pesticide exposure were also assigned a moderate weight. Exposure assessments that only crudely or subjectively classified pesticide exposures and ecologic and other proxy measures of exposure were assigned a low weight.

Many studies used questionnaire-based exposure assessments in which study participants (typically mothers) self-reported their exposures, in addition to, or instead of, quantifying OP pesticide biomarkers in samples of biological media. These exposure assessments typically include querying OP pesticide exposure directly, or asked study participants to report on behaviors and conditions associated with pesticide use (e.g., occupation, tasks). Such reporting likely misclassifies actual OP pesticide exposure. If conducted as part of a prospective exposure assessment, these errors are likely to be non-differential with respect to the outcome(s) of interest. In the context of a retrospective assessment in which the mother has knowledge of the outcome status of the child, these errors may be differential or non-differential.

Several studies used proxy measures (including ecological indicators) of pesticide exposure. These included, for example questions about occupational use and exposure to pesticides, distance from residence to fields where pesticides were applied, the proportion of land in a specified area dedicated to agricultural uses, and occurrence of pregnancy during the pesticide application season. Again, substantial non-differential exposure measurement error/misclassification of exposure is likely.

The CHARGE study (Shelton *et al.*, 2015) used a different method for exposure assessment. This study used geospatial analysis to focus on the residential proximity to OP exposure and the association of this exposure with autism spectrum disorders. OP exposure was assessed by Shelton *et al* (2015) using data from the California Department of Pesticide Regulation, with five OPs accounting for a total of 73% of the exposure and each accounting for 10% or more of

the exposure (chlorpyrifos, acephate, diazinon, bensulide, and dimethoate); eight OPs accounting for a total of 25% of the exposure and each accounting for 1% or more of the exposure (malathion, methyl parathion, azinphos-methyl, phosmet, oxydemeton-methyl, ethephon, naled, and methidathion); and eight OPs accounting for a total of 2% of the exposure and each accounting for 0.1% or more of the exposure (methamidophos, phorate, disulfoton, fenamiphos, coumaphos, parathion, ethoprop, and sulfotep).

Most of the studies reviewed herein assessed biomarkers of exposure quantified in samples of biological media (most often urine, but also blood, serum, and breast milk). These biomarkers were of three types: 1) OP pesticide residues, 2) metabolites of specific OP pesticides, and 3) non-specific OP metabolites.

Two studies focusing on neurodevelopmental outcomes measured OP exposure using both DAPs and malathion dicarboxylic acid (a metabolite of malathion) (Eskenazi *et al.*, 2007; Engel *et al.*, 2007), with TCPy exposure also being measured in one of these studies (Eskenazi *et al.*, 2007). Additionally, two studies focusing on birth outcomes measured OP exposure by testing for specific OPs, with Whyatt *et al.* (2004) measuring chlorpyrifos and diazinon; and Eskenazi *et al.* (2004) testing for DAPs and for seven pesticide specific metabolites (MDA - derived from malathion; PNP - derived from methyl parathion, parathion, and other nonpesticide chemicals; TCPy - from chlorpyrifos, chlorpyrifos methyl, and triclopyr; DEAMPY - from pirimiphos methyl; IMPY - from diazinon; CMHC - from coumaphos and coumaphos methyl; CIT - from isazophos and isazophos methyl). Finally, several method validation studies tested for specific OP pesticides, but did not evaluate the association between these exposures and specific adverse health outcomes (Whyatt *et al.*, 2007; Whyatt *et al.*, 2009; Bradman *et al.*, 2003).

The most commonly measured biomarkers were urinary DAPs. These non-specific markers are easily quantified using gas chromatography/mass spectrometry and related methods. Though objective, use of urinary DAPs as biomarkers for OP pesticide exposures has limitations, including substantial temporal variability, often varying substantially over short time scales (i.e., day-to-day). Quantification of DAPs in a single urine sample may not represent an individual's usual exposure to OP pesticides over the time period of interest (e.g., pregnancy) in their utility as biomarkers of OP pesticide exposure. Urinary DAP metabolite levels may also reflect exposure to ambient metabolites in addition to exposure to OP parent compounds. For example, Chen et al (2012) and Zhang et al (2008) have shown that direct exposure to DAPs can occur from consumption of DAPs in food. Lu et al. (2005) observed that OP pesticides in fortified fruit juice samples degraded into DAPs. In this literature, errors in DAP as a biomarker of OP pesticide exposure are likely to be non-differential with respect to outcomes. Epidemiologists often distinguish between two mechanisms or types of misclassification – those that are non-differential (or random) and those that are differential (non-random).

Total DAPs is a non-specific measure of OP exposure and is the sum of six separate molecules - three dimethyl alkylphosphate (DMAP) molecules of DMP, DMTP, DMDTP, and three diethyl alkylphosphate (DEAP) molecules of DEP, DETP, and DEDTP. Each metabolite is a breakdown product from multiple OPs (Table 2.2.4-2). Specifically, DMP, DMTP, and DMDTP are associated

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with 18, 13, and 5 OPs, whereas DEP, DETP, and DEDTP are associated with 10, 10, and 4 OPs, respectively. Thus, using DAPs as an exposure measure, it is not possible to separate the exposure and associated effects for single, specific OPs. For studies evaluating TCPy (e.g., Fortenberry *et al*., 2014; Eskenazi *et al*., 2007; Whyatt *et al*., 2009), this molecule is a metabolite of chlorpyrifos, chlorpyrifos-methyl, and the herbicide triclopyr and thus is not entirely specific to chlorpyrifos. TCPy can be found on directly food. Studies focusing solely on chlorpyrifos could assess exposure to only this OP (i.e., measure chlorpyrifos directly) (e.g., Lovasi *et al*., 2011; Whyatt *et al*., 2004; Rauh *et al*., 2011).

Table 2.2.4-2 CDC Table of Organophosphate Pesticides and Their Dialkyl Phosphate Metabolites (2008)

Pesticide	DMP	DMTP	DMDTP	DEP	DETP	DEDTP
Azinphos methyl	|	|	|			
Chlorethoxyphos				|	|	
Chlorpyrifos				|	|	
Chlorpyrifos methyl	|	|				
Coumaphos				|	|	
Dichlorvos (DDVP)	|					
Diazinon				|	|	
Dicrotophos	|					
Dimethoate	|	|	|			
Disulfoton				|	|	|
Ethion				|	|	|
Fenitrothion	|	|				
Fenthion	|	|				
Isazaphos-methyl	|	|				
Malathion	|	|	|			
Methidathion	|	|	|			
Methyl parathion	|	|				
Naled	|					
Oxydemeton-methyl	|	|				
Parathion				|	|	
Phorate				|	|	|
Phosmet	|	|	|			
Pirimiphos-methyl	|	|				
Sulfotepp				|	|	
Temephos	|	|				
Terbufos				|	|	|
Tetrachlorviphos	|					
Trichlorfon	|					

DMP = dimethylphosphate; DEP = diethylphosphate; DMTP = dimethylthiophosphate; DMDTP =

dimethyldithiophosphate; DETP = diethylthiophosphate; DEDTP = diethyldithiophosphate.

2.2.4.2.2 Neurological and Other Outcome Measures

With some exceptions, the outcomes assessed in this literature fall into three broad categories: 1) neurobehavioral and/or neurodevelopmental status; 2) birth outcomes; and 3) neurodevelopmental diseases and/or disorders (see Table 2.2-b).

There is a broad body of literature available on the use and interpretation of instruments designed to quantify neurological status that is beyond the scope of this summary. Many instruments were used in this literature to assess infant neurodevelopment and neurobehavior (see Table 2.2-b). Importantly, performance on some of these tests can be influenced by the administrator of the assessment. Also of concern is whether these assessments are sensitive enough to distinguish potentially subtle effects of OP pesticide exposure. Some degree of error in the assessment of neurological outcomes is likely in all of the studies, though the errors were unlikely to be related to exposure status in the well conducted studies.

The assessment of birth outcomes in this literature was primarily conducted by reviewing of medical records or birth certificates; these assessments are likely to have minimal errors, and errors that do arise are almost certainly non-differential with respect to exposure status. In some studies, however, birth outcomes were reported by the mother and in these instances, differential misclassification is possible.

The studies that evaluated specific diagnoses, autism spectrum disorders or developmental delay, for example, relied on existing medical records, with some effort to validate diagnoses in a subset of the investigations.

Studies that relied on standardized, validated instruments to assess neurodevelopment, medical records of birth outcomes, or validated diagnosis of disease states were weighted highly in the judgment of study quality. Those that used standardized instruments that had not been validated in the relevant population or screening assessments were given a moderate weight, while studies relying on selected sections of neurodevelopment assessment tools, maternal report of outcome status, or aggregated (ecological) outcome measures were given the lowest weight.

2.2.4.2.3. Statistical Analysis

Statistical analyses that were appropriate to the study question and study design, supported by adequate sample size, maximized the use of available data, and were well characterized in the report were weighted most highly. Acceptable statistical methods, moderate study power, and analytic choices that resulted in the loss of information or that were not clearly reported were given moderate weight. Reports with only minimal attention paid to the conduct and reporting of the statistical analyses were given the lowest weight.

2.2.4.2.4 Confounding

Risk factors for early life neurodevelopment perturbations that are associated with OP pesticide exposure, but not caused by pesticide exposure, are potential confounders in this literature. Socioeconomic determinants of child development (including, for example, maternal education and access to prenatal and early life health care), quantity and quality of parent-child interactions, and exposure to other environmental toxicants (e.g., lead, PCBs, other pesticides) are difficult to measure and were either not accounted for, or inadequately accounted for in many studies. That said, some studies were relatively homogeneous with respect to these factors and thus limited confounding by design, while others attempted to quantify these factors and adjust for them. Other important potential confounders, such as the child's sex, are easy to identify and adjust for analytically.

2.2.4.2.5 Risk of Bias

The internal validity of the studies reviewed was judged by noting the design strategies and analytic methods used in each study to constrain or eliminate selection bias, information bias, and confounding. Selection bias occurs when the sampling of the population by the investigator yields a study population that is not representative of the exposure and outcome distributions in the population sampled. Put simply, selection bias occurs if selection of the study sample yields a different estimate of the measure of association than that which would have been obtained, had the entire target population been evaluated. Although there are numerous sources of selection bias, there are several mechanisms that may have induced selection bias in the studies reviewed: less than 100% participation rates of eligible individuals due to non-responsiveness or refusal (self-selection bias); loss to follow-up (i.e. failure to retain all study participants initially enrolled in the study); and, in a case-control study, control selection bias arising because the exposure distribution in the control sample does not represent the exposure distribution of the study base (i.e., the population that gave rise to the cases or more formally, the person-time experience of that population).

Information bias (also referred to as observation bias) arises when study participants are incorrectly categorized with respect to their exposure or outcome status, or when errors arise in the measurement of exposure or outcome, in the case of continuously distributed measures. Epidemiologists often distinguish between two mechanisms or types of misclassification – those that are non-differential (or random) and those that are differential (non-random). Non-differential misclassification of exposure (or non-differential exposure measurement error) occurs when the probability or magnitude of error in the classification or measurement of exposure is independent of the outcome status of the study participants. Similarly, non-differential misclassification of outcome (or outcome measurement error) occurs when the probability or magnitude of error in the assignment of outcome status or level is independent of exposure status. In contrast, differential exposure misclassification (or measurement error) occurs when the error in the exposure assignment is not independent of the outcome status.

The mechanisms that cause non-differential misclassification in this literature include errors in the medical records, laboratory errors, sampling of biospecimens for biomarker assays, and errors in recall. The mechanisms that induce differential misclassification include recall bias,

and interviewer/observer bias. Note that mismeasurement of confounders can result in residual confounding of the association of interest, even when adjustment for that confounder has been conducted in the analysis.

Studies in which major sources of potential biases were not likely to be present, or in which potential sources of bias were present but effectively addressed and analyzed to maximize the study validity, and those in which sources of bias were unlikely to influence the magnitude and direction of the risk estimate were given a high weight. Studies where sources of bias were present and acknowledged by the authors but not addressed in the study and yet may influence the magnitude, but not direction of the association estimate received a moderate weight. A low weight was given to studies in which major biases were present and yet were not acknowledged or addressed in the study, such that they cannot be excluded as an alternative explanation for the study finding.

2.2.5 Review of Quality Results

Each of the studies in the 2015 review was judged to be of high, moderate, or low quality in each of five domains of study design and methodology effecting study quality as discussed above in Section 2.1. The results of the quality assessment are presented separately below for each group. The quality categories represent to the total evaluation. In Section 2.2.6, further evaluation of the study design and exposure assessment of the medium and high quality studies. This further evaluation led to additional studies being removed from the final analysis, with these excluded studies not being considered further in the remaining sections of this document.

2.2.5.1 "High" Quality Group

Fifteen articles assigned a high quality rating are shown in Tables 2.2.5.1-1 and 2.2.5.1-2. In general, these were prospective birth cohort studies with moderate to high sample size; exposure assessment was based on an objective biomarker measure, the outcome measurement(s) utilized standardized tests and trained data collectors, appropriate statistical analyses were performed, considering relevant covariates, and risks of bias were minimized to the extent possible. All of the studies from CCCEH, Mt. Sinai, CHAMACOS, and HOME cohorts are assigned a high ranking. Fortenberry *et al.* (2014, Table 2.2.5-1) reported on findings in the ELEMENT study population of 187 mother-child pairs, assessed third trimester maternal urinary TCPy as a biomarker of prenatal chlorpyrifos exposure, and employed a trained and experienced research team to administer a battery of validated ADHD/psychometric assessments (Conner's' Parental Rating Scales-Revised, Conner's' Continuous Performance Test, and the Behavior Assessment System for Children-Parental Rating Scales) that had been translated into Spanish.

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Table 2.2.5.1-1. High Quality Studies from 2015 and 2016 reviews: Summary of Study Design Elements Impacting Study Quality Assignment

Study	Study Location	Cohort Name / Description of Study Population	Study Design	Exposure Assessment	Outcome Assessment	Confounder / Covariate Control	Statistical Analysis	Risk of (other) Bias
Barr *et al*. (2010)	New Brunswick, New Jersey	Mother who underwent elective Cesarean delivery at term and their newborns, Saint Peter's University Hospital	Cross sectional birth cohort, modest sample numbers N=150 Mother-infant pairs	Objective measure, prenatal - parent CPF in maternal and cord serum at birth - exposures do not necessarily precede outcome	Birth outcomes from medical records	Generally appropriate; missing a few, e.g., maternal age, SES, nutrition, pre-natal care, race	Appropriate multivariate analysis	Convenience sample – deemed low probability of selection bias; low potential for exposure or outcome misclassification
Bouchard *et al*. (2010)	U.S. National Population	National Health and Nutrition Examination Survey (NHANES) Age 8-15 years	Cross-sectional, large sample size N=1139	Objective measure, postnatal – single non-OP specific child urinary DAPs	ADHD from DISC-IV diagnosis or medication in children 8-15 years old, standard protocol, trained interviewers	Appropriate: also included blood lead	Appropriate, accounted for NHANES multistage probability sampling	Low probability of selection bias; some potential for non-differential misclassification of exposure
Donauer et al. (2016).	Cincinnati, Ohio, USA	Health Outcomes and Measure of the Environment (HOME)	Prospective Birth Cohort Study – large sample size. N=327 Mother-infant pairs	Metabolite concentrations (total DAP, DM, and DE) of prenatal OP pesticide exposure quantified in two maternal spot urine samples provided at 16 and 26 weeks of gestation	For children, Bayley-II, MDI, and Psychomotor Development Index examinations at 1-3 years of age, and Clinical Evaluation of Language Fundamentals-Preschool, Second Edition, and the Wechsler Preschool and Primary Scale of Intelligence, at ages 4 and 5 were used.	Appropriate. Included household income, maternal intelligence, child's sex, maternal intelligence quotient, and maternal education; house inventory score, maternal age at birth, alcohol consumption during pregnancy, parity, and maternal lead levels	Appropriate. Bivariate and multivariable regression	Lack of frequent urine spot sampling, short half-life of DAP metabolites, and the sporadic exposure to DAP may have led to non-differential exposure misclassification; inability to determine exposure toxicity.
Engel et al. (2015)	Salinas Valley, California; Brown, Butler, Clermont, Hamilton, or Warren, Ohio; South Bronx, New York City; Manhattan, New York City	Pooled analysis of the CHAMACOS (1999-2000), Columbia (2000-2001), Mt. Sinai (1998-2002), and HOME (2003-2006) birth cohorts	Birth Cohort Study – large sample size N= 936 women (377 from CHAMACOS study, 265 from HOME study, 60 from Columbia study, and 234 from Mount Sinai study) Mother-infant pairs	Metabolite concentrations (total DAP, DMP, and DEP) of prenatal OP pesticide exposure quantified in two maternal spot urine samples provided at 13 and 26 weeks and 16 and 26 weeks of gestation (CHAMACOS and HOME studies) and in one maternal spot urine sample at 31 and 32 weeks (Mt. Sinai and Columbia studies) Blood samples from the mother and child (via the umbilical chord) were also collected and genotyped for the *PON1* genotype	For each child, Bayley Scales of Infant Development II (BSID-11) to generate a Mental Development Index (MDI) and a Psychomotor Development Index (PDI) to assess neurodevelopment in early childhood.	Appropriate. Included maternal education, marital status, and age at delivery, alcohol use during pregnancy, race/ethnicity, smoking and drug use during pregnancy, child sex, HOME environment score quartile, breastfeeding at least 3 months after birth, metabolite × center interaction terms, race/ethnicity, smoking and drug use during pregnancy.	Linear models with interaction analysis.	Lack of frequent urine spot sampling, short half-life of DAP metabolites, and the sporadic exposure to DAP may have led to non-differential exposure misclassification; inability to determine exposure toxicity; and hetergoenity of the study populations of the 4 cohorts and exposures may have effected the results
Furlong *et al*. (2014)	New York City, U.S.	Mount Sinai Children's Environmental Health Study	Prospective Cohort N=136 Age 7-9 years	Objective biomarker, prenatal OP pesticide exposure (DAP) quantified in single maternal spot urine sample provided during the 3rd trimester, simple imputation < lowest level of detection (LLOD)	Reciprocal social impairment at age 7-9 years assessed using the Social Responsiveness Scale, completed by mothers. Designed to assess reciprocal social behaviors in evaluating ASDs, used here as general indicator of impaired social responsiveness.	Appropriate. Included maternal and child demographic and SES indicators (age, education) and ETS (not other environmental toxicants).	Appropriate multivariate analysis	Selection bias possible due to considerable loss to follow-up; Residual confounding likely small in magnitude; some potential for non-differential misclassification of exposure possible explanation for null findings).
Fortenberry *et al*. (2014)	Mexico City, Mexico	Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT) study (3 cohorts 1994-1997 (cohort 1), 1997-	Prospective Birth Cohort Study – modest sample size	Objective measure - TCPy concentration was measured in third trimester maternal urine samples. In a subset of women	ADHD - LP - Conner's' Parent Rating Scales-Revised (CPRS-R), Conner's' Continuous Performance Test (CPT), and Behavior Assessment System for Children-Parental Rating Scales	Appropriate. Included continuous maternal IQ, education, socioeconomic status and blood lead one month after delivery, breast feeding (yes/no), child's sex,	Appropriate. Multivariable linear regression	History of maternal and paternal exposure to pesticides not included, use of a single urinary measure to estimate exposure, potential for differential exposure misclassification possible as mothers were

Study	Study Location	Cohort Name / Description of Study Population	Study Design	Exposure Assessment	Outcome Assessment	Confounder / Covariate Control	Statistical Analysis	Risk of (other) Bias
		2000 (cohort 2), and 2001-2005 (cohort 3))	N=187 Mother-infant pairs	randomly selected urinary TCPy in samples collected during all three trimesters of pregnancy was measured.	(BASC-PRS) – These are screening tools, not diagnostic tools. Standardization and quality control checks were conducted by reviews of videotaped evaluations.	continuous age at testing, birth length and head circumference at birth.		likely aware of the neurobehavioral status of their children
Fortenberry *et al.* (2014a)	Mexico City, Mexico	Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT) study	Prospective Birth Cohort Study – large sample size N=591 Mother-infant pairs	Objective measure - blood samples from mother–child pairs were analyzed for PON1. OP exposure was not explicitly assessed.	ADHD - LP - Conner's' Parent Rating Scales-Revised (CPRS-R), Conner's' Continuous Performance Test (CPT), and Behavior Assessment System for Children-Parental Rating Scales (BASC-PRS) - These are screening tools, not diagnostic tools.	Appropriate: Included continuous maternal IQ, education, socioeconomic status and blood lead one month after delivery, breast feeding (yes/no), child's sex, continuous age at testing, birth length and head circumference at birth.	Appropriate. Multivariable linear regression	Potential for differential exposure misclassification possible as mothers were likely aware of the neurobehavioral status of their children.
Harley et al. (2016)	Salinas Valley, California; Brown, Butler, Clermont, Hamilton, or Warren, Ohio; South Bronx, New York City; Manhattan, New York City	Pooled analysis of four CHAMACOS, Columbia, Mt. Sinai, and HOME birth cohorts	Birth Cohort Study – large sample size N= 1,235 women (484 from CHAMACOS study, 328 from HOME study, 82 from Columbia study, and 341 from Mount Sinai study) Mother-infant pairs	Metabolite concentrations (total DAP, DMP, and DEP) of prenatal OP pesticide exposure quantified in two maternal spot urine samples provided at 15.9 and 26.4 weeks of gestation (CHAMACOS and HOME studies) and in one maternal spot urine sample at 31.8 and 33.3 weeks (Mt. Sinai and Columbia studies) Blood samples from the mother and child (via the umbilical chord) were also collected and genotyped for the *PON1* genotype	For each child, head circumference, birth weight, and birth length were obtained from medical records	Appropriate. Included sex, race/ethnicity, cohort, country of origin, marital status, maternal education, smoking during pregnancy, parity, maternal age at delivery, and gestational age.	Appropriate. Multivariable linear regression and mixed-effect models to test the effect modification for sex, maternal education, race/ethnicity, and child *PON1* genotype.	Lack of frequent urine spot sampling, short half-life of DAP metabolites, and the sporadic exposure to DAP may have led to non-differential exposure misclassification; inability to determine exposure toxicity; and hetergoenity of the study populations of the 4 cohorts and exposures may have effected the results
Quirós-Alcalá *et al.* (2011)	Salinas Valley, California, USA	Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS)	Prospective Birth Cohort Study Outcomes assessed at: N=149 (6 months) N=149 (1 year) N=97 (3.5 years) N= 274 (5 years)	Objective biomarker, prenatal OP pesticide exposure (DAP) quantified in two maternal spot urine samples at 14 and 27 weeks gestation (on average), and in single child urine sample provided at time of each outcome assessment	Autonomic nervous system dysregulation at ages 6 months, 1 year, 3.5 years, and 5 years; standard protocol, trained, bilingual research staff, appropriate to age of child. May not be sensitive to resolve subtle effects of OP pesticide exposure.	Appropriate. Included maternal and child demographic and SES indicators (age, education).	Appropriate multivariate analysis	Selection bias possible due to considerable loss to follow-up; Residual confounding likely small in magnitude; some potential for non-differential misclassification of exposure (possible explanation for null findings).
Raanan *et al.* (2015)	Salinas Valley, California	CHAMACOS (Mothers and their children. Children were followed from through age 7)	Prospective Birth Cohort N = 364 children	Diethyl (DE) and dimethyl(DM) phosphate metabolites and other dialkyl phosphate (DAP) metabolites of OP pesticides measured in mother's urine during pregnancy and from children during childhood	Mother's report of child's respiratory symptoms and child's exercise-induced coughing at 5 and 7 years of age	Models were adjusted for child's sex, child's age, maternal smoking during pregnancy, secondhand tobacco smoke exposure during early childhood, season of birth, mean daily fine particulate concentration during first 3 months of life (PM2.5), breastfeeding, mold and cockroaches in home, and home distance from highway in first year.	Generalized estimating equations (GEE) with repeated measures of respiratory symptoms	Risk of misclassification of exposure, due to nonspecificity of DAP metabolite used as biomarker which may reflect exposure to parent compound (pesticide) or preformed DAPs in environment, and also due to potential for daily fluctuations in DAP levels due to high variability in OP pesticide exposures.

Study	Study Location	Cohort Name / Description of Study Population	Study Design	Exposure Assessment	Outcome Assessment	Confounder / Covariate Contro	Statistical Analysis	Risk of (other) Bias
Rauch *et al*. (2012)	Cincinnati, Ohio, USA	Health Outcomes and Measure of the Environment (HOME) Age: newborns	Prospective Birth Cohort Study – large sample size. N=306	Objective biomarker of prenatal OP pesticide exposure (DAP) quantified in two maternal spot urine samples provided at 16 and 26 weeks gestation	Abstracted birth weight from medical records, calculated gestational age from mother's self-reported date of last menstrual period. When gestational age not available, results of an ultrasound was used or a Ballard examination performed just after delivery.	Appropriate. Included income, education, maternal depressive symptoms, maternal IQ, insurance status, area of residence, prenatal care, PON1 genotype, and gestational exposure to alcohol, lead, and tobacco.	Appropriate. multivariable regression	Unable to rule out the possibility that differences in DAP concentrations partially reflect individual variation in metabolism, Recall error
Rauh et al. (2015)	New York City, New York	Columbia Center for Children's Environmental Health (CCCEH) (Children of Black and Dominican mothers, aged 9 – 13.9 years old)	Prospective Birth Cohort Study N = 271	Chlorpyrifos (CPF) concentrations in sample of umbilical cord blood collected at delivery Chlorpyrifos (CPF) concentrations in sample of maternal blood collected within 2 days postpartum	Neurodevelopmental assessment via Archimedes spirals, a drawing exercise, to test for tremor	Models were adjusted for sex, exact age at testing, ethnicity, and medications	Chi-square was to assess crude associations between high CPF exposure and presence of mild to moderate tremor. Binary logistic regression was used to estimate associations between high exposure level and presence of tremor	Risk of misclassification of exposure, particularly due to lack of CPF exposure biomarkers to assess postnatal and childhood exposure. Misclassification may also occur in the outcome assessment due to the use of a clinical scale, based on observer's rating to assess tremor
Stein et al. (2016)	Salinas Valley, California area	CHAMACOS Study (Pregnant women and 7 year-old children)	Prospective longitudinal cohort study N = 329 Mother-infant pairs	Biomarker of prenatal OP pesticide exposure (DAPs) quantified in two maternal spot urine sample collected at 13 and 26 weeks of gestation. Mothers were also interviewed following birth at 6 months, 1, 2, 3.5, 5, and 7 years to collect data on early adversities and assess the home environment using the HOME test	Children's IQ was analyzed using the Wechsler Intelligence Scale for Children	Appropriate. All models were adjusted for maternal IQ score at 6m assessed by Peabody Picture Vocabulary Test. Full-Scale and Verbal Comprehension IQ models were also adjusted for language of neurological assessment. Models in which adversity measures did not include H.O.M.E. subscores were adjusted for long term H.O.M.E. score average; models in which adversity measures did not include parental educational attainment were adjusted for maternal education.	Appropriate. Multivariate linear regression model and three-way interaction model	Selection bias probable; adversity measures calculated via *a priori* hypotheses, instead of incorporating using more advanced statistical methods; lack of frequent maternal urine spot sampling and due to the short half-life of DAP metabolites may have led to exposure misclassification; low concentrations of DAP detected in the urine; lack of study details regarding the urine collection methods
Wolff *et al*. (2007)	New York City, U.S.	Mount Sinai Children's Environmental Health Study	Prospective Cohort – moderate sample size N=404 Mother-infant pairs	Objective biomarker, prenatal OP pesticide exposure (DAP), malathion (MDA) quantified in single maternal spot urine sample provided during the 3rd trimester	Birth outcomes from computerized perinatal database	Appropriate: different covariates for each outcome, e.g., weight: maternal age, race/ethnicity, maternal BMI*pregnancy weight gain, infant sex, gestational age, creatinine	Appropriate PROC GLM, with varying covariates	Selection bias unlikely; some potential for non-differential misclassification of exposure
Yolton et al. (2013)	Cincinnati, Ohio, USA	Health Outcomes and Measure of the Environment (HOME)	Prospective Birth Cohort Study – large sample size. N=350 Mother-infant pairs	Metabolite concentrations (total DAP, DM, and DE) of prenatal OP pesticide exposure quantified in two maternal spot urine samples provided at 16 and 26 weeks of gestation	For children, a neurobehavioral assessment was conducted via the NICU Network Neurobehavorial Scale (NNNS) at ~ 5 weeks of age	Appropriate. Included sex, birthweight, infant weight change from birth to exam, parity, maternal age at delivery, marital status, education, employment during pregnancy, household income, body mass index (BMI) at 16 weeks gestation, weight gain per week during pregnancy, moderate to severe depression (score > 13) measured on the Beck Depression Inventory-II during pregnancy and at 5 weeks post-delivery, reported marijuana and alcohol use during pregnancy, whole blood lead and	Appropriate. Bivariate and multivariable regression	Low concentrations of DAP detected in the urine; lack of frequent urine spot sampling during pregnancy.

						folate during pregnancy, serum cotinine, and reported fruit and vegetable consumption during pregnancy		

Table 2.2.5.1-2 High Quality Studies from 2012/2014 literature search of Children's Environmental Health Epidemiology Studies

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
Whyatt *et al.* (2004) Columbia U. (N=314)	Birth length, Birth weight, head circumference	4 cord plasma chlorpyrifos exposure groups and 4 chlorpyrifos and diazinon exposure groups. Chlorpyrifos only categories, Group 1: levels below LOD (32% of participants); Group 2: lowest 1/3 of detectable levels (20 %); Group 3: middle 1/3 (24%), Group 4: highest 1/3 (25%.) Chlorpyrifos and diazinon together: Group 1: 26%, Group 2: 22 %, Group 3: 26%, Group 4: 26%.	Gestational age, maternal pre-pregnancy weight, maternal net pregnancy weight gain, gender of newborn, parity, race/ethnicity, ETS in home, season, cesarean section	For each log unit increase in cord plasma chlorpyrifos levels, birth weight decreased by 42.6 g (95% CI: –81.8 to –3.8) and birth length decreased by 0.24 cm (95% CI: –0.47 to –0.01). Birth weight averaged 186.3 g less (95% CI: –375.2 to –45.5) among newborns with the highest compared with lowest 26% of exposure levels (p = 0.01).	Associations between birth weight and length and cord plasma chlorpyrifos were statistically significant (p ≤ 0.007) among newborns born before the January 2001 policy change. Among newborns born after January 2001, exposure levels were substantially lower, and no associations with fetal growth outcomes were observed (p > 0.8).	Strengths: prospective nature of the study; direct measurement of chlorpyrifos in cord blood and personal air samples, rather than non-specific markers of organophosphate pesticide exposure; consideration of other pesticides and environmental contaminants as covariates in the multivariate models. Limitations: single exposure sampling period; the authors did not present nor discuss regression diagnostics to assess the degree to which their models met or violated the assumptions implicit in linear models.
Berkowitz *et al.*(2004) Mt. Sinai (N=404)	Birth length, birth weight, head circumference, gestational age	LOD: 11 ug/L (57% <LOD TCPy)	Race/ethnicity, infant sex, and gestational age. The authors also controlled for birth weight or birth length in their assessment of head circumference and pesticide exposure.	Mean levels of birth weight, length, head circumference, and gestational age did not differ between those with urinary pesticide metabolite levels below and above the level of detection. Similarly, no statistically significant associations were observed between reported pesticide	PON1 activity also predictor of smaller head circumference; creatinine corrected	Very well conducted study with numerous strengths and very few weaknesses. The questionnaire-based pesticide exposure questions are subject to imperfect recall. Errors would, on average, attenuate associations between these exposure metrics and fetal development.

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
				exposure and mean indices of fetal growth and gestational age.		Recall-based exposure assessments were fortified by objective measures of pesticides/pesticide metabolites. A metabolite specific for chlorpyrifos (TCPy) was assessed. Statistical analysis was appropriate. Observed mean reductions in the outcome parameters appear to be small in magnitude and may be of little clinical significance. Assessment and control for confounding were appropriate. However, confounding by unmeasured (and mismeasured) risk factors for abnormal growth that are related to pesticide exposure would bias the results in this study. Such factors may be related to socioeconomic status of the study participants, a cofactor which is difficult to define, no less measure in an epidemiologic study. Limited external validity (generalizability) due to the particular study population recruited and the numerous exclusion criteria applied.
Eskenazi *et al*.	Birth length, birth	Total DAPs:	Gestational age, gestational age	Decreases in gestational	Maternal urine collection	Strengths in the study

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Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
(2004) CHAMACOS (N=488)	weight, head circumference, Gestation Length, Ponderal index	median 136 nmol/L (range: 10–6,854); DEP: median 22 nmol/L (range: 2–680 nmol/L); TCPy: median 3.3 nmol/L (range: 0.2–56.1nmol/L) (76% >LOD)	squared, maternal age, pregnancy weight gain, week of initiating prenatal care, parity, infant sex, mother's country of birth, body mass index, family income, poverty level, smoking, alcohol, illicit drug use, environmental tobacco smoke, caffeine, history of low birth weight, and history of pre-term delivery.	duration associated with two measures of in utero pesticide exposure: levels of metabolites of dimethyl phosphate pesticide compounds and whole blood ChE.	averaged weeks 14, 26, not creatinine-corrected	design include the longitudinal design, the use of multiple exposure biomarkers, including quantification of non-specific (DAPs), chlorpyrifos-specific (TCPy) metabolites, and other environmental co-exposures. A reasonable set of exclusion criteria were applied.The selection of the CHAMACOS population, which consists mostly of children from low-income families, served to increase the relative statistical efficiency of the study, as this population is at high risk of neurodevelopmental deficits, compared to the general population.The statistical analysis used to assess the associations between the markers of exposure and neurodevelopment were appropriate.Errors in the assignment of exposure in this prospective study will likely have resulted in attenuation of observed associations.
Harley *et al*. (2011) CHAMACOS (N=329)	Birth length, birth weight, head circumference, gestational age	The geometric mean for the DAP concentrations during pregnancy (for the average of the two sampling periods) was 146 nmol/L (95% CI: 133,	Maternal intelligence (Peabody Picture Vocabulary Test (PPVT)), measures of how stimulating the environment is, and known or suspected neurotoxins were measured prenatally. To measure the quality and extent of stimulation available to a child in the home environment,	The authors observed evidence of an association between prenatal exposure to OP pesticides as measured by urinary DAP metabolites in women during pregnancy, is associated with decreased cognitive functioning in	Infants whose PON1 genotype and enzyme activity levels suggested that they might be more susceptible tothe effects of OP pesticide exposure had decreased fetal growth and length of gestation. PON1 may be a contributing	This study has many strengths, the longitudinal design, the measurement of urinary DAP at multiple time points and following children to age seven when tests of cognitive function are reportedly more reliable. The authors were

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
		160); of this, a larger proportion was DMP metabolites (GM = 109 nmol/L; 95% CI =98, 120) than DEPs (GM= 23 nmol/L; 95% CI = 21, 25). Allele frequencies: PON1 192 Q allele= 50%; PON1 -108 T allele= 46%.Mean arylesterase activity: For infants: 33.6 U/mL (SD = 16) For mothers: 136.6 U/mL (SD = 44). Mean paraoxonase activity: For infants: 256.6 U/L (SD = 165); For mothers: 989.0 U/L (SD = 616).	the Infant-Toddler HOME (Home Observation for Measurement of the Environment) inventory was completed at the 6-month, 1, 2, 3.5, 5, and 7 year visits; known or suspected neurotoxicants, polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), p,p'-dichlorodiphenyltrichlorethane (DDT), p,p'-dichlorodiphenyltrichlorethylene (DDE), and lead.	children at age 7.	factor to preterm or low birth weight birth.	able to adjust for or consider many factors related to cognitive function, such as prenatal exposure to other environmental agents, socioeconomic indicators, maternal intelligence and education, and child stimulation. The cohort had a relatively homogenous socioeconomic profile, reducing the potential for uncontrolled confounding.
Engel *et al*. (2007) Mt. Sinai (N=311)	Brazelton Neonatal Behavioral Assessment Scale (BNBAS), primitive reflexes (neurological integrity) measured before hospital discharge.	DEP: 24.7 nmol/L; Total DAP: 82 nmol/L	Maternal age, race, marital status, education, cesarean delivery, delivery anesthesia, infant age at examination, infant gender, infant jaundice, smoking (yes/no), alcohol consumption, caffeine consumption, illicit drug use during pregnancy, and the examiner.	No adverse associations were found for DAPs and any measured behavior. Relative to the first quartile, quartiles 2–4 of total DEPs, DMPs, and DAPs were associated with an increased proportion of abnormal reflexes, although the associations did not increase monotonically and varied in their strength and precision.	Used non-specific biomarker DEP/DAP	This was a well conducted prospective study conducted in a young, predominantly minority population. The study design, analytic approach, and statistical analyses were appropriate. Pesticide metabolites evaluated are not specific for chlorpyrifos. The BNBAS was administered before hospital discharge only on a subset of children in the cohort (n =311/404). Factors related to weekend

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
						delivery (e.g., fewer inductions) would be underrepresented among the tested subjects, and may induce bias, reduce the degree of precision with which associations were estimated, and limit the generalizability of the study findings.The statistical analysis was largely appropriate. Imputing of missing data may affect precision of association estimates and result in attenuated effect estimates as a result of exposure measurement error. Assessment and control for confounding were appropriate. However, confounding by unmeasured (and mis-measured) risk factors for abnormal growth that are related to pesticide exposure would bias the results in this study. Such factors may be related to socioeconomic status of the study participants, a cofactor which is difficult to define, no less measure in an epidemiologic study.
Young *et al*. (2005) CHAMACOS (N=381)	Neurodevelopment, Brazelton Neonatal Behavioral AssessmentScale (BNBAS), abnormal reflexes	DAP (average during pregnancy): median 222nmol/L (range: 7–21,867 nmol/L); DEP (average during pregnancy):	Maternal age, BMI, any smoking/alcohol/drug use during pregnancy, gestational age at which prenatal care was initiated, total number of prenatal care visits, mean pregnancy blood pressure, parity, method of delivery, general anesthesia used during	Among the >3 day old infants, increasing average prenatal urinary metabolite levels were associated with both an increase in number of abnormal reflexes (total DAP: adjusted beta = 0.53, 95% CI = 0.23, 0.82; dimethyls: adjusted beta =	Associations seen pre-natal OP, not post-natal OP exposure, Maternal urine collection averaged weeks 14, 26	Strengths: Longitudinal design, measurement and consideration of many confounders, the prenatal exposure measures were, with some exceptions, the average of two measurements, and thus may better reflect chronic

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
		median 21 nmol/L (range: 2–680 nmol/L)	delivery, breastfeeding initiated after delivery, poverty level, infant sex, age in days at BNBAS, minutes since last feed at BNBAS, and BNBAS examiner.	0.41, 95% CI = 0.12, 0.69; diethyls: adjusted beta = 0.37, 95% CI = 0.09, 0.64), and the proportion of infants with more than three abnormal reflexes (total DAP: adjusted OR = 4.9, 95% CI = 1.5, 16.1; dimethyls: adjusted OR = 3.2, 95% CI = 1.1, 9.8; diethyls: adjusted OR = 3.4, 95% CI = 1.2, 9.9).		exposure during the pregnancy Weaknesses: Potential for residual confounding, potential for exposure misclassification, potential selection bias (not all children had the outcome measures), study population not generalizable to the whole US.
Rauh *et al*. (2006) Columbia U. (N=254)	Neurodevelopment: The Bayley Scales of Infant Development II (BSID-II), Mental Development Index (MDI) and Psychomotor Development Index (PDI) at 12, 24, and 36 months of age. • Behavior: Child Behavior Checklist (CBCL) at 12, 24, and 36 months. • Quality of the child-care environment: The Home Observation for Measurement of the Environment (HOME)	Exposure levels were categorized as low (≤ 6.17 pg/g) or high (>6.17 pg/g)	Data were collected regarding lead exposure, demographics, education and occupational history, income, active and passive smoking, alcohol and drug use during pregnancy, and residential pesticide use. Final models included prenatal environmental tobacco smoke (ETS) exposure, gender, ethnicity, gestational age at birth, quality of home care-taking environment, maternal education, and maternal IQ.	At the 36 month milestone, the likelihood of highly exposed children developing mental delays were 2.4 times greater (95% CI: 1.12-5.08, p = 0.02) and motor delays were 4.9 greater (95% CI: 1.78-13.72; p = 0.002) than those with lower prenatal exposure. The GLM analysis for PDI scores showed a significant effect of chlorpyrifos exposure over time with an estimated deficit of approximately 7 points by age 36 months (p = 0.01).	The authors summarize three main findings: 1) by age 3, the more highly exposed children demonstrated mental and motor delays; 2) the observed developmental trajectories for PDI and MDI scores confirmed that the adverse impact on cognitive and motor development increased over time; and 3) by age 3, highly exposed children were more likely to demonstrate clinically significant attention problems.	• Only 53% of the children reached the three year milestone with study data collected. It is unclear what percentage of these children did not survive, were lost to follow-up, or too sick to participate. • Reliance on a single exposure level (prenatal/cord blood.) • No control for exposure over the subsequent 3 years • Creation of a dichotomous exposure variable brings limitations due to the amount of within-group variation. • Limitations of the sensitivity and predictive validity of the developmental tests, especially among children less than 3 years of age. • No discussion of whether this 7-point deficit is clinically relevant. • Due to the pervasive, non-specific nature of neurological effects, it is difficult to attribute

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
						causality.
Lovasi *et al*. 2011 Columbia U. (N=266)	Bayley scores (MDI/PDI) 12 months, 24 months, 36 months	N/A	Neighborhood characteristics: The percentage of housing units without complete plumbing, the percentage of vacant housing units, the percentage of residents below the federal poverty line, the percentage of residents older than 25 years of age who completed high school, the percentage of households receiving public assistance, the percentage of housing units with one or more residents per room, racial composition, the percentage of residents born outside the United States, the percentage of Spanish-speaking residents, and the percentage of residents who were linguistically isolated	Neighborhood characteristics did not confound the observed association between chlorpyrifos levels and cognitive development.	Hierarchal regression analysis of potential confounding by SES	Direct measurement of chlorpyrifos.The statistical analyses were generally appropriate. Missing data on covariates were estimated using multiple imputation, and the variance estimates presented appropriately reflect the degree of uncertainty caused by missing covariate data. Robust standard errors were used.The setting of the investigation in a sample drawn from low-income African American and Dominican communities is both a strength (increases the power, restriction of confounders) and a limitation of the study (reduced generalizability).
Engel *et al*. (2011) Mt. Sinai (N=276)	Bayley scores (MDI/PDI) at 12 months, 24 months.	DEP: 24.7 nmol/L; Total DAP: 82 nmol/L (same as Engel 2007)	Maternal age, race/ethnicity, marital status, education, breast-feeding, child sex, alcohol, smoking, or drug use during pregnancy, maternal IQ, a score based on assessment of the home environment (HOME), season of urine collection, language spoken in the home, age at testing, examiner, and urinary creatinine level.	An observed association between prenatal total dialkylphosphate metabolite level and a decrement in mental development at 12 months among blacks and Hispanics.	Used non-specific biomarker DEP/DAP; some evidence of effect modification by PON1 genotype	Limitations include use of non-specific markers of chlorpyrifos pesticide exposure (DAPs), use of only a single (third-trimester) urine sample, and the large proportion of loss to follow-up. Statistical analysis was appropriate. Imputing of missing data may affect precision of association estimates and result in attenuated effect estimates as a result of exposure measurement error, although these are offset by the further

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
						categorization of the exposure levels (at the median). However, binning of exposure levels reduces precision, relative to a continuously distributed measure of exposure. Assessment and control for confounding were appropriate. However, confounding by unmeasured (and mismeasured) risk factors for abnormal growth that are related to pesticide exposure would bias the results in this study. Such factors may be related to socioeconomic status of the study participants, a cofactor which is difficult to define, no less measure in an epidemiologic study.
Eskenazi *et al*. (2007) CHAMACOS (N=372)	Neurodevelopment, Bayley Index (MDI, PDI), Maternal behavior checklist at 6, 12, and 24 months	DEP: geom. Mean in mother 18.1 nmol/L (95% CI = 16.7–19.7); DEP geometric mean in child at 24 months 10.5 nmol/L (95% CI =8.8–12.6); TCPy median 3.54 ug/l	Psychometrician, location of assessment, exact age at assessment, sex, breast-feeding duration (months), HOME score, and household income, parity, maternal PPVT, maternal age, education, depressive symptoms, active/passive smoking exposure during pregnancy, regular alcohol use during pregnancy, marital status, father's presence in home, housing density, maternal work status, ≥ 15 hours out-of-home childcare/week, birth weight, gestational age, abnormal reflexes, PCBs, lead, DDT, β-hexachlorocyclohexane, and hexa-chlorobenzene	DAP metabolite levels during pregnancy, particularly from dimethyl phosphate pesticides, may be negatively associated at 24 months with mental development (MDI) on the Bayley Scales and an increase in risk of maternally reported PDD.	No strong associations identified with DE or TCPy, Maternal urine collection averaged weeks 14, 26	Strengths: Longitudinal design, measurement and consideration of many confounders (including other environmental chemicals); the prenatal exposure measures were, with some exceptions, the average of two measurements, and thus may better reflect chronic exposure during the pregnancy Weaknesses: Potential for residual confounding, potential for exposure misclassification, potential selection bias (not all children had the outcome

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
						measures), study population not generalizable to the whole US.
Eskenazi *et al.* (2010) CHAMACOS (N=371)	Neurodevelopment, Bayley Index (MDI, PDI), Maternal behavior checklist at 6, 12, and 24 months, PON1 gene and enzyme levels	The geometric mean for the DAP concentrations during pregnancy (for the average of the two sampling periods) was 146 nmol/L (95% CI: 133, 160); of this, a larger proportion was DMP metabolites (GM = 109 nmol/L; 95% CI =98, 120) than DEPs (GM= 23 nmol/L; 95% CI = 21, 25). Allele frequencies: PON1 192 Q allele= 50%; PON1 -108 T allele= 46%.Mean arylesterase activity: For infants: 33.6 U/mL (SD = 16) For mothers: 136.6 U/mL (SD = 44). Mean paraoxonase activity: For infants: 256.6 U/L (SD = 165); For mothers: 989.0 U/L (SD = 616).	Psychometrician, location of assessment, exact age at assessment, sex, breast-feeding duration (months), HOME score, and household income, parity, maternal PPVT, maternal age, education, depressive symptoms, active/passive smoking exposure during pregnancy, regular alcohol use during pregnancy, marital status, father's presence in home, housing density, maternal work status, ≥ 15 hours out-of-home childcare/week, birth weight, gestational age, abnormal reflexes, PCBs, lead, DDT, β-hexachlorocyclohexane, and hexa-chlorobenzene	Decrease MDI (24 months) $PON1_{108TT}$ –5.7 (–9.0 to –2.5) 9=0.01; Decrease PDI (24 months) $PON1_{108TT}$ –2.8 (–5.7 to 0.2) p=0.07; increased odds PDD 2.0 (0.8 to 5.1) p=0.14; no association $PON1_{192}$; no association PON1 activity measured newborn, 2 years, maternal and MDI, PDI, PDD. Evidence of decreasing MDI score by number of $PON1_{108}$ variant alleles: $PON1_{108CC}$ –3.2 (–9.8 to 3.5), CT –3.7 (–8.0 to 0.6), TT –5.5 (–11.1 to 0.1), p-interaction 0.98.	In this study population, evidence PON1 may influence MDI score, but not PDI or PDD risk at two-years. Non-significant evidence of decreasing MDI score by increasing DAP levels across strata of the number of $PON1_{108}$ variant alleles, interaction non-significant. Similar trend with prenatal DEP levels and MDI, PDI by PON108 alleles, but less pronounced. Overall, limited, non-definitive evidence of effect modification by PON1 status in the relation between mental and psychomotor effects and prenatal DAPs.	Strengths: Longitudinal design, measurement and consideration of many confounders (including other environmental chemicals); the prenatal exposure measures were, with some exceptions, the average of two measurements, and thus may better reflect chronic exposure during the pregnancy Weaknesses: Potential for residual confounding, potential for exposure misclassification, potential selection bias (not all children had the outcome measures), study population not generalizable to the whole US. Study may be under-powered to evaluate effect modification by *PON1* status.
Marks *et al.* (2010) CHAMACOS	CBCL; K-CPT; ADHD confidence index;	DAP (geometric mean) pregnancy	Psychometrician, exact age at assessment, sex, maternal	Prenatal DAPs were non-significantly associated	Marked effect modification by gender: 11-fold increase	Strengths: Longitudinal design, measurement and

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
(N=348)	Hillside behavioral rating scale; composite ADHD indicator	109.0 nmol/L; DEP 17.7 nmol/L	education, depressive symptoms, PPVT (continuous), ≥ 15 hr out-of-home child care/week, breast feeding duration (months), maternal age, parity, marital status, active/passive smoking exposure and regular alcohol use during pregnancy, presence of father in home, maternal work status, and household income	with maternal report of attention problems and ADHD at age 3.5 years, but were significantly related at age 5 years [CBCL attention problems: β = 0.7 points; 95% confidence interval (CI), 0.2-1.2; ADHD: β = 1.3; 95% CI, 0.4-2.1].	ADHD composite indicator in boys, less than 2-fold in girls, however unstable estimates; weak evidence of association DAPs at 3.5, 5 years and attention	consideration of many confounders (including other environmental chemicals); the prenatal exposure measures were, with some exceptions, the average of two measurements, and thus may better reflect chronic exposure during the pregnancy Weaknesses: Potential for residual confounding, potential for exposure misclassification, potential selection bias (not all children had the outcome measures), study population not generalizable to the whole US.
Rauh *et al.* (2011) Columbia U. (N=265)	• Wechsler Scales of Intelligence for Children (WISC-IV) • Child Behavior Checklist (CBCL).	• Chlorpyrifos levels in umbilical cord blood samples, N=256 newborns • If no cord blood (12% of subjects), levels were imputed from mothers' values. • Values for samples with non-detectable chlorpyrifos levels (N=115, 43%) were imputed by using assay-specific limit of detection (LOD) values to impute an approximate	Data were collected regarding lead exposure, demographics, education and occupational history, income, active and passive smoking, alcohol and drug use during pregnancy, and residential pesticide use. Final models included prenatal environmental tobacco smoke (ETS) exposure, gender, ethnicity, gestational age at birth, quality of home care-taking environment, maternal education, and maternal IQ.	Full-Scale IQ: (B) of -0.003, CI = 0.006, 0.001, p= 0.064 Working Memory Index: (B) of -0.006, CI = 0.009, 0.002, p<0.001. The investigators articulated these results as showing that a 1 pg/g increase in chlorpyrifos exposure was associated with a 0.006 point decrease in the log-transformed Working Memory score and a 0.003 point decrease in the log-transformed Full-Scale IQ score. The investigators concluded that for each standard deviation increase in exposure (4.61pg/g) there is a 1.4% reduction in Full-Scale IQ and a 2.8%	For each standard deviation increase in exposure (4.61pg/g) there is a 1.4% reduction in Full-Scale IQ and a 2.8% reduction in Working Memory.	Strengths • Direct assessment of chlorpyrifos levels using maternal serum and cord blood. • Analysis using a continuous CPF level, which, in contrast to dichotomous CPF levels, provides a more meaningful look at potential threshold effects and dose-response trends. • The investigators rigorously evaluated their methods for imputing values for undetectable CPF levels which in the end, were validated. • The authors describe an elegant and

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
		level.		reduction in Working Memory.		methodologically sound statistical analysis, addressing many of the potential shortcomings of their exposure data and covariates. Weaknesses: The use of a single snapshot of prenatal chlorpyrifos exposure may not be an accurate surrogate for full prenatal exposure levels. • There is no control for exposure over the subsequent 7 years which may be critical, especially as the process of neurocognitive development is fluid and rapid during these early childhood years. • Possibility of that an increased awareness of the risks of pesticide exposures could disproportionately affect postnatal exposure behavior.• Complicating this analysis is the pervasive, non-specific nature of neurological effects and the difficulty in attributing causal pathways. • when closely reviewed, the 95% CI for Full Scale IQ for both techniques contain 0 (LASSO: -0.006, 0.001, p=0.064; fully-adjusted: -0.006, 0.001, p=0.048• The authors do not address the clinical relevance of the 1.4% and 2.8% reductions

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
						and how this may impact a child or his/her psychological or educational plans.
Engel *et al.* (2011) Mt. Sinai (N=169)	Wechsler Preschool and Primary Scale of Intelligence, 3rd edition (WPPSI-III) at ages < 7 years; Wechsler-IV Intelligence Scale (verbal comprehension; perceptual reasoning, working memory, processing speed, full scale intelligence) at age 7-9 years	DEP: 24.7 nmol/L; Total DAP: 82 nmol/L (same as Engel 2007)	Maternal age, race/ethnicity, marital status, education, breast-feeding, child sex, alcohol, smoking, or drug use during pregnancy, maternal IQ, a score based on assessment of the home environment (HOME), season of urine collection, language spoken in the home, age at testing, examiner and urinary creatinine level.	At age 6-9 years, non-statistically significant reductions in full scale IQ, perceptual reasoning, verbal comprehension, working memory and processing speed with increasing DAP, more profound with DEP than DMP	Used non-specific biomarker DEP/DAP; some evidence of effect modification by PON1 genotype	Limitations include use of non-specific markers of chlorpyrifos pesticide exposure (DAPs), use of only a single (third-trimester) urine sample, and the large proportion of loss to follow-up. The statistical analysis was largely appropriate. Imputing of missing data may affect precision of association estimates and result in attenuated effect estimates as a result of exposure measurement error, although these are offset by the further categorization of the exposure levels (at the median). Assessment and control for confounding were appropriate. However, confounding by unmeasured (and mis-measured) risk factors for abnormal growth that are related to pesticide exposure would bias the results in this study. Such factors may be related to socioeconomic status of the study participants, a cofactor which is difficult to define, no less measure in an epidemiologic study.

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
Bouchard *et al.* (2011) CHAMACOS (N=329)	Wechsler-IV Intelligence Scale (verbal comprehension; perceptual reasoning, working memory, processing speed, full scale intelligence) measured at age 7 years	Total DAPs (quintiles): Q1 (39 nmol/L); Q2 75 nmol/L; Q3 126 nmol/L; Q4 221 nmol/L; Q5 508 nmol/L. Geometric mean DAP 131 nmol/L	Maternal intelligence, measures of how stimulating the environment is, and known or suspected neurotoxins were measured prenatally. Maternal intelligence was assessed via the Peabody Picture Vocabulary Test (PPVT). To measure the quality and extent of stimulation available to a child in the home environment, the Infant-Toddler HOME (Home Observation for Measurement of the Environment) inventory was completed at the 6-month, 1, 2, 3.5, 5, and 7 year visits; known or suspected neurotoxicants, polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), p,p'-dichlorodiphenyltrichlorethane (DDT), p,p'-dichlorodiphenyltrichlorethylene (DDE), and lead.	The authors observed evidence of an association between prenatal exposures to OP pesticides as measured by urinary DAP metabolites in women during pregnancy, and decreased cognitive functioning in children at age 7.	Prenatal measures taken later half of pregnancy more significantly associated intelligence than early; little evidence post-natal OP exposure associated with intelligence; 7 point reduction in full scale intelligence DAP Q5/Q1 (SS)	Strengths: the longitudinal design, the measurement of urinary DAP at multiple time points and following children to age seven when tests of cognitive function are reportedly more reliable. The authors were able to adjust for or consider many factors related to cognitive function, such as prenatal exposure to other environmental agents, socioeconomic indicators, maternal intelligence and education, and child stimulation. The cohort had a relatively homogenous socioeconomic profile, reducing the potential for uncontrolled confounding.
Whyatt *et al.* (2007) Columbia U. (N=102)	None	Geometric mean, 6.9 ± 17.0 ng/m^3; range < 0.4–171 ng/m^3. Personal air monitor: median 2.8 ng/m^3, mean 6.2 ± 11.1 ng/m^3, range < 0.4–83.4 ng/m^3	N/A	There was little within-home variability and no significant difference in air concentrations within homes over time ($p \geq 0.2$); between-home variability accounted for 88% of the variance in the indoor air levels of propoxur, 92% in chlorpyrifos, 94% in diazinon, and 62% in piperonyl butoxide ($p < 0.001$). Indoor and maternal personal air insecticide levels were highly correlated ($r = 0.7$–0.9, $p < 0.001$).	Indoor and maternal personal air insecticide levels were highly correlated ($r = 0.7$–0.9, $p < 0.001$).	Strengths: study design and exposure assessment techniques, Limitations: only those cohort participants enrolled after 2011 were included in the analysis (most likely due to the lack of serial data from the earlier years.)
Whyatt *et al.*	None	The limit of	N/A	Meconium TCPy	TCPy in maternal urine	Comprehensive exposure

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Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
(2009) Columbia U. (N=102)		detection (LOD) of chlorpyrifos in blood samples was 0.5–1 pg/g plasma. The LOD of TCPy in urine samples was 0.26 ng/mL urine. The LOD for TCPy in meconium was 0.2 ng based on a sample weighing 0.5 g. Exposure marker levels below the LOD were given a value of half the level of detection, and were then log10 transformed.		concentrations were positively correlated with chlorpyrifos in maternal and cord blood ($r = 0.25–0.33$, $p < 0.05$) and with TCPy in maternal urine ($r = 0.31$, $p < 0.01$).	samples was not reliable, but the maternal and cord blood chlorpyrifos as well as the TCPy levels in meconium were reliable measures of exposure	assessment including actual blood chlorpyrifos levels, the repeated sampling, and the environmental sampling. Weaknesses: only included participants recruited in the post-cancellation period, use of nonparametric, rank-based statistics is appropriate but the large number of observations below the level of detection receiving equal rank, may be problematic; no dietary assessment
Rauh et al.(2012), (n=40)	Morphological change in the pediatric brain in regions of the brain known to be associated with learning, cognition and social behavior	Tertile 3 (≥4.39 pg/g), compared to Tertiles 0, 1, 2 (<4.39 pg/g, including those not exposed to CPF)	Age, sex	Authors report differences in brain structure (regional cerebral size and thickness) by CPF exposure groups, and the differences (high>low CPF) in regional brain size is likely due to enlargement of underlying white matter. Statistical interaction by gender reported.	Authors concluded that the evidence from the study illustrated changes in brain morphology in association with higher CPF exposure, and that changes observed were in areas of the brain that subserve those learning, cognition and social behavioral, supported by previous observational and experimental literature.	Study supports general hypothesis of CPF influence on brain morphology, but lacks specific hypotheses regarding particular areas of the cerebrum affected; limited and somewhat unbalanced depiction of the available rodent experimental data; statistical methods appropriate, correction for multiple statistical comparisons a strength; MRI image readers blinded to exposure status enhances study validity; lack of information on other validation practices; small sample size, pilot study, low statistical power; external validity

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
						limited; one time measure of pre-natal exposure

2.2.5.2 "Moderate" Quality Group

Fifteen articles were assigned a moderate quality rating, as shown in Table 2.2.5.2-1. In general, these were cross-sectional or prospective cohort studies with small to high sample size; exposure assessment was based on a non-specific biomarker measure or current self-report, the outcome measurement(s) utilized standardized tests or screening tools, appropriate statistical analyses were performed, considering some but maybe not all relevant covariates, and risks of bias were minimized to some extent. For example, Guodong *et al*. (2012) cross-sectionally evaluated the relationship between DAPs concentrations in urine sampled from 301 young children as an objective, non-specific marker of prenatal OP pesticide exposure and Developmental Quotients based on the Gesell Developmental Schedules adapted for a Chinese population.

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Table 2.2.5.2-1. Moderate Quality Studies: Summary of Study Design Elements Impacting Study Quality Assignment

Study	Study Location	Cohort Name / Description of Study Population	Study Design	Exposure Assessment	Outcome Assessment	Confounder / Covariate Control	Statistical Analysis	Risk of (other) Bias
Andersen *et al* (2015)	Denmark	School age children living in Denmark	Prospective Birth Cohort, small sample size N=177 (112 maternal occupational pesticide exposure during pregnancy; 65 without) Age: 6-11 years	Prenatal occupational pesticide exposure ascertained by maternal interview at enrollment	Objective standardized clinical exam; neurophysiological status (heart rate variability); Previously validated, neuropsychological testing with demonstrated sensitivity to environmental pollutants; administered and scored by single neuropsychologist	Appropriate. Self-reported by mother. Included age, maternal demographics and risk factors, SES indicator (broad categories), maternal smoking and alcohol use. Possibility of recall errors (residual confounding). Self-reported (by mother)	Appropriate multivariate analysis. Evaluated numerous hypotheses without adjustment for multiple comparisons. Also constructed structural equation model of interaction between child sex and prenatal pesticide exposure on general intellectual ability (parameterized as a latent variable)	Selection bias possible due to loss to follow-up; Residual confounding likely, small in magnitude; potential for non-differential misclassification of exposure
Guodong *et al*. (2012)	Shanghai, China	2-year old children visiting community hospitals	Cross-sectional, large sample size N=301 Age 23-25 months	Objective biomarker, OP exposure (DAP) quantified in single child spot urine sample, simple imputation <LLOD; OP exposure also assessed via questionnaire administered to mothers after delivery	Developmental Quotients based on Gesell Developmental Schedules, adapted for Chinese population	Appropriate. Assessed via mother report via questionnaire, included child sex, maternal demographics and risk factors, SES indicators (maternal education, household income), maternal smoking and alcohol use; Possibility of recall errors	Appropriate multivariate analysis. Assumed linear relationship between log-transformed DAP level and DQ Scores	Selection bias unlikely; Residual confounding likely, small in magnitude; potential for non-differential misclassification of exposure
Handal *et al*. (2007)	Cayambe-Tabacundo region, Ecuador	EcoSalud Project: infants and young children 3 to 61 months of age in lower-altitude communities A&B dominated largely by cut-flower production and in more traditional rural, higher altitude community C.	Cross-sectional, modest sample size from census N=283 Age 3-61 months - 154 high exposure - 129 low exposure	Objective proxy measure: Community of residence	Ages and Stages Questionnaire (ASQ), adapted into local vernacular, 2 trained testers – considered a screening tool	Appropriate: child health status (anemia, stunting) and other characteristics of the home environment (stimulation by 2 methods)	Appropriate: Multiple linear regressions to evaluate associations between community of residence and delayed development, Pairwise t-tests and chi-square to assess mean difference in ASQ score; Effect size Cohen's d	Selection bias unlikely; Residual confounding likely, small in magnitude; potential for non-differential misclassification of exposure
Handal *et al*. (2008)	Cayambe-Tabacundo region, Ecuador	EcoSalud Project: Children 3 to 23 months of age in lower-altitude communities A&B dominated largely by cut-flower production and in more traditional rural, higher altitude community C.	Cross-sectional, modest sample size N:121 Age 3-23 months	Proxy: Distance home to farm, parental employment, pesticide use on domestic crops & within home, child play activities	Ages and Stages Questionnaire (ASQ) (ages 24-61 months), a screening tool; Visual Motor Integration (VMI) Test (ages 48-61 months); two trained testers.	Appropriate: As above	Appropriate: As above	Selection bias unlikely; Residual confounding likely, small in magnitude; potential for non-differential misclassification of exposure

Table 2.2.5-2. Moderate Quality Studies: Summary of Study Design Elements Impacting Study Quality Assignment (continued)

Study	Study Location	Cohort Name / Description of Study Population	Study Design	Exposure Assessment	Outcome Assessment	Confounder / Covariate Control	Statistical Analysis	Risk of (other) Bias
Handal *et al*.(2007B)	Cayambe-Tabacundo region, Ecuador	EcoSalud Project: Children 24 to 61 months of age in lower-altitude communities designated A & B were dominated largely by cut-flower production than in more traditional rural, higher altitude community C.	Cross-sectional, modest sample size N: ASQ - 142 Age 24-61 months VMI - 57 Age 48-61 months	Proxy: Maternal employment during pregnancy, child plays outdoors, Pesticide use on domestic crops , inside home	Ages and Stages Questionnaire (ASQ) (3-23 months) – screening tool; Reach-and-grasp, UC Berkeley Preferential Looking Test Cards; trained tester	Appropriate: As above	Appropriate: As above; Prehension- Logistic regression models, generalized estimation equations (GEE) to account for between-test dependence	Selection bias unlikely; Residual confounding likely, small in magnitude; potential for non-differential misclassification of exposure
Llop *et al*. (2013)	Spain	INMA (Environment and Childhood) Project	Prospective Birth Cohort, large sample size N=1980 Age 14 months	Maternal self-report of prenatal and postnatal indoor pesticide use (pesticide spray or use of a plug-in device assessed via questionnaire)	Mental and psychomotor development at 14 months assessed using validated instrument (Bayley Scales of Infant Development)	Appropriate. Self-reported by mother. Included maternal demographics and risk factors (Age, BMI) SES indicators (maternal education, occupation), maternal smoking and alcohol use. Also childcare behaviors (breast feeding, number of siblings, day care) Possibility of recall errors (residual confounding)	Appropriate multivariate analysis	Selection bias unlikely; Residual confounding likely, small in magnitude; potential for non-differential misclassification of exposure
Oulhote and Bouchard (2013)	Canadian National Population	Canadian Health Measures Survey (CHMS; cycle 1, 2007–2009);	Cross-sectional Study, large national sample size n = 779 children Age 6-11 years	Child urinary DAP, DMP, DEP collected within 2 weeks of survey questionnaire completion by the parents.	Behavioral problems in children based on the parent version of the Strengths and Difficulties Questionnaire (SDQ) (Goodman 1997) - SDQ is a validated screening questionnaire and accepted by parents.	Appropriate: Included sex, age, race/ethnicity, family income, parental education, blood lead levels, maternal smoking during pregnancy, birth weight, maternal age at child's birth, child's BMI, and fasting status (fasting duration at urine collection > 10 hour/≤ 10 hour)	Appropriate: Logistic Regression	Selection bias unlikely; Study population not mixed, single urine sample
Petit *et al*. (2010)	Brittany, France	Newborn children in the PELAGIE Study.	Prospective Birth Cohort, large sample size N= 3,159 Age: newborns	Ecological, proxy indicator of exposure (proportion of municipality devoted to agricultural activity	Objectively measured birth outcomes assessed using hospital records	Appropriate. Maternal report via questionnaire. Included maternal demographics (age, BMI) and pregnancy risk factors (gestational age, hypertension, diabetes, season of pregnancy) SES indicators (district of residence, maternal education, occupation), maternal smoking and alcohol use. Also childcare behaviors (breast feeding, number of siblings, day care); Possibility of recall errors	Appropriate multivariate analysis. Multiple comparisons were conducted for this evaluation, and the authors did not adjust for multiple testing	Selection bias unlikely; Residual confounding likely, small in magnitude; potential for substantial non-differential misclassification of exposure; Misclassification of outcomes unlikely

Table 2.2.5-2. Moderate Quality Studies: Summary of Study Design Elements Impacting Study Quality Assignment (continued)

Study	Study Location	Cohort Name / Description of Study Population	Study Design	Exposure Assessment	Outcome Assessment	Confounder / Covariate Control	Statistical Analysis	Risk of (other) Bias
Ruckart *et al*. (2004)	Mississippi, USA (29 counties), Ohio (one multi-family facility)	Children who were 6 years or younger in MS and OH when homes sprayed with MP	Retrospective cohort, moderate sample size in 2 states, participating/ nonparticipating similar in sex and age MS: N=365 (147 exposed, 218 unexposed) OH: N=287 (104 exposed, 183 unexposed) Age: 1.9-12.5 years old at testing	Specific OP measures: Household methyl parathion (wipe) by approved labs, OR highest urinary PNP level in household, analyzed by CDC	Variety of neuro measures: PENTB, Parenting Stress Index (PSI), Personality Inventory for Children (PIC), Vineland Adaptive Behavior Scales (VABS)	Appropriate: Income, race, site term, ethnicity, mother's use of chemicals at work, mother health/pregnancy conditions, report that child had lead or mercury poisoning (MS only); Raw scores were child age-adjusted	Appropriate: Linear regression (continuous scores), logistic regression (dichotomous scores)	Selection bias possible due to loss of follow-up; Residual confounding likely; substantial potential for differential exposure misclassification (time between spraying and testing, frequency and duration unknown)
Shelton *et al*. (2014)	California, USA	CHARGE Study (3 year-old children)	Case-control, large sample size N = 970 Age 3 years N=486 (ASD) N=168 (DD) N=316 (Controls)	Proxy indicator of prenatal OP pesticide exposure (residential proximity to agricultural pesticide applications defined using ecological pesticide use data)	Clinical outcomes – validated within the study	Appropriate adjustment for demographics (place of birth, race), SES indicators (paternal education) and vitamin intake during pregnancy	Appropriate multivariate analysis.	Selection bias probable; Residual confounding likely; substantial potential for differential misclassification of exposure. Outcome misclassification unlikely.
Suarez-Lopez *et al*. (2012)	Pedro Moncayo County, Pichincha, Ecuador	Secondary Exposure to Pesticides Among Infants, Children and Adolescents (ESPINA) Study, plus new volunteers	Cross-sectional, moderate sample size, from prior census N=277 Exposed: (n=158) Unexposed: (n=119) Age 4-9 years	Proxy: Cohabitation with flower worker >1yr, Adult questionnaire	Child AChE level using commercial kit	Appropriate: Sex, age, height-for-age, hemoglobin concentration, income, pesticide use within household lot, pesticide use by neighbors, examination date, residence, distance to nearest flower plantation	Appropriate: Multiple linear regression (continuous) and logistic regression (polychotomous variables), adjusted models	Selection bias possible due to loss but deemed low; Residual confounding likely small in magnitude; potential for differential exposure misclassification
Suarez-Lopez *et al*. (2013a)	Pedro Moncayo County, Pichincha, Ecuador	Secondary Exposure to Pesticides Among Infants, Children and Adolescents (ESPINA) Study, plus new volunteers	Cross-sectional, moderate sample size, from prior census N=307 Age 4-9 years	Non-specific measures: Child AChE level using commercial kit; Proxy: Cohabitation with flower worker >1yr, Adult questionnaire	Heart rate (HR), Systolic blood pressure (SBP), Diastolic blood pressure (DBP) by standard methods	Appropriate: Sex, age, height-for-age, hemoglobin concentration, income, pesticide use within household lot, pesticide use by neighbors, examination date, residence, distance to nearest flower plantation	Appropriate: Multiple linear regression (continuous), adjusted.	Selection bias possible due to loss but deemed low; Residual confounding likely small in magnitude; potential for differential exposure misclassification
Suarez-Lopez *et al*. (2013)	Pedro Moncayo County, Pichincha, Ecuador	Secondary Exposure to Pesticides Among Infants, Children and Adolescents (ESPINA) Study, plus new volunteers	Cross-sectional, moderate sample size, from prior census N=271 Age 4-9 years	Non-specific measures: Child AChE level using commercial kit	NEPSY-II test (trained examiners): general assessment battery	Model defined a priori: hemoglobin, age, sex, race, height-for-age z score, household income, maternal education, and flower worker cohabitation status	Logistic models (dichotomous and polychotomous) and linear regression models, adjusted; Effect modification according to sex among significant associations; Some imputed values	Selection bias possible due to loss but deemed low; Residual confounding likely small in magnitude; potential for differential exposure misclassification
Wang *et al*. (2012)	Shanghai, China	Pregnant women and newborn children	Cross-sectional Birth Cohort Study N=187 Age: newborns	Objective biomarker of prenatal OP pesticide exposure (DAP) in single maternal spot urine sample provided at onset of labor. Simple imputation of observations < LLOD. OP pesticide exposure also assessed via questionnaire administered after delivery	Gestational age and pre-term delivery appropriately defined and assessed using medical records	Appropriate. Included maternal anthropomorphic, demographic, and SES indicators (income, occupation), predictors of high-risk pregnancy (pregnancy weight gain, gestational age)	Appropriate multivariate analysis	Selection bias unlikely; Residual confounding likely small in magnitude; potential for differential misclassification of exposure
Zhang *et al*. (2014)	Shenyang, China	Newborn children in a birth cohort study	Prospective Cohort, n=249 mother-infant pairs Age: newborns	Biomarker of prenatal OP pesticide exposure (DAP) quantified in single maternal spot urine sample provided at delivery. Many observations < LLOD	Neonatal neurodevelopment assessed using validated instrument (Neonatal Behavioral Neurological Assessment) by trained examiners	Appropriate. Included maternal demographic and SES indicators (age, education), predictors of high-risk pregnancy (BMI, gestational age) and environmental toxicant exposure (cord blood lead); Results stratified by sex	Appropriate multivariate analysis	Selection bias unlikely; Residual confounding likely small in magnitude; considerable potential for non-differential misclassification of exposure (unlikely to account for non-null findings)

2.2.5.3 "Low" Quality Group

Seventeen articles were assigned a low quality rating, as shown in Appendix 3. In general, these were small or pilot studies; exposure assessment was based on a proxy measure(s), outcome measurement(s) utilized screening tools or self-report, limited statistical analyses were performed, relevant covariates were not included or discussed, or risks of bias were possible. For example, Acosta-Maldonado *et al.* (2009) conducted a cross-sectional pilot study among 54 women, only nine of whom were considered to have had prenatal exposure to pesticides (defined based on either an exposure history profile or AChE level in blood sampled at the time of admission into the hospital for delivery. The outcome assessed in this study was a standardized but partially subjective assessment of placental maturity (the Placental Maturity Index, PMI). Covariates adjustment of estimated associations between prenatal exposure and PMI in this study was minimal and limited to placental characteristics. Cartier et al (2016) is from the PELAGIE prospective cohort study and used DAP biomarkers as the exposure biomarkers. However, despite these strengths, the urine samples were collected within the home instead of a laboratory or clinic and were returned via the local mail, which could have potentially led to several sampling errors due to inadequate collection and storage methods.

The remaining sections of this document do not discuss further studies identified in the 'low' category. Due to limitations in these studies, they do not provide reliable information evaluating associations between OP exposure and neurodevelopmental outcomes.

2.2.6 Assessment of Epidemiological Studies for Relevance to Analysis

Using the criteria summarized in Section 2.2.4, a total of 47 literature articles were identified in the 2015 literature review and were judged as high, moderate, or low quality. Overall, 15 articles, 15 articles, and 17 articles were judged to be of high, moderate, or low quality, respectively. For the 30 high and moderate quality studies, additional evaluation was conducted as described in this section.

While all of the moderate quality studies had strengths including sample design and outcome assessment, six of these moderate quality studies did not have sufficient exposure assessment methods to determine whether exposure to OPs actually occurred. These studies were conducted on study populations in Spain (Llop *et al.*, 2013), Ecuador (Handal *et al.*, 2007; 2007b; 2008), Denmark (Andersen *et al.*, 2015), and France (Petit *et al.*, 2010). In these studies, participants were considered exposed or unexposed to pesticides based on non-specific exposure measures, such as self-reported occupational exposure, home pesticide spraying, and proportion of municipality devoted to agricultural activity. For all of these proxy exposure assessments, the pesticides used may have included not only OPs, but also pyrethroids, fungicides, and growth regulators. Given the uncertainty about whether OP exposure actually occurred in these studies and whether observed outcomes are associated with OP exposure or with other pesticides, these studies are excluded from further analysis.

The focus of this epidemiological literature review is on the neurodevelopmental outcomes from exposures to low levels of OPs (i.e. below exposures which would result in 10% or more AChE inhibition). Three studies conducted in Ecuador focused on child AChE inhibition and the

potential association of AChE inhibition with other measures including parental occupation (Suarez-Lopez *et al.*, 2012), as well as clinical autonomic nervous system (ANS) outcomes such as blood pressure and heart rate (Suarez-Lopez *et al.*, 2013a), and neurodevelopmental outcomes (Suarez-Lopez *et al.*, 2013). The range of AChE activity levels are lower in the first tertile (range of 1.44 to 2.93 U/mL) compared to the third tertile (range from 3.33 to 4.69 U/mL). Therefore, due to the outcomes assessed and the potentially toxic cholinergic effects that were associated with these outcomes, these studies are not considered to be relevant to this review and are not discussed further here.

In a retrospective cohort study of children exposed to methyl parathion (MP) before age 6 years in Mississippi and Ohio (Ruckart *et al.* 2004), as assessed by household urinary PNP or wipe samples, exposed children performed worse than unexposed children on a few of numerous neurobehavioral development tests conducted. Specifically, participants classified as having had MP exposure had more difficulty with short term memory and attention tasks, and parents reported more behavioral and motor skill problems, relative to unexposed children. However, upon closer inspection of the results across the MS and OH study sites, these neurobehavioral outcomes are not seen consistently. These inconsistencies may be due to differences in how the exposure occurred across the sites, including the fact that MS participants and OH participants were tested 2.5 and 4.5 years after MP spraying in the home. When comparing exposed and unexposed children using general intelligence testing, integrated visual and motor skills testing, and multistep processing, they did not see any differences. The exposure scenario associated with these observations is a critical element in assessing the utility and reliability of this study. Samples were collected in locations from OH and MS where illegal spraying of methyl parathion is known to have occurred during the 1994-1996 time period. Based on the "Revised Organophosphorous Pesticide Cumulative Risk Assessment" (USEPA, 2006), methyl parathion is known to be among the more potent OPs. It is unknown whether study participants were exposed to MP levels that would have induced cholinergic effects. Therefore, given the uncertainty around this illegal use combined with the high potency for cholinergic toxicity, the agency is not emphasizing this study further in the analysis.

In addition, two high quality studies from the CHAMACOS birth cohort (Quirós-Alcalá *et al.* 2011; Ranaan *et al.*, 2015) assessed the association between DAPs and autonomic nervous system (ANS) outcomes and respiratory symptoms, respectively. The ANS outcomes assessed included heart rate and respiratory sinus arrhythmia. Overall, while there was some evidence of ANS dysregulation for infants at 6 months, these results were not consistently observed for the other assessed child (1 year, 3.5 years, and 5 years) and maternal OP exposures. Ranaan et al (2015) reported that total DAPs and DEAP metabolites in urine from the second half of pregnancy were significantly associated with increased odds of respiratory symptoms in children. Ranaan *et al.* l (2015) also reported exercise-induced coughing at 5 and 7 years of age was significantly associated with total DAPs, DEAPs, and DMAPs in children's urine collected between the ages of 6 months and 5 years (AUC). The authors concluded that early-life exposure to OP pesticides was associated with respiratory symptoms consistent with possible asthma in childhood. There is not a body of literature to compare these results against, making it difficult to put them into context. Furthermore, these studies did not focus on

neurodevelopmental outcomes, which is the focus of this analysis. Consequently, Quirós-Alcalá *et al.* (2011) and Raanan *et al.* (2015) are not being evaluated further.

2.2.7 Birth Outcome Epidemiologic Studies

Four identified studies, three high quality and one moderate quality, focused solely on OP exposure and adverse birth outcomes related to fetal growth (Barr *et al.*, 2010; Rauch *et al.*, 2012; Wang *et al.*, 2012; Wolff *et al.*, 2007). Harley et al (2016) is a meta-analysis of data from the HOME, Mt. Sinai, CCCEH, and CHAMACOS cohorts which allowed for a better assessment of race and genetic variability given the larger sample size in the pooled analysis.

The birth outcomes assessed included birth weight, birth length, head circumference at birth, and gestational age. The exposure assessment for these studies was conducted using objective measures or biomarkers such as maternal urinary DAPs (Rauch *et al.*, 2012; Wang *et al.*, 2012; Wolff *et al.*, 2007), maternal urinary malathion (MDA) (Wolff *et al.*, 2007), or maternal and cord blood levels for specific OPs (Barr *et al.*, 2010). The results from these studies are generally inconsistent, with some studies documenting statistically significant associations between OP exposure and birth outcomes whereas others do not. For example, maternal and cord blood serum levels of TCPy were inversely associated in univariate analyses conducted among participants in a prospective cohort study conducted in New Jersey (Barr *et al.* 2010), though the associations did not persist after adjusting for gravidity, maternal pre-pregnancy BMI, infant sex, and gestational age. Chlorpyrifos levels were near the lower limit of detection in this study, though detectable in 98.6% of maternal serum and 62.8% of cord serum samples. Overall, birth length was not associated with third-trimester DAP in the Mount Sinai Cohort (Wolff *et al.* 2007). However, among those with slow-activity paraoxonase-1 (PON1) or PON192, urinary total DMP (but not total DAP or DEP) was statistically significantly associated with shorter birth length (p= 0.032). Birth length was also not associated with maternal urinary DAP sampled at delivery in the cross-sectional investigation conducted in Shanghai, China (Wang *et al.* 2012). Similarly, inconsistent results were observed across these studies for birth weight, gestational age, and head circumference at birth.

For the pooled analysis in Harley et al (2016), no evidence of association was observed between OPs exposure via the ∑DAP, ∑DEP, and ∑DMP urinary metabolites and birth outcomes for birth weight, birth length, and head circumference, as all of the 95% CIs encompassed the null value of 0 and all of the p-values were > 0.05 (p-values ranged from 0.46 to 0.98), respectively. For the interaction analysis, no evidence of a statistically significant interaction between the four cohorts and exposure to urinary metabolites (∑DAP, ∑DEP, and ∑DMP) relative to the birth outcomes (birth weight, birth length, and head circumference) were observed since all of the p for interaction values were above > 0.10, respectively. A statistically significant interaction was observed between the PON1-108 genotype (specifically the PON1-108CC gene) and the urinary metabolite ∑DEP relative to pooled birth weight (p = 0.08), and between the PON1-108 genotype (specifically the PON1-108CC genes) and the urinary metabolites ∑DAP and ∑DMP relative to pooled birth length (p for interaction = 0.07; p = 0.02) among children. With the PON1192 genotype, a statistically significant interaction was observed between the urinary

metabolite ∑DAP relative to pooled head circumference (p for interaction = 0.05). For mothers, a statistically significant interaction was observed between the PON1-108 genotype and the urinary metabolites ∑DAP and ∑DMP relative to pooled head circumference (p for interaction = 0.09 for both), respectively. No additional statistically significant interactions were observed for any other genotypes among children and mothers, and no statistically significant interactions were observed for race, sex or maternal education.

Overall, in this 2015/2016 literature review, inconsistent evidence of OP exposure and association with adverse birth outcomes/fetal growth was observed (Barr *et al.*, 2010; Rauch *et al.*, 2012; Wang *et al.*, 2012; Wolff *et al.*, 2007). This lack of consistency in the literature was also observed for birth outcomes in the recent chlorpyrifos HHRA (USEPA, 2014) which notes that researchers from CCCEH, Mt. Sinai, and CHAMACOS also investigated the possible role of prenatal OP exposure and fetal growth. These results were not consistent across these cohorts. Authors with CCCEH observed evidence of an inverse association, *i.e.*, increasing cord blood chlorpyrifos was associated with decreased measures of birth weight and length, while authors with the Mt. Sinai and CHAMACOS cohorts reported either no association, or evidence of a *positive* relationship, respectively [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Inconsistent results may be due to differences across study groups in exposure profiles as well as dissimilar methods of prenatal OP exposure assessment [ADDIN EN.CITE <EndNote><Cite><Author>Needham</Author><Year>2005</Year><IDText>Assessing exposure to organophosphorus pesticides by biomonitoring in epidemiologic studies of birth outcomes</IDText><DisplayText>(Needham, 2005)</DisplayText><record><dates><pub-dates><date>Apr</date></pub-dates><year>2005</year></dates><keywords><keyword>California/epidemiology</keyword><keyword>Chlorpyrifos/blood/metabolism/urine</keyword><keyword>Cohort Studies</keyword><keyword>Environmental Monitoring/methods</keyword><keyword>Female</keyword><keyword>Humans</keyword><keyword>Insecticides/blood/metabolism/urine</keyword><keyword>Maternal Exposure</keyword><keyword>New York City/epidemiology</keyword><keyword>Pregnancy</keyword><keyword>Pregnancy Outcome</keyword></keywords><urls><related-urls><url>http://www.ncbi.nlm.nih.gov/pubmed/15811842</url></related-urls></urls><isbn>0091-6765 (Print) 0091-6765 (Linking)</isbn><work-type>Comparative Study</work-type><titles><title>Assessing exposure to organophosphorus pesticides by biomonitoring in epidemiologic studies of birth outcomes</title><secondary-title>Environ Health Perspect</secondary-title><short-title>Assessing exposure to organophosphorus pesticides by biomonitoring in epidemiologic studies of birth outcomes</short-title><alt-title>Environmental health perspectives</alt-title></titles><pages>494-8</pages><number>4</number><contributors><authors><author>Needham, L. L.</author></authors></contributors><edition>2005/04/07</edition><language>eng</language><added-date format="utc">1334673025</added-date><ref-type name="Journal Article">17</ref-type><custom3>A</custom3><auth-address>Centers for Disease Control and Prevention, Atlanta, Georgia 30341-3724, USA. lneedham@cdc.gov</auth-address><remote-database-provider>Nlm</remote-database-provider><rec-number>3349</rec-number><last-updated-date format="utc">1334673025</last-updated-date><accession-

num>15811842</accession-num><volume>113</volume></record></Cite></EndNote>].

Given the lack of consistency among cohorts for the fetal growth metrics, the proposed link between fetal growth and OP exposure is tenuous. Therefore, consistent with previous evaluations for chlorpyrifos, EPA is focusing the remainder of this document on neurodevelopmental outcomes. Although the agency is not evaluating these birth outcome studies further at this time, the agency will continue to monitor the scientific literature for advances in this line of research.

2.2.8 Neurodevelopment Outcome Epidemiologic Studies

From an initial total of 30 high or moderate quality studies, with the exclusion of six studies for insufficient exposure assessment, three studies with measurable AChE inhibition and potential cholinergic toxicity which are outside the scope of this analysis, one study from an illegal use of a highly potent OP (MP) where cholinergic toxicity cannot be ruled out, five studies with birth outcome as the only assessment, two with outcomes not related to neurodevelopmental outcomes, and one study assessing only PON1 genotype expression and neurobehavioral outcomes (Fortenberry et al, 2014a), a total of twelve studies from the 2015/2016 literature review focusing on neurodevelopmental outcomes remain to be evaluated. Eight are considered high quality (Bouchard *et al.*, 2010; Fortenberry *et al.*, 2014; Furlong *et al.*, 2014; Donauer et al. (2016), Stein et al. (2016), Rauh et al, (2015), Engel et al (2015), and Yolton et al. (2013). Four are considered moderate quality studies (Guodong *et al.*, 2012; Oulhote and Bouchard, 2013; Zhang *et al.*, 2014; Shelton *et al.*, 2014).

Furlong et al (2014) is from the Mt. Sinai cohort study; Stein et al (2016) is from CHAMACOS, and Rauh et al (2015) is from CCCEH--- thus these studies share many of the same strengths and uncertainties as other studies from these cohorts.

Most of these studies used biomarker measures for their exposure assessment, including child or maternal urinary DAPs (Bouchard *et al.*, 2010; Furlong *et al.*, 2014; Guodong *et al.*, 2012; Oulhote and Bouchard, 2013; Zhang *et al.*, 2014; Yolton et al, 2013; Donauer et al, 2016; Stein et al, 2016, Engel et al, 2015), child urinary DMP and DEPs (Oulhote and Bouchard, 2013; Zhang *et al.*, 2014; Guodong *et al.*, 2012), or maternal TCPy (Fortenberry *et al.*, 2014). Shelton *et al.* (2014) used pesticide use data from the California Department of Pesticide Regulation and geospatial methods to map the specific pesticide use pattern to the participant residence. Engel et al. (2015) reports on the results of a pooled analysis from HOME, CCCEH, CHAMACOS, and Mt. Sinai to evaluate the association between prenatal urinary DAPs and neurodevelopmental outcomes at 24 months only.

The study populations include national in scope (Bouchard *et al.*, 2010; Oulhote and Bouchard, 2013); mainly urban (Fortenberry *et al.*, 2014; Fortenberry *et al.*, 2014a; Furlong *et al.*, 2014; Guodong *et al.*, 2012; Zhang *et al.*, 2014; Rauh et al, 2015, Shelton *et al.*, 2014); suburban (Yolton et al, 2013; Donauer et al, 2016); and agricultural (Stein et al, 2016).

2.2.8.1 CCCEH, CHAMACOS, & Mt. Sinai Cohorts

The CCCEH, Mt. Sinai, and CHAMACOS studies reflect different types of exposed groups in the total population which strengthens the weight of the evidence considerations regarding this stream of information. The CCCEH Mother's and Newborn study and the Mt. Sinai Child Growth and Development study participants were likely exposed to OPs through the diet and through residential use of the pesticide for indoor pest control. In the residential setting, study populations were most likely exposed through indoor residential use of the pesticide during the study time period and additionally exposed to OPs via the oral route through ingesting residues in the diet and from hand-to-mouth contact with in-home surfaces, as well as possible dermal or inhalation exposure through contact with treated areas in the home environment [ADDIN EN.CITE ADDIN EN.CITE.DATA]. In contrast, CHAMACOS cohort participants were employed as farm laborers or were residing in homes with farm laborers. The CHAMACOS study participants likely experienced exposure to OPs through the diet and from occupational exposure (primarily inhalation and dermal routes), as well as probable indirect take-home exposures (the "tracking in" of pesticide residues through shoes and clothing, augmented by poor hygiene practices) [ADDIN EN.CITE ADDIN EN.CITE.DATA]. In each of these three US children's health cohorts, the biological measurements in these cohorts were comparable to the general population NHANES.

These cohort studies each enrolled pregnant women during roughly the same time period, measured both environmental exposure to the pesticide during pregnancy and also measured biomarkers representing internal dose during pregnancy and at delivery, and prospectively assessed associations in their newborns and young children through age 7 years. Each study includes several hundred (approximately 100-400) mother-infant pairs; these sample sizes are sufficient to perform statistically valid analyses. Investigators from each study cohort utilized a similarly strong study design (prospective birth cohort); measured pesticide exposure using several different methods including environmental indicators as well as specific and non-specific biomarkers of OPs; ascertained developmental outcomes using validated assessment tools well-established in both clinical and research settings; and, measured, analyzed, selected and statistically adjusted for potentially confounding variables including socio-economic status and other environmental exposures using reasonable and appropriate methods. Limitations exist as well. These studies utilized a one-time measure (or the average of two measures) of chlorpyrifos or OP exposure to assess prenatal pesticide exposure throughout the gestational period, were unable to assess the influence of mixtures (co-occurring exposures in the relevant biological time window), and reflect a small sample size to fully evaluate the effect of more than one simultaneous exposure on neurodevelopment, *i.e.*, evidence of effect modification.

As noted, two major uncertainties in environmental epidemiology studies are the accurate and reliable measurement of exposure and potential confounding variables such as the influence of mixtures. The researchers with each of the three cohorts have provided supplemental methodological research to address these areas to the extent possible. Across the three children's health cohorts, study authors measured biomarkers of OP exposure. There is

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uncertainty as to the extent measurement of non-specific metabolites of OP or chlorpyrifos accurately reflects OP exposure; CCCEH and Mt. Sinai studies do not estimate post-natal exposure to chlorpyrifos among child participants, therefore the influence of early life and childhood OP exposure is unaccounted for in these analyses. The CHAMACOS cohort measured urinary levels of DAPs in young children and with the exception of pervasive developmental disorder (PDD) [per 10-fold increase in prenatal and post-natal DAP [prenatal: odds ratio (OR) = 2.3, $p = 0.05$; child DAPs OR = 1.7, $p = 0.04$]. did not observe negative significant associations in relation to neurodevelopment from post-natal exposure [ADDIN EN.CITE ADDIN EN.CITE.DATA]. The CHAMACOS cohort investigators also measured AChE and butyl ChE as supplemental indicators of OP exposure.

Potential confounding bias is another major uncertainty within environmental epidemiology studies. Confounding variables, exposures that could be related to OP exposure and neurodevelopmental outcomes such as blood lead, may result in an incorrect epidemiological risk estimate. Across these cohort studies, investigators collected relevant information concerning demographic characteristics and other environmental exposures, and were, to the extent possible with the existing information, able to effectively hold constant the influence of these other variables when estimating the association between prenatal chlorpyrifos and adverse neurodevelopmental outcomes. Control of these variables is important to reduce the chances of a false positive study result. Overall, statistical analyses were judged to be appropriate and reasonable (not overly large number of statistical model variables) to the research question by EPA and expert Panel reviews (FIFRA SAP 2008 and 2012).

Researchers with both the Mt. Sinai and CHAMACOS cohorts evaluated neonatal neurological functioning in association with prenatal OP exposure; CCCEH did not conduct these measurements. To measure indices of abnormal neonatal behavior and/or neurological integrity authors used outcome measures derived from the Brazelton Neonatal Behavioral Assessment Scale (BNBAS), a neurological assessment of 28 behavioral items and 18 primitive reflexes. This tool was administered to infants 2-5 days post-partum by trained neonatologists in the hospital setting using similar environmental conditions. The authors with both study groups observed an increased number of abnormal reflexes in relation to increasing measures of OP exposure [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Among the other 27 measures in the BNBAS, neither study group reported evidence of any other positive associations. The authors also observed evidence of potential effect modification by PON1 activity level in the relation between DAPs and neonatal neurodevelopment in which infants of mothers who are slower metabolizers have greater risk of abnormal reflexes (Young *et al.* 2005; Engel *et al.* 2007). However, EPA notes these studies are likely under-powered to make a statistically robust estimate of this statistical interaction.

Researchers across the three children's health cohorts utilized the Bayley Scales of Infant Development II (BSID-II) to generate a Mental Development Index (MDI) and a Psychomotor Development Index (PDI) to assess neurodevelopment in early childhood. In the CCCEH Mothers and Newborn study, Rauh *et al.* (2006) investigated MDI and PDI at 12, 24, and 36 months of age. Children were categorized as having either high (>6.17pg/g) or low ($\leq$6.17pg/g)

prenatal chlorpyrifos exposure, using categories informed by results of the previous study on birth characteristics [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Authors reported that the difference in MDI scores was "marginally significant" (p = 0.06) between the "high" and "low" exposed groups; the high exposed group scoring an average of 3.3 points lower than the low exposed [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Regarding the PDI score (motor skills), none of the 12 or 24 month PDI scores showed significant effects, but the 36 month score was significantly related to chlorpyrifos exposure. Researchers noted that the effects were most pronounced at the 36 month testing period.

CCCEH study authors (Rauh et al., 2015) evaluated the relationship between prenatal chlorpyrifos exposure and motor development/movement among 271 of the cohort participants who had reached the age of approximately 11 years. When comparing children in the upper tertile of exposure (>6.17 pg/g; N=43) to those in the lower tertiles (N=228), they observed statistically significant associations between prenatal chlorpyrifos exposure and mild to moderate tremor in the dominant arm, both arms, either arm, and a marginally statistically significant association in the non-dominant arm. The specific OR calculated associated with these elevated risks of arm tremor are as follows: dominant arm (OR=3.2; 95% CI=l .3-8. l; p=O.O 15); both arms (OR=3.3 ; 95% CI=l .1-9.4; p=0.027); either arm (OR=2.2; 95% CI=l .1-4.6; p=0.028); and non-dominant arm (OR=2. l; 95% CI=0.99-4.3; p=0.055). These associations were observed even after controlling for potential confounding factors such as medication, sex, and ethnicity.

Within the 36 month testing period, the likelihood of highly exposed children developing mental delays were significantly greater (MDI: 2.4 times greater (95% CI: 1.12-5.08, p = 0.02) and PDI: 4.9 times greater (95% CI: 1.78-13.72; p = 0.002)) than those with lower prenatal exposure [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Within the Mt. Sinai study, authors administered the BSID-II to participating children at 12 and 24 months and observed that prenatal total DAP metabolite level was associated with a decrement in mental development at 12 months among blacks and Hispanic children; however, these associations either attenuated or were non-existent at the 24-month visit [ADDIN EN.CITE
<EndNote><Cite><Author>Engel</Author><Year>2011</Year><IDText>Prenatal exposure to organophosphates, paraoxonase 1, and cognitive development in childhood</IDText><DisplayText>(Engel et al., 2011)</DisplayText><record><dates><pub-dates><date>Aug</date></pub-dates><year>2011</year></dates><keywords><keyword>Aryldialkylphosphatase/ genetics</keyword><keyword>Child</keyword><keyword>Child, Preschool</keyword><keyword>Cognition/ drug effects</keyword><keyword>Female</keyword><keyword>Genotype</keyword><keyword>Humans</keyword><keyword>Infant</keyword><keyword>Perception/drug effects</keyword><keyword>Phosphoric Acid Esters/ toxicity</keyword><keyword>Pregnancy</keyword><keyword>Prenatal Exposure Delayed Effects</keyword></keywords><isbn>1552-9924 (Electronic)
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title>Environ Health Perspect</secondary-title><alt-title>Environmental health perspectives</alt-title></titles><pages>1182-8</pages><number>8</number><contributors><authors><author>Engel, S. M.</author><author>Wetmur, J.</author><author>Chen, J.</author><author>Zhu, C.</author><author>Barr, D. B.</author><author>Canfield, R. L.</author><author>Wolff, M. S.</author></authors></contributors><edition>2011/04/22</edition><language>eng</language><added-date format="utc">1329668765</added-date><ref-type name="Journal Article">17</ref-type><auth-address>University of California-Berkeley, Center for Environmental Research and Children's Health, School of Public Health, Berkeley, California, USA.</auth-address><remote-database-provider>NLM</remote-database-provider><rec-number>1180</rec-number><last-updated-date format="utc">1396923587</last-updated-date><accession-num>21507778</accession-num><electronic-resource-num>10.1289/ehp.1003183</electronic-resource-num><volume>119</volume></record></Cite></EndNote>]. In the CHAMACOS cohort, Eskenazi *et al.* [ADDIN EN.CITE ADDIN EN.CITE.DATA] observed that prenatal DAP levels were adversely associated with MDI, and at 24 months of age these associations reached statistical significance. In this study, neither prenatal DAPs nor maternal TCPy were associated with PDI (motor skills), nor did authors observe evidence of different risk by PON1 status [ADDIN EN.CITE ADDIN EN.CITE.DATA].

With respect to the findings related to the autism spectrum, from CCCEH, Rauh *et al.* (2006) reported a large odds ratio for PDD (OR=5.39; 95% CI: 1.21-24.11) when comparing high to low chlorpyrifos exposure groups. Among 7-9 years old children in the Mount Sinai Cohort (Furlong *et al.* 2014), there was no overall statistically significant association between maternal third trimester urinary DAP metabolite levels and reciprocal social responsiveness. However, some evidence of modification of the association between prenatal OP pesticide exposure and impaired social responsiveness in early childhood was observed by both race/ethnicity and child sex, with an association between DEAP and poorer social responsiveness observed among black participants and boys. No association was observed among whites or Hispanics, among girls, or for DAP or DMAP biomarker levels. In the CHAMACOS cohort, Eskenazi *et al.* (2010) reported non-significant, but suggestive, increased odds of PDD of 2.0 (0.8 to 5.1; p=0.14), whereas Eskenazi et al (2007) reported a statistically significant association between total DAP exposure and increased odds of PDD.

With respect to attention problems, Rauh *et al.* [ADDIN EN.CITE ADDIN EN.CITE.DATA] also investigated 36-month child behavior checklist (CBCL) (behavioral) scores. Significant differences were observed between the high and low chlorpyrifos exposure groups in the general category of attention-problems (p=0.010), and in the more specific DSM-IV scale for ADHD problems (p = 0.018). The CHAMACOS cohort also investigated attention problems in early childhood using three different assessment tools: maternal report of child behavior at 3.5 and 5 years of age; direct assessment of the child at 3.5 and 5 years; and by a psychometrician's report of the behavior of the child during testing at 5 years. In this study population, higher concentrations of OP metabolites in the urine of pregnant women were associated with

increased odds of attention problems and poorer attention scores in their children at age 5 years [ADDIN EN.CITE ADDIN EN.CITE.DATA].

To measure intelligence among school aged children, authors from each of the three children's health cohorts used the Wechsler Intelligence Scale for Children, 4th edition (WISC-IV). The instrument measures four areas of mental functioning: the Verbal Comprehension Index, the Perceptual Reasoning Index, the Working Memory Index, and the Processing Speed Index. A Full-Scale IQ score combines the four composite indices. WISC-IV scores are standardized against U.S. population-based norms for English and Spanish-speaking children. In the CCCEH Mothers and Newborn Study, Rauh *et al.* [ADDIN EN.CITE <EndNote><Cite ExcludeAuth="1"><Author>Rauh</Author><Year>2011</Year><IDText>Seven-year neurodevelopmental scores and prenatal exposure to chlorpyrifos, a common agricultural pesticide</IDText><DisplayText>(2011)</DisplayText><record><dates><pub-dates><date>Aug</date></pub-dates><year>2011</year></dates><keywords><keyword>Adolescent</keyword><keyword>Adult</keyword><keyword>Child</keyword><keyword>Chlorpyrifos/toxicity</keyword><keyword>Female</keyword><keyword>Humans</keyword><keyword>Intelligence/drug effects</keyword><keyword>Male</keyword><keyword>Memory/drug effects</keyword><keyword>Pesticides/toxicity</keyword><keyword>Pregnancy</keyword><keyword>Prenatal Exposure Delayed Effects</keyword><keyword>Young Adult</keyword></keywords><isbn>1552-9924 (Electronic)
0091-6765 (Linking)</isbn><custom2>3237355</custom2><titles><title>Seven-year neurodevelopmental scores and prenatal exposure to chlorpyrifos, a common agricultural pesticide</title><secondary-title>Environ Health Perspect</secondary-title><alt-title>Environmental health perspectives</alt-title></titles><pages>1196-201</pages><number>8</number><contributors><authors><author>Rauh, V.</author><author>Arunajadai, S.</author><author>Horton, M.</author><author>Perera, F.</author><author>Hoepner, L.</author><author>Barr, D. B.</author><author>Whyatt, R.</author></authors></contributors><edition>2011/04/22</edition><language>eng</language><added-date format="utc">1329668161</added-date><ref-type name="Journal Article">17</ref-type><auth-address>Heilbrunn Center for Population and Family Health, Mailman School of Public Health, New York, NY 10032 USA. var1@columbia.edu</auth-address><remote-database-provider>NLM</remote-database-provider><rec-number>1079</rec-number><last-updated-date format="utc">1396923707</last-updated-date><accession-num>21507777</accession-num><electronic-resource-num>10.1289/ehp.1003160</electronic-resource-num><volume>119</volume></record></Cite></EndNote>] evaluated the relationship between prenatal chlorpyrifos exposure and neurodevelopment among 265 of the cohort participants who had reached the age of 7 years and had a complete set of data including prenatal maternal interview data, prenatal chlorpyrifos marker levels from maternal and/or cord blood samples at delivery, postnatal covariates, and neurodevelopmental outcome data [ADDIN EN.CITE <EndNote><Cite><Author>Rauh</Author><Year>2011</Year><IDText>Seven-year neurodevelopmental scores and prenatal exposure to chlorpyrifos, a common agricultural

pesticide</IDText><DisplayText>(V. Rauh et al., 2011)</DisplayText><record><dates><pub-dates><date>Aug</date></pub-dates><year>2011</year></dates><keywords><keyword>Adolescent</keyword><keyword>Adult</keyword><keyword>Child</keyword><keyword>Chlorpyrifos/toxicity</keyword><keyword>Female</keyword><keyword>Humans</keyword><keyword>Intelligence/drug effects</keyword><keyword>Male</keyword><keyword>Memory/drug effects</keyword><keyword>Pesticides/toxicity</keyword><keyword>Pregnancy</keyword><keyword>Prenatal Exposure Delayed Effects</keyword><keyword>Young Adult</keyword></keywords><isbn>1552-9924 (Electronic)
0091-6765 (Linking)</isbn><custom2>3237355</custom2><titles><title>Seven-year neurodevelopmental scores and prenatal exposure to chlorpyrifos, a common agricultural pesticide</title><secondary-title>Environ Health Perspect</secondary-title><alt-title>Environmental health perspectives</alt-title></titles><pages>1196-201</pages><number>8</number><contributors><authors><author>Rauh, V.</author><author>Arunajadai, S.</author><author>Horton, M.</author><author>Perera, F.</author><author>Hoepner, L.</author><author>Barr, D. B.</author><author>Whyatt, R.</author></authors></contributors><edition>2011/04/22</edition><language>eng</language><added-date format="utc">1329668161</added-date><ref-type name="Journal Article">17</ref-type><auth-address>Heilbrunn Center for Population and Family Health, Mailman School of Public Health, New York, NY 10032 USA. var1@columbia.edu</auth-address><remote-database-provider>NLM</remote-database-provider><rec-number>1079</rec-number><last-updated-date format="utc">1396923707</last-updated-date><accession-num>21507777</accession-num><electronic-resource-num>10.1289/ehp.1003160</electronic-resource-num><volume>119</volume></record></Cite></EndNote>]. While models were developed using continuous measures of both prenatal chlorpyrifos exposure and Wechsler scores, for ease of interpretation, investigators reported that for each standard deviation increase in exposure (4.61 pg/g) there is a 1.4% reduction in Full-Scale IQ and a 2.8% reduction in Working Memory. In the Mt. Sinai study, prenatal maternal DEP urinary metabolite concentrations were associated with slight decrements in Full Scale Intelligence Quotient (FSIQ), Perceptual Reasoning, and Working Memory between the ages of 6 and 9 years, and difference in intelligence measures by putative PON1 status were also noted [ADDIN EN.CITE <EndNote><Cite><Author>Engel</Author><Year>2011</Year><IDText>Prenatal exposure to organophosphates, paraoxonase 1, and cognitive development in childhood</IDText><DisplayText>(Engel et al., 2011)</DisplayText><record><dates><pub-dates><date>Aug</date></pub-dates><year>2011</year></dates><keywords><keyword>Aryldialkylphosphatase/genetics</keyword><keyword>Child</keyword><keyword>Child, Preschool</keyword><keyword>Cognition/ drug effects</keyword><keyword>Female</keyword><keyword>Genotype</keyword><keyword>Humans</keyword><keyword>Infant</keyword><keyword>Perception/drug effects</keyword><keyword>Phosphoric Acid Esters/toxicity</keyword><keyword>Pregnancy</keyword><keyword>Prenatal Exposure Delayed

Effects</keyword></keywords><isbn>1552-9924 (Electronic)
0091-6765 (Linking)</isbn><custom2>3237356</custom2><titles><title>Prenatal exposure to organophosphates, paraoxonase 1, and cognitive development in childhood</title><secondary-title>Environ Health Perspect</secondary-title><alt-title>Environmental health perspectives</alt-title></titles><pages>1182-8</pages><number>8</number><contributors><authors><author>Engel, S. M.</author><author>Wetmur, J.</author><author>Chen, J.</author><author>Zhu, C.</author><author>Barr, D. B.</author><author>Canfield, R. L.</author><author>Wolff, M. S.</author></authors></contributors><edition>2011/04/22</edition><language>eng</language><added-date format="utc">1329668765</added-date><ref-type name="Journal Article">17</ref-type><auth-address>University of California-Berkeley, Center for Environmental Research and Children's Health, School of Public Health, Berkeley, California, USA.</auth-address><remote-database-provider>NLM</remote-database-provider><rec-number>1180</rec-number><last-updated-date format="utc">1396923587</last-updated-date><accession-num>21507778</accession-num><electronic-resource-num>10.1289/ehp.1003183</electronic-resource-num><volume>119</volume></record></Cite></EndNote>]. Similarly, in the CHAMACOS cohort, Bouchard *et al*. [ADDIN EN.CITE <EndNote><Cite ExcludeAuth="1"><Author>Bouchard</Author><Year>2011</Year><IDText>Prenatal exposure to organophosphate pesticides and IQ in 7-year-old children</IDText><DisplayText>(2011)</DisplayText><record><dates><pub-dates><date>Aug</date></pub-dates><year>2011</year></dates><keywords><keyword>Child</keyword><keyword>Female</keyword><keyword>Humans</keyword><keyword>Intelligence/ drug effects</keyword><keyword>Intelligence Tests</keyword><keyword>Pesticides/ toxicity</keyword><keyword>Phosphoric Acid Esters/ toxicity/urine</keyword><keyword>Pregnancy</keyword><keyword>Prenatal Exposure Delayed Effects</keyword></keywords><isbn>1552-9924 (Electronic)
0091-6765 (Linking)</isbn><custom2>3237357</custom2><titles><title>Prenatal exposure to organophosphate pesticides and IQ in 7-year-old children</title><secondary-title>Environ Health Perspect</secondary-title><alt-title>Environmental health perspectives</alt-title></titles><pages>1189-95</pages><number>8</number><contributors><authors><author>Bouchard, M. F.</author><author>Chevrier, J.</author><author>Harley, K. G.</author><author>Kogut, K.</author><author>Vedar, M.</author><author>Calderon, N.</author><author>Trujillo, C.</author><author>Johnson, C.</author><author>Bradman, A.</author><author>Barr, D. B.</author><author>Eskenazi, B.</author></authors></contributors><edition>2011/04/22</edition><language>eng</language><added-date format="utc">1329668160</added-date><ref-type name="Journal Article">17</ref-type><auth-address>University of California-Berkeley, Center for Environmental Research and Children's Health, School of Public Health, Berkeley, California 94704, USA.</auth-address><remote-database-provider>NLM</remote-database-provider><rec-number>1045</rec-number><last-updated-date format="utc">1396923587</last-updated-date><accession-num>21507776</accession-

num><electronic-resource-num>10.1289/ehp.1003185</electronic-resource-num><volume>119</volume></record></Cite></EndNote>] observed evidence of an association between prenatal exposures to OPs as measured by urinary DAP (total DAP, DEP, and DMP) metabolites in women during pregnancy, and decreased cognitive functioning in children at age 7. In this study, children in the highest quintile of maternal DAP concentrations had a statistically significant 7-point difference in IQ points compared with those in the lowest quintile.

To determine if early childhood social adversities modify the association between maternal prenatal OP exposure and cognition in children (measured using the WISC-IV results), Stein et al. (2016) showed negative associations between maternal prenatal exposure to DAP metabolites and cognition in children were observed to be stronger among children who reported more adversity, relative to children who experienced less adversity. For example, for boys who experienced a more adverse learning environment, a statistically significant decrease was observed between prenatal DAP concentrations and Full-Scale IQ (β = -13.3; 95% CI: -19.9; -6.7; $p < 0.01$), relative to boys who lived in a less adverse learning environment (β = 4.2; 95% CI: -4.2; 12.5; $p < 0.01$). A similar observation was reported for perceptual reasoning and processing speed (β = -9.8; 95% CI: -17.4; -2.2; $p < 0.01$; β = -12.7; 95% CI: -19.5; -6.0; $p = 0.01$), respectively. For girls who experienced more economic adversity, a stronger effect on the association between prenatal DAP concentrations and Full-Scale IQ was observed, relative to girls who experienced less economic adversity (β = -8.5; 95% CI: -16.7, -0.4 vs. β = -4.7 95% CI: -12.8, 3.4; $p = 0.18$). This was also observed for working memory for girls (β = -5.9; 95% CI: -13.7, -1.8 vs. β = -2.6 95% CI: -10.0, 4.8; $p = 0.05$). In addition, similar findings were observed for girls whose mother's had more maternal adversity (β = -11.5; 95% CI: -18.5, -4.4 vs. β = -1.5 95% CI: -7.9, 4.9; $p = 0.07$).

To ascertain whether observed differences in neurodevelopment after prenatal chlorpyrifos exposure may be explained by differences in brain morphology between exposed groups, investigators compared MRI brain images between high and low chlorpyrifos exposed child study participants [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Authors determined there were distinct morphological differences in brain areas associated with these neurodevelopmental outcomes. The pilot study included 40 child participants due to strict inclusion and exclusion criteria, and the high cost of performing the imaging studies on each child. EPA convened a Federal Panel of experts to perform a written peer-review of this study.[5] The Federal Panel concurred with the authors' conclusions in general; however, the Federal Panel also noted that significantly greater and more sophisticated MRI imaging studies would be needed to link the morphological changes indicated in this study with specific functional outcomes noted in the CCCEH IQ study. Therefore, while generally supportive of the epidemiologic findings, additional study is needed to make specific links with areas of brain development change.

In sum, across these three children's environmental health studies, authors consistently identified associations with neurodevelopmental outcomes in relation to OP exposure. There is

[5] [HYPERLINK "http://www.regulations.gov/" \l "!documentDetail;D=EPA-HQ-OPP-2008-0850-0170"].

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evidence of delays in mental development in infants (24-36 months), attention problems and autism spectrum disorder in early childhood, and intelligence decrements in school age children who were exposed to chlorpyrifos or OPs during gestation. Investigators reported strong measures of statistical association across several of these evaluations (odds ratios 2-4 fold increased in some instances), and observed evidence of exposures-response trends in some instances, *e.g.*, intelligence measures.

EPA has considered the strengths and limitations of these studies, and believes that random or systematic errors in the design, conduct or analysis of these studies were unlikely to fully explain observed positive associations between *in utero* OP exposure and adverse neurodevelopmental effects observed at birth and through childhood (age 7 years). EPA believes these are strong studies which support a conclusion that OPs likely played a role in these outcomes.

2.2.8.2 HOME Cohort

Two studies from the HOME cohort on neurodevelopmental outcomes have been reported (Yolton et al, 2013; Donauer et al, 2016). The HOME study is a prospective cohort study conducted from March 2003 to February 2006, and the study population included pregnant women living in the Cincinnati, Ohio area who successfully delivered live-born infants. The HOME cohort is a more recent US cohort compared to CHAMACOS, Mt. Sinai and CCCEH and represents exposure from post mitigation activities on many OPs which occurred during tolerance reassessment. Thus, the exposure pattern to the mothers in the HOME cohort and the study demographics (i.e. suburban, middle class study population) are likely different relative to studies from CHAMACOS, Mt. Sinai and CCCEH. Moreover, the demographic data reported in the HOME cohort indicate that mothers tended to have a higher socioeconomic status and were more likely to eat and live a healthier lifestyle relative to mothers involved in CHAMACOS, Mt. Sinai and CCCEH. As a result, these life habits were potentially protective towards the OP exposures during pregnancy.

Exposure was assessed twice during pregnancy—at 16 ± 4 weeks and 26 ± 4 weeks of gestation—via spot urine samples that were stored and later laboratory tested. The urine samples were measured for six DAPs.

In the Yolton et al (2013) study, a neurobehavioral assessment, the NICU Network Neurobehavioral Scale (NNNS), was conducted at approximately 5 weeks of age, and each infant was scored based on several categories (or subscales) of the NNNS. A total of 350 mother/ infants were included within this study, and a statistically significant association ($p < 0.05$) between maternal metabolite urinary concentrations (DE and DAP) and the following NNNS subscales for infant performance was observed: attention, lethargy, hypotonia, and autonomic stress. After adjusting the model for covariates, evidence of an association was observed between increased mean urinary DE metabolites and increased attention ($\beta = 0.066$, $p < 0.05$), with no statistically significant covariates. Decreased lethargy and hypotonia were also associated with increased urinary DE metabolites at 16 weeks of gestation ($\beta = -0.069$, $p = 0.04$;

$\beta = -0.101$, $p = 0.03$), with black race and birthweight as statistically significant covariates ($\beta = -0.462$, $p = 0.03$; $\beta = -0.044$, $p = 0.01$). For DAP metabolite concentrations, evidence of an association was observed between increased DAP concentrations at 26 weeks of gestation and decreased autonomic stress (β coefficient = -0.010, $p = 0.01$), with birthweight of infants and maternal blood lead levels during gestation as statistically significant covariates ($\beta = -0.003$, $p = 0.02$; $\beta = 0.031$, $p = 0.02$), respectively.

In Donauer et al, 2016, offspring were assessed through neurodevelopmental examinations conducted yearly, at ages 1-5 years. The Bayley-II assessed mental and motor advancement for children ages 1-3 years, and the MDI and PDI were used to analyze cognitive, language, and motor development. For 4 year-old children, language skills were assessed by the Clinical Evaluation of Language Fundamentals-Preschool, and the WPPSI was used to assess intelligence (IQ) including verbal, performance, and full-scale for children 5 years of age. Of the 327 maternal-child cases, evidence of a significant association between maternal levels of urinary metabolites (total DAP and total DM) and verbal IQ of the child from the Wechsler Preschool and Primary Scale of Intelligence test was observed in both the unadjusted and adjusted models at the $p < 0.2$ level (unadjusted model: $\beta = 0.005$, $p = 0.034$, $\beta = 0.003$, $p = 0.144$; adjusted model: $\beta = 0.003$, $p = 0.179$, $\beta = 0.003$, $p = 0.144$), respectively. Although evidence of a positive association was observed for total DAP and total DM metabolites and full-scale IQ (unadjusted model: $\beta = 0.004$, $p = 0.111$; $\beta = 0.004$, $p = 0.124$, when the model was adjusted, no evidence of a positive association was observed (adjusted model: $\beta = 0.001$, $p = 0.494$; $\beta = 0.002$, $p = 0.441$). No evidence of a positive association was observed between performance IQ of the child and total DAP, DM, and DE, respectively. For Bayley-II MDI, evidence of a positive association was observed for maternal exposure to total DM and total DE at the child's 2-year visit (unadjusted model: $\beta = 0.003$, $p = 0.16$; $\beta = 0.003$, $p = 0.16$); however, when the model was adjusted, no evidence of a positive association was observed for total DM and total DE (adjusted model: $\beta = 0.002$, $p = 0.28$; $\beta = 0.002$, $p = 0.37$). No evidence of a positive association was observed between maternal exposure (for total DAP, total DM, and total DE) and the Bayley-II Psychomotor Developmental Index for children aged 1-3 years.

2.2.8.3 Meta-analysis (Engel et al, 2015)

Engel et al. (2015) reports on the results of a pooled analysis from four cohorts (N=936) to evaluate the association between prenatal DAPs and neurodevelopmental outcomes at 24 months only. In addition, researchers in this study assessed the impact on these associations of the specific cohort, race/ethnicity, and the PON I genotype of study participants. Researchers across the four children's health cohorts utilized the BSID-11 to generate MDI and PDI to assess neurodevelopment in early childhood. The four cohorts include CHAMACOS (N=377), HOME (N=265), Mt. Sinai (N=234), CCCEH (N=60), It is noted that the CCCEH participants included in this analysis are from women enrolled in 2000 to 2001, a time period which is during the phase out of chlorpyrifos in residential settings.

The results of this pooled study are relatively consistent with those seen in the individual cohorts at 24 months. After controlling for race/ethnicity, smoking, and drug use during pregnancy, a statistically significant association was observed in the pooled population between

total DAPs exposure and MDI decrements, but not with PDI decrements. Consistent with the results from Eskenazi et al. (2007), the strongest evidence of an association was observed for the CHAMACOS cohort, with statistically significant associations for both total DAPs and total DMAPs exposure and MDI decrements. No significant associations were seen within the Mt. Sinai and CCCEH cohorts, a result which is basically consistent with the previous observations at 24 months in these cohorts (Engel et al., 2011; Rauh et al.,2006). The study authors observed significant heterogeneity from combining the cohorts, especially with regards to race/ethnicity, and noted that impacts on specific subpopulations may be lost when looking at the pooled results.

2.2.8.4 Other Neurodevelopmental Studies

In the first of two Chinese studies focusing on generic neurodevelopmental outcomes, Zhang *et al.* (2014) investigated prenatal exposure to OPs and neurobehavioral development of neonates in a birth cohort study in Shenyang, China. The authors reported that consistent statistically significant associations were observed between all of the quantified urinary biomarkers of prenatal OP pesticide exposure and neonatal neurodevelopment deficits assessed 3 days after birth. A 10-fold increase in total DAPs concentration was associated with an average decrease in Neonatal Behavioral Neurological Assessment (NBNA) summary scores of 1.78 points (95% CI: -2.12 to -1.45). No evidence of departure from linearity of the exposure-response relationship between maternal DAP concentrations and NBNA scores was observed. In the second Chinese study focusing on neurodevelopmental outcomes, Guodong *et al.* (2012) conducted a cross-sectional study, and did not identify any statistically significant associations between the children's urinary DAP metabolite levels and any of the DQ (Developmental quotients) scores. The authors mentioned that their results should be interpreted with caution since OP exposure was quantified in single spot urine sample from children, and should be followed up with a longitudinal study with repeated measurement of exposure levels in urine samples.

Two studies focused on impaired social responsiveness, autism spectrum disorders, or developmental delays (Furlong *et al.*, 2014; Shelton *et al.*, 2014). Among 7-9 years old children in the Mount Sinai Cohort (Furlong *et al.* 2014), there was no overall statistically significant association between maternal third trimester urinary DAP metabolite levels and reciprocal social responsiveness (a measure linked to many neuropsychiatric conditions that involve impaired social functioning (Constantino and Gruber 2005)). However, some evidence of modification of the association between prenatal OP pesticide exposure and impaired social responsiveness in early childhood was observed by both race/ethnicity and child sex, with an association between DEP and poorer social responsiveness observed among black participants and boys. No association was observed among whites or Hispanics, among girls, or for DAP or DMP biomarker levels.

Shelton *et al.* (2014), investigated autism spectrum disorders and developmental delay (DD) in relation to gestational residential proximity to agricultural pesticide applications utilizing the

California population-based Childhood Autism Risks from Genetics and Environment (CHARGE) study. The investigators reported that children with autism spectrum disorder were 60% more likely to have OPs applied near the home [1.25 km distance; adjusted OR (aOR) = 1.60; 95% CI: 1.02–2.51] than mothers of normally developing children. They added that as the buffer distance grew larger, these associations became lesser, indicating an exposure-response effect. The authors also mentioned that each 100-lb (45.4 kg) increase in the amount applied over the course of pregnancy (within 1.5 km of the home) was associated with a 14% higher prevalence of autism spectrum disorder (aOR = 1.14; 95% CI: 1.0, 1.32), but no association was identified with DD.

A total of three studies focused on OP exposure and behavioral, memory, or attention/ADHD outcomes (Oulhote and Bouchard, 2013; Bouchard *et al.*, 2010; Fortenberry *et al.*, 2014). In a national cross-sectional study of Canadian children 2007-2009 data for age 6-11 years (Oulhote and Bouchard, 2013), there were no overall statistically significant associations observed between child urinary DAP, DMP, or DEP metabolite levels and parentally reported behavioral problems. In contrast, Bouchard *et al.* (2010), looking at U.S. children age 8-15 years in the 2000-2004 National Health and Nutrition Examination Survey (NHANES),[6] observed a positive association between attention and behavior problems and DAPs and DMPs, but not DEPs. For example, even after controlling for potential confounders such as sex, age, ethnicity, and creatinine concentration, they found that a 10-fold increase in DMAP concentration was associated with a 55 to 72% increased odds of ADHD.

Fortenberry *et al.* (2014) evaluated the relationship between pesticide exposure and ADHD in school age Mexican children, recruiting 187 mother-child pairs from a prospective birth cohort, ELEMENT (Early Life Exposures in Mexico to Environmental Toxicants). The authors reported that, there were no statistically significant associations between tertiles of maternal third trimester urinary TCPy and measures of attention and hyperactivity in children. However, there was suggestive evidence for increases in the ADHD index in relation to TCPy tertiles among boys (the highest TCPy tertile was associated with an ADHD index score that was 5.55 points higher than children in the lowest tertile; p-value = 0.06).

3.0 Weight of Evidence Analysis: Integration Across Multiple Lines of Evidence

OPP's 2016 "Framework for Incorporating Human Epidemiologic & Incident Data in Risk Assessments for Pesticides" provides the foundation for evaluating multiple lines of scientific evidence (U.S. EPA, 2016c). OPP uses a WOE analysis for evaluating epidemiology and human incident data, such that conclusions are made on the preponderance of the information rather than relying on any one study. OPP uses the modified Bradford Hill criteria like those in the MOA/human relevance framework as a tool for organizing and integrating information from different sources (Hill, 1965; U.S. EPA, 1999, 2005; Sonich-Mullin et al., 2001; Meek et al., 2003;

[6] http://www.cdc.gov/nchs/nhanes.htm

OECD AOP Wiki Users Handbook[7]). It is important to note that the Bradford Hill Criteria are not intended as a check box approach but instead are points to consider when evaluating the totality of evidence. In addition, the availability of a fully elucidated MOA/AOP is a not requirement for using epidemiology studies in human health risk assessment. The Bradford Hill Criteria explicitly considers such concepts as strength, consistency, dose response, temporal concordance and biological plausibility in a weight of evidence analysis; sections 3.1-3.3 below summarize the available evidence based on these principles.

The agency's 2002 guidance on "Determination of the Appropriate FQPA Safety Factor(s) in Tolerance Assessment" contains a discussion of the relative weighing of animal and human data with respect to pre- and post-natal toxicity, dose-response, toxicokinetics, and mode of action. The guidance specifically states that

> "potential for pre- and postnatal toxicity can be determined from human and animal studies. Although human studies are seldom available, human data are the most relevant data for assessing potential health risks. *When sufficient human data are available to judge that an adverse developmental outcome is related to exposure, the degree of concern increases* (p. 33, emphasis added)."

Table 1 of the 2002 FQPA Safety Factor guidance notes that when effects are found in humans related to exposure that these data receive "Increasing Weight". This table lists the following factors as receiving increasing weight in the WOE analysis with respect to pre- and post-natal toxicity:

- Effects found in humans related to exposure
- Same types of effects seen in more than one species
- Effects of a different type with greater potential consequences in young compared to adults
- Persistence or relatively longer recovery of effects in young compared to adults

The agency notes that each of these factors applies to the database of studies for OPs. As described in detail above, epidemiology studies from multiple investigators representing multiple locations and different populations show associations between OP exposure and adverse neurodevelopmental outcomes. Regarding multiple laboratory species in the database of laboratory studies, a range of effects on the developing nervous system has been shown in mouse (e.g., Braquenier et al., 2010; Venerosi et al., 2015), rat (e.g., Carr et al., 2015; Vatanparas et al., 2013), and guinea pig (e.g., Mamczarz et al., 2016). Although very high levels of OP exposure can lead to coma, paralysis, or even death, the 10% AChE point of departure represents a precursor event believed to be protective of this downstream cholinergic neurotoxicity. The 10% AChE PoD is much lower than these exposure levels and thus is believed to be health protective of cholinergic neurotoxicity. In contrast, neurodevelopmental effects such as reduced IQ, autism spectrum disorder, and ADHD are adverse health outcomes

[7] https://aopwiki.org/wiki/index.php/Main_Page#OECD_User_Handbook

of significant public health consequence. Moreover, these types of neurodevelopment effects are likely to persist through life. Further evidence of the long term consequence of such exposure is found in Rauh et al (2015) who have shown even at age 11 children exposed to chlorpyrifos in the home environment were more likely to exhibit mild or mild to moderate arm tremor.

3.1 Dose-Response Relationships & Temporal Concordance

Since the MOA(s)/AOP(s) is/are not established for neurodevelopmental outcomes (USEPA, 2012, 2014), it is not possible to describe the concordance in key events or biological steps leading to neurodevelopmental outcomes. As such, the quantitative linkages between molecular initiating events (MIE)s, intermediate steps, and ultimately the adverse outcome (i.e., neurodevelopmental effects) cannot be determined. (See Appendix 6)

With respect to the timing of exposure, across the epidemiology database of studies the maternal urine, cord blood and other (meconium) measures provide evidence that exposure did occur to the fetus during gestation, but the actual level of such exposure during the critical window(s) of susceptibility is not known. While significant uncertainties remain about the actual exposure levels experienced by mothers and infant participants in the children's health cohorts, it is unlikely that these exposures resulted in AChE inhibition. As part of the CHAMACOS study, Eskenazi *et al.* (2004) measured AChE activity and showed that no differences in AChE activity were observed. The biomarker data from the Columbia University studies are supported by the agency's dose reconstruction analysis using the PBPK-PD model. Following the recommendation of the FIFRA SAP (2012), the agency conducted a dose reconstruction analysis of residential uses available prior to 2000 for pregnant women and young children inside the home (USEPA, 2014). Based on the output from the PBPK-PD model, for the highest exposure considered (i.e., indoor broadcast use of a 1% chlorpyrifos formulation), <1% RBC AChE inhibition in pregnant women would be expected. While uncertainty exists as to actual OP exposure at (unknown) critical windows of exposure, EPA believes it is unlikely individuals in the epidemiology studies experienced RBC AChE inhibition.

Preamble to the Integrated Science Assessments (ISAs) which serve as a scientific foundation for the review of EPA's National Ambient Air Quality Standards (NAAQS) notes that strong evidence for causality can be provided through "natural experiments" when a change in exposure is found to result in a change in occurrence or frequency of health (USEPA, 2015c). Within the Columbia University epidemiology studies, the relationship in time between prenatal chlorpyrifos exposure and adverse neurodevelopmental outcomes is concordant. The time period under study within the Columbia University (CCCEH) study, spanned the point in time in which pesticide manufacturers voluntarily cancelled the use of chlorpyrifos in the home environment, and researchers were able to show the change in exposure before (high use period) and after (low/no use period) the period of removal of chlorpyrifos products from the residential marketplace. Moreover, prior to the voluntary cancellation there were >80% detectable levels of chlorpyrifos in cord blood but in the time period after the cancellation only

16% of the measured values were greater than the level of detection (LOD); there was only one child born in the time period subsequent to the voluntary cancellation of chlorpyrifos in the residential marketplace for whom the cord blood chlorpyrifos level was in the upper-tertile of pre-cancellation exposure levels. The significantly reduced proportion of measured values greater than the LOD as well as the observation of an absence of an association between prenatal chlorpyrifos exposure among infants born after the voluntary cancellation of chlorpyrifos and neurodevelopmental effects support the hypothesis that chlorpyrifos is related to these outcomes. However, as noted by study authors, EPA and the FIFRA SAP (2012), this could also be due to inadequate sample size to detect a small to modest effect among the group of infants born after the voluntary cancellation. It is notable that epidemiology studies from other research groups have not included analyses across different years of exposure.

3.2 Strength, Consistency & Specificity

In making a weight-of-evidence analysis, it is important to consider the strength of the statistical measures of association between OP exposure and adverse neurodevelopmental outcomes through childhood (epidemiology) and possibly into adulthood (animal studies). It is also important to consider the strength of the integrated qualitative and quantitative evidence, the consistency of the observed associations across epidemiology studies and considering both animal and human data support the conclusion that chlorpyrifos plays a role in adverse neurodevelopmental outcomes.

3.2.1 Strength

As noted in the 2016 epidemiology and incident framework, findings of large, precise risks increases confidence that the association is not likely due to chance, bias, or other factors (USEPA, 2016c). In the case of the CCCEH study, there are a number of notable associations. Regarding infant and toddler neurodevelopment, the CCCEH authors reported statistically significant deficits of 6.5 points on the PDI at 3 years of age when comparing high to low exposure groups (Rauh et al., 2006). Notably these decrements in PDI persist even after adjustment for group and individual level socioeconomic variables (Lovasi et al., 2011). These investigators also observed increased odds of mental delay (OR=2.4; 95% CI: 1.1-5.1) and psychomotor delay (OR=4.9; 95% CI: 1.8-13.7) at age three when comparing high to low exposure groups (Rauh et al., 2006). Rauh et al (2006) also reported extremely large odds ratios for attention disorders (OR=11.26; 95% CI: 1.79-70.99), ADHD (OR=6.50; 95% CI: 1.09-38.69), and PDD (OR=5.39; 95% CI: 1.21-24.11) when comparing high to low chlorpyrifos exposure groups (Rauh et al., 2006). EPA notes that the magnitude of these results are so large that they are unlikely to be affected by residual confounding although limited sample sizes resulted in imprecise estimates.

From the CHAMACOS cohort, Eskanazi et al (2007) reported that both prenatal and postnatal DAPs were associated with risk of PDD (per 10-fold increase in prenatal DAPs: OR = 2.3, p = 0.05; child DAPs OR = 1.7, p = 0.04). Marks et al (2010) reported prenatal DAPs were associated

with scores on the K-CPT ADHD Confidence Index > 70th percentile (OR = 5.1; 95% CI: 1.7–15.7 and with a composite ADHD indicator of the various measures (OR = 3.5; 95% CI: 1.1–10.7). Some outcomes exhibited evidence of effect modification by sex, with associations found only among boys. Children's concurrent total DAP and DMP metabolite levels at 3.5 years and 5 years were unrelated to attention outcomes, and but child DEP concentrations at 5 years were adversely associated with the composite measure of attention (OR = 2.0; 95% CI:1.1–3.6).

In a recent evaluation by Stein et al (2016) from the CHAMACOS cohort, boys who experienced a more adverse learning environment, a statistically significant decrease was observed between prenatal DAP concentrations and Full-Scale IQ (β = -13.3; 95% CI: -19.9; -6.7; $p < 0.01$), relative to boys who lived in a less adverse learning environment (β = 4.2; 95% CI: -4.2; 12.5; $p < 0.01$). A similar observation was reported for perceptual reasoning and processing speed (β = -9.8; 95% CI: -17.4; -2.2; $p < 0.01$; β = -12.7; 95% CI: -19.5; -6.0; $p = 0.01$), respectively. For girls who experienced more economic adversity, a stronger effect on the association between prenatal DAP concentrations and Full-Scale IQ was observed, relative to girls who experienced less economic adversity (β = -8.5; 95% CI: -16.7, -0.4 vs. β = -4.7 95% CI: -12.8, 3.4; $p = 0.18$). This was also observed for working memory for girls (β = -5.9; 95% CI: -13.7, -1.8 vs. β = -2.6 95% CI: -10.0, 4.8; $p = 0.05$).

3.2.2 Consistency

The Preamble to the ISA's note that an inference of causality is strengthened when a pattern of elevated risks is observed across several independent studies. The reproducibility of findings constitutes one of the strongest arguments for causality. Moreover, statistical significance is not the sole criterion by which the presence or absence of an effect is determined (USEPA, 2015c).

Published and submitted laboratory animal studies have been reviewed for OPs. The >30 papers on chlorpyrifos provide evidence of long-lasting neurodevelopmental disorders in rats and mice; however, there was no clear consistency in terms of pattern, timing, or dose response for these effects. The additional toxicological literature and guideline DNT studies with the other OPs provide more evidence for the same conclusions, with again the same caveats and uncertainties. While overall cognitive function and motor activity appeared to be altered the most often, it is apparent that these behaviors were also the most often evaluated.

Across the various epidemiology studies within the scope of this analysis and with adequate quality in design and exposure assessment, it is important to note that these studies used study methods that were highly variable, including different exposure measurement, outcome assessment, study design, and geographical location. These differences make it challenging to compare the results across studies. Among the epidemiology studies, two of the cohorts (CCCEH and ELEMENT) have focused on chlorpyrifos whereas the other studies (Mt. Sinai cohort, CHAMACOS cohort, HOME cohort, CHARGE study, Bouchard *et al.*, 2010) have focused on less specific biomarkers (i.e., DAPs) and are not specific to any particular OP. When considered in concert, the epidemiology studies provide consistent findings for some outcomes.

In comparison to the studies from CCCEH, Mt. Sinai, and CHAMACOS, results from the ELEMENT and CHARGE studies provide some supportive evidence for the findings. However, the results from the HOME cohort show different results; namely positive associations between neurodevelopmental outcomes and maternal DAPs (Yolton et al, 2013; Donauer et al, 2016). The HOME study represents a different demographic profile (higher socioeconomic status, suburban, middle class) and different time period (2003-2006) compared to CCCEH, Mt. Sinai, and CHAMACOS which are made up of women with much lower socioeconomic status and who experienced different exposure patterns.

In the two Chinese studies, EPA does not know how the OP exposures from these studies relate to the currently registered use pattern for OPs used in the U.S./North America. In the case of these two Chinese studies (Guodong *et al.,* 2012; Zhang *et al.*, 2014), there may be differences in the study population and outcome measurements that may account for the observed differences in study results, with Zhang *et al.* (2014) documenting statistically significant associations for total DEAPs, total DMAPs, and total DAPs and Guodong *et al.* (2012) observing no association with these exposures. The Zhang *et al.* (2014) study was conducted in Shenyang, with a study population reported as 87% urban and 13% rural, whereas the Guodong *et al.* (2012) study was conducted in Shanghai with a 99% urban and 1% suburban study population. Given the higher percentage of study participants from rural areas, the study participants from Zhang *et al.* (2014) may have had different pattern and magnitude of OP exposures compared to those from Guodong *et al.* (2012). In addition, it is noted that different outcome measurements were made in these studies, with Guodong *et al.* (2012) assessing 23-25 month old children using a developmental quotient score and Zhang *et al.* (2014) assessing 3 day old infants using a Neonatal Behavioral Neurological Assessment. Given the different outcome assessments, exposure potential, study designs (cohort vs. cross-sectional), and ages of the participants in these two studies, it is difficult to draw conclusions on how these study results compare. It is notable that the results from the Zhang *et al.* (2014) study focusing on neonates are consistent with those from other studies which reported statistically significant associations between delayed neurological development measured in newborns and total DEAP, total DMAP, and total DAP exposure (Engel *et al.*, 2007; Young *et al.*, 2005). However, it is noted that neurological development was measured within a few days of birth for Zhang *et al.* (2014) and Engel *et al.* (2007), whereas Young *et al.* (2005) conducted their measurements within two months of birth.

The CHAMACOS and Mt. Sinai cohorts that measured neurological effects at birth (the Brazelton index), observed a putative association with OPs [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Yolton et al (2013) used the NNNS tool at approximately 5 weeks of age in the HOME cohort and reported evidence of a positive association between increased mean urinary DE metabolites and increased attention and decreased autonomic stress.

Researchers across CCCEH, Mt. Sinai, CHAMACOS, and HOME studies utilized the Bayley Scales of Infant Development II (BSID-II) to generate a MDI and a PDI to assess neurodevelopment in early childhood. In the CCCEH study, Rauh *et al.* (2006) investigated MDI and PDI at 12, 24, and

36 months of age. Children were categorized as having either high (>6.17pg/g) or low (≤6.17pg/g) prenatal chlorpyrifos exposure, using categories informed by results of the previous study on birth characteristics [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Authors reported that the difference in MDI scores was "marginally significant" (p = 0.06) between the "high" and "low" exposed groups; the high exposed group scoring an average of 3.3 points lower than the low exposed [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Regarding the PDI score (motor skills), none of the 12 or 24 month PDI scores showed significant effects, but the 36 month score was significantly related to chlorpyrifos exposure.

Within the 36 month testing period, the likelihood of highly exposed children developing mental delays were significantly greater (MDI: 2.4 times greater (95% CI: 1.12-5.08, p = 0.02) and PDI: 4.9 times greater (95% CI: 1.78-13.72; p = 0.002)) than those with lower prenatal exposure [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Within the Mt. Sinai study, authors administered the BSID-II to participating children at 12 and 24 months and observed that prenatal total DAP metabolite level was associated with a decrement in mental development at 12 months among blacks and Hispanic children; however, these associations either attenuated or were non-existent at the 24-month visit [ADDIN EN.CITE
<EndNote><Cite><Author>Engel</Author><Year>2011</Year><IDText>Prenatal exposure to organophosphates, paraoxonase 1, and cognitive development in childhood</IDText><DisplayText>(Engel et al., 2011)</DisplayText><record><dates><pub-dates><date>Aug</date></pub-dates><year>2011</year></dates><keywords><keyword>Aryldialkylphosphatase/ genetics</keyword><keyword>Child</keyword><keyword>Child, Preschool</keyword><keyword>Cognition/ drug effects</keyword><keyword>Female</keyword><keyword>Genotype</keyword><keyword>Humans</keyword><keyword>Infant</keyword><keyword>Perception/drug effects</keyword><keyword>Phosphoric Acid Esters/ toxicity</keyword><keyword>Pregnancy</keyword><keyword>Prenatal Exposure Delayed Effects</keyword></keywords><isbn>1552-9924 (Electronic)
0091-6765 (Linking)</isbn><custom2>3237356</custom2><titles><title>Prenatal exposure to organophosphates, paraoxonase 1, and cognitive development in childhood</title><secondary-title>Environ Health Perspect</secondary-title><alt-title>Environmental health perspectives</alt-title></titles><pages>1182-8</pages><number>8</number><contributors><authors><author>Engel, S. M.</author><author>Wetmur, J.</author><author>Chen, J.</author><author>Zhu, C.</author><author>Barr, D. B.</author><author>Canfield, R. L.</author><author>Wolff, M. S.</author></authors></contributors><edition>2011/04/22</edition><language>eng</language><added-date format="utc">1329668765</added-date><ref-type name="Journal Article">17</ref-type><auth-address>University of California-Berkeley, Center for Environmental Research and Children's Health, School of Public Health, Berkeley, California, USA.</auth-address><remote-database-provider>NLM</remote-database-provider><rec-number>1180</rec-number><last-updated-date format="utc">1396923587</last-updated-date><accession-num>21507778</accession-num><electronic-resource-num>10.1289/ehp.1003183</electronic-resource-

num><volume>119</volume></record></Cite></EndNote>]. In the CHAMACOS cohort, Eskenazi *et al.* [ADDIN EN.CITE ADDIN EN.CITE.DATA] observed that prenatal DAP levels were adversely associated with MDI, and at 24 months of age these associations reached statistical significance. In this study, neither prenatal DAPs nor maternal TCPy were associated with PDI (motor skills), nor did authors observe evidence of different risk by PON1 status [ADDIN EN.CITE ADDIN EN.CITE.DATA]. In contrast to the findings of CCCEH, Mt. Sinai, and CHAMACOS, the HOME study (Donauer et al, 2016) reported evidence of a positive association for MDI was observed for maternal exposure to total DM and total DE at the child's 2-year visit; however, when the model was adjusted, no evidence of a positive association was observed for total DM and total DE (adjusted model ($\beta = 0.002$, $p = 0.28$; $\beta = 0.002$, $p = 0.37$). No evidence of an association was observed between maternal exposure (for total DAP, total DM, and total DE) and the Bayley-II Psychomotor Developmental Index for children aged 1-3 years.

With respect to attention problems, Rauh *et al.* [ADDIN EN.CITE ADDIN EN.CITE.DATA] also investigated 36-month child behavior checklist (CBCL) (behavioral) scores. Significant differences were observed between the high and low chlorpyrifos exposure groups in the general category of attention-problems ($p=0.010$), and in the more specific DSM-IV scale for ADHD problems ($p = 0.018$). The CHAMACOS cohort also investigated attention problems in early childhood using three different assessment tools: maternal report of child behavior at 3.5 and 5 years of age; direct assessment of the child at 3.5 and 5 years; and by a psychometrician's report of the behavior of the child during testing at 5 years. In this study population, higher concentrations of OP metabolites in the urine of pregnant women were associated with increased odds of attention problems and poorer attention scores in their children at age 5 years [ADDIN EN.CITE ADDIN EN.CITE.DATA].

Of the four new studies focusing on ADHD and OP exposure, three found statistically significant associations, with only Oulhote and Bouchard (2013) finding no association with total DMAP, DEAP, or total DAP exposure. In contrast, Bouchard *et al.* (2010) observed an association with total DMAP and total DAP exposure and ADHD. Fortenberry *et al.* (2014) found suggestive, but not statistically significant, evidence of an association with TCPy and ADHD in boys. Overall, the Fortenberry and Bouchard study results are consistent with that of earlier studies from the CCCEH (Rauh *et al.*, 2006) and CHAMACOS [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Specifically, statistically significant associations were observed by Rauh *et al.* (2006) with chlorpyrifos exposure and ADHD, Eskenazi *et al.* (2007) with total DMAPs and total DAPs and ADHD, and Marks *et al.* (2010) with total DEAP, DMAP, and total DAP exposure.

It is important to put into context the specific outcome measures used in the assessment of attention and neurobehavioral problems. For example, Bouchard *et al.* (2010) identified statistically significant associations between OP exposure and ADHD/behavioral problems, whereas Oulhote and Bouchard (2013) did not. It is valuable to compare these studies given that they are both cross-sectional studies using large population level datasets with biomarker information, with Oulhote and Bouchard (2013) using a Canadian dataset and Bouchard et al (2010) using a U.S. dataset. Bouchard *et al.* (2010) used criteria for ADHD from DSM-IV, whereas Oulhote and Bouchard (2013) used a "Strengths and Differences Questionnaire

(SDQ)," with the SDQ being a more generic assessment of mental health status than the DSM-IV criteria. When Oulhote and Bouchard (2013) compared their results to Bouchard *et al.* (2010), they noted that their outcome measurements may not have been as sensitive and that this may account for the difference in study results.

Across epidemiology studies looking at ADHD/behavioral problems, a suggestive or statistically significant positive association was observed in multiple studies between OP exposure and these neurobehavioral outcomes (Bouchard *et al.*, 2010; Fortenberry *et al.*, 2014; Rauh *et al.*, 2006, Eskenazi *et al.*, 2007). While these studies have differences in the years that the exposure occurred, study design, exposure assessment, and outcome assessment, the commonality in their results is striking.

Several studies have now documented suggestive or positive associations between OP exposure and autism spectrum disorders (Rauh *et al.*, 2006; Shelton *et al.*, 2014; Furlong *et al.*, 2014; Eskenazi *et al.*, 2007; Eskenazi et al, 2010). Specifically, Furlong *et al.* (2014) reported suggestive, but not statistically significant, evidence of an association between total DEAP exposure and reciprocal social responsiveness among black participants and boys. These results are consistent with previous studies conducted on the CCCEH and CHAMACOS cohorts, with these studies also showing statistically significant associations between OP exposure and autism spectrum disorders (Rauh *et al.*, 2006; Eskenazi *et al.*, 2007).[8] Specifically, Eskenazi *et al.* (2007) reported a statistically significant association between PDD and total DAP exposure, whereas Rauh *et al.* (2006) showed a significant association between PDD and specifically chlorpyrifos exposure. Both PDD and reciprocal social responsiveness are related to the autism spectrum disorder. Using a different exposure assessment method (geospatial analysis and residential proximity to total OP exposure), Shelton *et al.* (2014) also documented statistically significant associations between total OP exposure and autism spectrum disorders. While these studies vary in the magnitude of the overall strength of association, they have consistently observed a positive association between OP exposure and autism spectrum disorders.

Finally, CCCEH, Mt. Sinai, and CHAMACOS observed an inverse relation between the respective prenatal measures of OPs and intelligence measures at age 7 years. To measure intelligence among school aged children, authors from each of the three children's health cohorts used the WISC-IV. Rauh *et al.* [ADDIN EN.CITE <EndNote><Cite ExcludeAuth="1"><Author>Rauh</Author><Year>2011</Year><IDText>Seven-year neurodevelopmental scores and prenatal exposure to chlorpyrifos, a common agricultural pesticide</IDText><DisplayText>(2011)</DisplayText><record><dates><pub-dates><date>Aug</date></pub-dates><year>2011</year></dates><keywords><keyword>Adolescent</keyword><keyword>Adult</keyword><keyword>Child</keyword><keyword>Chlorpyrifos/

[8] The DSM-V defines ASD (autism spectrum disorder) which now encompasses several disorders that were different diagnoses in DSM-IV, including PDD (pervasive developmental disorder, a catch-all where the other categories didn't fit). Depending on when the study was conducted, the authors may use the PDD or ASD criteria and terminology.

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toxicity</keyword><keyword>Female</keyword><keyword>Humans</keyword><keyword>Intelligence/drug effects</keyword><keyword>Male</keyword><keyword>Memory/drug effects</keyword><keyword>Pesticides/ toxicity</keyword><keyword>Pregnancy</keyword><keyword>Prenatal Exposure Delayed Effects</keyword><keyword>Young Adult</keyword></keywords><isbn>1552-9924 (Electronic)
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0091-6765 (Linking)</isbn><custom2>3237356</custom2><titles><title>Prenatal exposure to organophosphates, paraoxonase 1, and cognitive development in childhood</title><secondary-title>Environ Health Perspect</secondary-title><alt-title>Environmental health

perspectives</alt-title></titles><pages>1182-8</pages><number>8</number><contributors><authors><author>Engel, S. M.</author><author>Wetmur, J.</author><author>Chen, J.</author><author>Zhu, C.</author><author>Barr, D. B.</author><author>Canfield, R. L.</author><author>Wolff, M. S.</author></authors></contributors><edition>2011/04/22</edition><language>eng</language><added-date format="utc">1329668765</added-date><ref-type name="Journal Article">17</ref-type><auth-address>University of California-Berkeley, Center for Environmental Research and Children's Health, School of Public Health, Berkeley, California, USA.</auth-address><remote-database-provider>NLM</remote-database-provider><rec-number>1180</rec-number><last-updated-date format="utc">1396923587</last-updated-date><accession-num>21507778</accession-num><electronic-resource-num>10.1289/ehp.1003183</electronic-resource-num><volume>119</volume></record></Cite></EndNote>]. Similarly, in the CHAMACOS cohort, Bouchard *et al.* [ADDIN EN.CITE <EndNote><Cite ExcludeAuth="1"><Author>Bouchard</Author><Year>2011</Year><IDText>Prenatal exposure to organophosphate pesticides and IQ in 7-year-old children</IDText><DisplayText>(2011)</DisplayText><record><dates><pub-dates><date>Aug</date></pub-dates><year>2011</year></dates><keywords><keyword>Child</keyword><keyword>Female</keyword><keyword>Humans</keyword><keyword>Intelligence/ drug effects</keyword><keyword>Intelligence Tests</keyword><keyword>Pesticides/ toxicity</keyword><keyword>Phosphoric Acid Esters/ toxicity/urine</keyword><keyword>Pregnancy</keyword><keyword>Prenatal Exposure Delayed Effects</keyword></keywords><isbn>1552-9924 (Electronic)
0091-6765 (Linking)</isbn><custom2>3237357</custom2><titles><title>Prenatal exposure to organophosphate pesticides and IQ in 7-year-old children</title><secondary-title>Environ Health Perspect</secondary-title><alt-title>Environmental health perspectives</alt-title></titles><pages>1189-95</pages><number>8</number><contributors><authors><author>Bouchard, M. F.</author><author>Chevrier, J.</author><author>Harley, K. G.</author><author>Kogut, K.</author><author>Vedar, M.</author><author>Calderon, N.</author><author>Trujillo, C.</author><author>Johnson, C.</author><author>Bradman, A.</author><author>Barr, D. B.</author><author>Eskenazi, B.</author></authors></contributors><edition>2011/04/22</edition><language>eng</language><added-date format="utc">1329668160</added-date><ref-type name="Journal Article">17</ref-type><auth-address>University of California-Berkeley, Center for Environmental Research and Children's Health, School of Public Health, Berkeley, California 94704, USA.</auth-address><remote-database-provider>NLM</remote-database-provider><rec-number>1045</rec-number><last-updated-date format="utc">1396923587</last-updated-date><accession-num>21507776</accession-num><electronic-resource-num>10.1289/ehp.1003185</electronic-resource-num><volume>119</volume></record></Cite></EndNote>] observed evidence of an association between prenatal exposures to OPs as measured by urinary DAP (total DAP, DEP, and DMP) metabolites in women during pregnancy, and decreased cognitive functioning in

children at age 7. In this study, children in the highest quintile of maternal DAP concentrations had a statistically significant 7-point difference in IQ points compared with those in the lowest quintile. The CHAMACOS results with the WISC-IV were furthered in Stein et al (2016) who evaluated potential impact from adverse learning environment. In contrast, to the findings of CCCEH, Mt. Sinai, and CHAMACOS, the HOME study (Donauer et al, 2016) reported evidence of a significant positive association between maternal levels of urinary metabolites (total DAP and total DM) and verbal IQ of the child from the WPPSI was observed in both the unadjusted and adjusted models. Although evidence of a positive association was observed for total DAP and total DM metabolites and full-scale IQ (unadjusted model: $\beta = 0.004$, $p = 0.111$; $\beta = 0.004$, $p = 0.124$, when the model was adjusted, no evidence of a positive association was observed (adjusted model: $\beta = 0.001$, $p = 0.494$; $\beta = 0.002$, $p = 0.441$).

As stated in the EPA neurotoxicity guidelines[9], direct extrapolation of developmental neurotoxicity results from laboratory animals to humans is limited by the lack of knowledge about underlying toxicological mechanisms and the relevance of these results to humans. EPA notes consistencies across the databases of *in vivo* laboratory animal studies and epidemiology studies, although challenges of making a direct comparison between neurodevelopmental domain inter-species remain. It can be assumed that developmental neurotoxicity effects in animal studies indicate the potential for altered neurobehavioral development in humans, although the specific types of developmental effects seen in experimental animal studies may not be the same as those that may be produced in humans. However, considering the toxicological and epidemiological data in the context of three major neurodevelopmental domains (specifically, cognition, motor control, and social behavior), insights can be gained. Previously reviewed studies of chlorpyrifos in rats and/or mice reported impaired cognition, changes in locomotor activity levels, altered social interaction, and to a lesser extent, changes in neuromotor function (FIFRA SAP 2012; USEPA, 2014). While there are fewer studies for all the other OPs, behavioral effects in the same functional domains were again reported. The most commonly reported outcome in the laboratory animal studies was cognitive dysfunction, and although it was overall consistent there were again differences in cognitive specificity, gender differences, or dose response. Quite a few studies also report changes in motor activity and sensory function in offspring, but there generally fewer laboratory animal studies that assess social interactions for OPs other than chlorpyrifos. It is notable that the laboratory animal studies vary in experimental designs such as species, strain, gender, dosing regimens (age, routes, vehicle), and test parameters (age, protocol). Likewise, observational epidemiology studies vary by population characteristics (race/ethnicity, SES, and pesticide use/exposure profile), co-exposures (mix of chemicals, windows of exposure), and method of exposure and outcome assessment. Given the differences across laboratory animal and epidemiology studies, the qualitative similarity in research findings is striking.

In contrast, quantitatively, there are notable differences between animals and humans. Specifically, in animals, the doses most often used in these studies are sufficient to elicit ≥10% brain and RBC inhibition depending on the study design, age of the animal, and sampling time.

[9] http://www.epa.gov/raf/publications/pdfs/NEUROTOX.PDF

In the epidemiology studies, based on the comparisons with biomonitoring data, reported AChE data from CHAMACOS and the results of the chlorpyrifos dose-reconstruction analysis, it is unlikely that RBC AChE would have been inhibited by any meaningful or measurable amount, if any at all, and most likely none in the brain. This key difference in dose response between the experimental toxicology and epidemiology studies poses challenges in interpreting such data. There are a number of possible hypotheses such as: 1) limitations of experimental laboratory studies which have limited statistical power due to relatively small sample sizes; 2) humans display a broader array of behaviors and cognitive abilities than rats, thus limiting the sensitivity of the rat studies; and 3) in the epidemiology studies, the timing of OP application and blood collections are not coupled—thus higher levels of blood OPs were likely missed.

3.2.3 Specificity

In considering the FQPA 10X Safety Factor, the statute indicates that both pre- and post-natal toxicity and exposure be considered. As such, it is appropriate to consider the degree specificity of with regard to lifestage. There are numerous animal studies in the literature which vary in their study design but all involve gestational and/or early postnatal dosing with behavioral evaluation from adolescence to adulthood. The data provide support for the susceptibility of the developing mammalian brain to chlorpyrifos exposure through gestation and early in life, with adverse outcomes in several neurological domains including cognitive, anxiety and emotion, social interactions, and neuromotor function. The studies have not shown that any specific developmental period is critical overall to the long-term outcomes, since similar effects are observed with different exposure periods. For example, cognitive changes in one laboratory using a radial arm maze were observed following gestational and early, but not late, postnatal exposure (Aldridge *et al.*, 2005; Icenogle *et al.*, 2004; Levin *et al.*, 2002; Levin *et al.*, 2001), whereas other laboratories cognitive deficits in a Morris water maze were reported following both gestational and late-postnatal exposure (Billauer-Haimovitch *et al.*, 2009; Turgeman *et al.*, 2011). Likewise, some changes in anxiety and social behaviors were reported at both gestational and postnatal exposure periods. Overall, these data do not clearly show specific critical periods of exposure but support a conclusion that early life (pre- and post-natal) represent susceptible lifestages.

As discussed above, numerous epidemiological investigations have observed a link between prenatal exposure to chlorpyrifos or OPs (measured as chlorpyrifos, TCPy, or DAPs) and adverse effects on neurodevelopment through age seven years, with additional more limited evidence up through approximately age eleven. As noted previously, for these epidemiology studies chlorpyrifos was only assessed directly in the CCCEH study, with the Mexican cohort study (Fortenberry *et al.*, 2014) assessing the chlorpyrifos metabolite TCPy, and the CHAMACOS, HOME, and Mt. Sinai cohorts focusing on the DAPs.

The majority of epidemiological studies investigated only prenatal exposures. Specifically, with respect to biomarkers representing prenatal exposures, CCCEH, Mt. Sinai, and CHAMACOS each reported evidence of impaired mental and psychomotor development, albeit not consistent by

age at time of testing (ranging from 6 month to 36 months across the three cohorts). Statistically significant or suggestive associations between chlorpyrifos or DAPs exposure and attentional problems/ADHD were reported by multiple prospective cohorts (Rauh *et al.*, 2006; Eskenazi *et al.*, 2007; Marks *et al.*, 2010; and Fortenberry *et al.*, 2014) with additional support from a case control study, Bouchard *et al.* (2010). In addition, the three US cohorts and the CHARGE study have reported suggestive or positive associations between OP exposure and autism spectrum disorders (Rauh *et al.*, 2006; Shelton *et al.*, 2014; Eskenazi *et al.*, 2007; Furlong *et al.*, 2014). While the studies from CCCEH, Mt. Sinai, and CHAMACOS studies vary in the magnitude of the overall strength of association, they have consistently observed a positive association between OP exposure and autism spectrum disorder. Several studies have also documented statistically significant association between prenatal DAP exposure and abnormal motor development in neonates (reflexes, Brazelton score or similar measure; Young *et al.*, 2005; Engel *et al.*, 2007; Zhang *et al.*, 2014). Finally, CCCEH, Mt. Sinai and CHAMACOS have reported an inverse relation between the respective prenatal measures of chlorpyrifos or DAPs and intelligence measures at age 7 years (Rauh *et al.*, 2011; Engel *et al.*, 2011; Bouchard *et al.*, 2011).

With regards to effects pre-natal compared to post-natal, a small number of studies have assessed postnatal exposure to DAPs. Postnatal exposure to DAPs has been assessed in the CHAMACOS cohort (Eskenazi *et al.*, 2007; Young *et al.*, 2005; Bouchard *et al.*, 2011) and three cross-sectional studies (Guodong *et al.*, 2012; Bouchard *et al.*, 2010; Oulhote and Bouchard, 2013). With the exception of Bouchard *et al.* (2010) and findings of PDD increase in post-natal DAP from CHAMACOS (Eskenazi *et al.*, 2007; Marks et al, 2010), no adverse neurodevelopmental associations were found between postnatal urinary metabolite levels and any of the developmental outcomes. Bouchard *et al.* (2010) looked at U.S. children age 8–15 years in the 2000–2004 National Health and Nutrition Examination Survey (NHANES), and observed a positive association between attention and behavior problems and total DAPs and DMAPs, but not DEAPs. Postnatal exposures have not been assessed in the CCCEH and Mt. Sinai studies (Rauh *et al.*, 2011; Engel *et al.*, 2011); as such, there are no studies included in this analysis which directly assessed the potential for postnatal chlorpyrifos exposure and associations with neurodevelopmental effects. However, given that the major source of exposure (residential use) was cancelled partway through the CCCEH study which substantially reduced and largely removed chlorpyrifos from the home environment, this limits the ability of the CCCEH study to inform the impacts of long-term postnatal exposure to chlorpyrifos on neurodevelopment from the current uses of chlorpyrifos.

In sum, given that the extensive experimental laboratory animal database suggests that the post-natal period is a potential susceptible time, the lack of postnatal exposure assessment in the CCCEH and Mt. Sinai studies is a source of uncertainty in the epidemiology database

3.3 Biological Plausability & Coherence

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EPA's cancer guidelines (2005) includes guidance which are also applicable to this current evaluation of OPs. In fact, the Guidelines indicate:

"*evaluation of the biological plausibility of the associations observed in epidemiologic studies reflects consideration of both exposure-related factors and toxicological evidence relevant to identification of potential modes of action (MOAs). Similarly, consideration of the coherence of health effects associations reported in the epidemiologic literature reflects broad consideration of information pertaining to the nature of the biological markers evaluated in toxicologic and epidemiologic studies*. [p. 39]."

The Cancer Guidelines further state that "*lack of mechanistic data, however, is not a reason to reject causality* [p. 41]."

At this time, a MOA(s)/AOP(s) has/have not been established for neurodevelopmental outcomes. This growing body of literature does demonstrate, however, that OPs are biologically active on a number of processes that affect the developing brain. Multiple *in vitro* studies on endpoints relevant to the developing brain have identified outcomes in picomole concentrations, including concentrations lower than those that elicit AChE inhibition *in vitro*.

- 1 pM chlorpyrifos oxon decreased axon length (~50%) in superior cervical ganglion cell cultures (AChE inhibition starting at 1000pM in same cell system): Howard et al., 2005
- 0.03 pM oxon (30 fM) increased in CREB levels (~50%) primary cortical neuron cultures (AChE inhibition starting at 100 pM in same cell system): Schuh et al., 2002
- 10 pM oxon decreased axon length (~40%) in dorsal root ganglion cell cultures (AChE inhibition starting at 100 pM in same cell system): Yang et al., 2008

With regard to coherence, there is a large body of *in vivo* laboratory studies which show long-term behavioral effects from early life exposure, albeit at doses which cause AChE inhibition. EPA considers the results of the toxicological studies relevant to the human population, as qualitatively supported by the results of epidemiology studies. The lack of established MOA/AOP pathway does not undermine or reduce the confidence in the findings of the epidemiology studies. When all the evidence is considered together, there are uncertainties with respect to a number of factors such as exposure assessment, lack of ability to make strong causal linkages, and unknown window(s) of susceptibility. The epidemiology studies reviewed in the 2012/2014 and 2015/2016 literature reviews represent different investigators, locations, points in time, exposure assessment procedures, and outcome measurements. Despite all these uncertainties and differences in study design, multiple investigators have identified associations with neurodevelopmental outcomes such as ADHD/behavioral problems and autism spectrum, in relation to OP exposure. There is evidence of delays in mental development in infants (24-36 months), attention problems and autism spectrum disorder in early childhood, and intelligence decrements in school age children who were exposed to OPs during gestation. Investigators reported strong measures of statistical association across several

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of these evaluations (odds ratios 2-4 fold increased in some instances), and observed evidence of exposures-response trends in some instances, *e.g.*, intelligence measures. Thus, with respect to biological plausibility and coherence, although uncertainties remain, these uncertainties are diminished in the context of the qualitative similarity between the epidemiology studies.

4.0 10X FQPA Safety Factor for Infants and Children

As section 408(b)(2)(C) of the FFDCA instructs EPA, in making its "reasonable certainty of no harm" finding, that in "the case of threshold effects, an additional tenfold margin of safety for the pesticide chemical residue and other sources of exposure shall be applied for infants and children to take into account potential pre- and postnatal toxicity and completeness of data with respect to exposure and toxicity to infants and children." Section 408 (b)(2)(C) further states that "the Administrator may use a different margin of safety for the pesticide chemical residue only if, on the basis of reliable data, such margin will be safe for infants and children." Given the totality of the evidence, there is sufficient uncertainty in the human dose-response relationship for neurodevelopmental effects which prevents the agency from reducing or removing the statutory 10X FQPA Safety Factor. For the human health risk assessments for the OPs, a value of 10X will be applied. Similarly, a database uncertainty factor of 10X will be retained for occupational risk assessments. The agency will continue to evaluate the epidemiology studies and pursue approaches for quantitative or semi-quantitative comparisons between doses which elicit AChE inhibition and those which are associated with neurodevelopmental outcomes prior to a revised human health risk assessment. **The FQPA 10X Safety Factor or database uncertainty factor will be retained for OPs for the population subgroups that include infants, children, youths, and women of childbearing age for all exposure scenarios.**

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6.0 Appendices

1. Table of *In Vivo* Developmental Neurotoxicity Studies of OPs.
2. Summary of Guideline DNT Studies Submitted to the Agency for OPs other than Chlorpyrifos.
3. Low Quality Studies: Summary of Study Design Elements Impacting Study Quality Assignment
4. Table of Systematic Review Analysis: Initial Search Research & Initial Exclusion Criteria
5. Table of Systematic Review Analysis: Second Tier Exclusion Criteria
6. Plausible hypotheses on MOA/AOP for neurodevelopmental outcomes (Extracted from Section 4.4.3. RHHRA for chlorpyrifos)

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Appendix 1. Table of *In Vivo* Developmental Neurotoxicity Studies of OPs.

(Effects described are only those measured after weaning. Bold indicates functional domains that were reported to show treatment effects.)

OP	Study	Species & strain	Dose, route, vehicle	Dosing period	ChE inhibition	Domain	Age of testing	Outcomes	NOEL, LOEL	Notes & Study Problems
Chlormephos	Ceh *et al.*, 2012	mouse BALB/c	3.5, 0.35 ug/ml in drinking water of dams ~ 0.6, 0.06 mg/kg/d (@ 5ml/d, 30 g)	7 day premating to weaning	No	**Anxiety & Emotion**	PND70-80	Increased time in closed arms & decreased time in open arms in elevated plus maze, ~0.6 mg/kg/d, M&F	NOEL=0.35 ug/ml in water, ~0.06 mg/kg/d	Litter not unit of statistical analysis No pup allocation described but had to have used some littermates M & F responses appear similar but not statistically compared
Diazinon	Roegge *et al.*, 2008	rat Sprague Dawley	0.5, 2 mg/kg/d sc DMSO	PND1-4	Cites Slotkin *et al.* (2006): 0.5 mg/kg/d produced <10% brain inhibition on PND5, 2 mg/kg/d produced 25-30% brain inhibition 2 hr after dose on PND4, and 10-20% inhibition on PND5	**Anxiety & Emotion**	PND52-56	Decreased time in open arms in elevated plus maze, 2 mg/kg/d, M only	No NOEL LOEL=0.5 mg/kg/d	Pups & dams redistributed daily No effect in F Accepts p<0.1 as significant for interactions No dose-response in feeding or milk preference studies Abstract misstates sex differences
							PND64-67, 78-79	Decreased latency to eat in novelty suppressed but not home-cage feeding, 0.5 & 2 mg/kg/d, M only		
							PND73-74	Decreased chocolate milk preference, 0.5 mg/kg/d only, M only		
							PND86-87	No effect on forced swim test		
Diazinon	Spyker and Avery, 1977	mouse F2 hybrid (NCTR cross bred)	0.18, 9 mg/kg/d in peanut butter	GD1-birth	No	**Sensory**	PND38	Increased errors on visual cliff, 0.18 mg/kg/d only, F only No effect acoustic startle or olfactory responses	No NOEL LOEL=0.18 mg/kg/d	No pup allocation described but had to have used littermates Not clear when both sexes tested and/or compared Statistics not described Maternal weight gain lowered at both doses Weight gain of high dose pups decreased Preweaning testing: decreased contact placing, 0.18 mg/kg/d only No dose-response for some measures Twice as many controls as treated Looks like decreased rotarod endurance PND65, not significant due to high variability
						Neuromotor	PND50, 60, 65,75	No effect swimming ability Increased rod cling endurance, 0.18 & 9 mg/kg/d, sex not specified Decreased inclined plane performance, 0.18 & 9 mg/kg/d, sex not specified		
						Activity	PND75-76	No effect open field		
						Cognition	PND87	No effect errors in Lashley maze		
Diazinon	Timofeeva *et al.*, 2008	rat Sprague Dawley	0.5, 2 mg/kg/d sc DMSO	PND1-4	Cites Slotkin *et al.* (2006): 0.5 mg/kg/d produced <10% brain inhibition on PND5, 2 mg/kg/d produced	Activity	PND28-42	No effect in figure-8 chamber, M&F	No NOEL LOEL=0.5 mg/kg/d	Littermates of those used in Roegge *et al.* 2008 Pups & dams redistributed daily Accepts p<0.1 as significant for interactions
						Sensory	PND77-84	Decreased prepulse inhibition, 0.5 & 2 mg/kg/d, M only		

ED_002896_00064133-00112

					25-30% brain inhibition 2 hr after dose on PND4, and 10-20% inhibition on PND5	**Cognition**	PND28-35, 91-126	No effect on T-maze spontaneous alternation, M&F Increased working memory errors, 0.5 mg/kg/d only, M&F		No mention of sex effects in T-maze No dose-response for some measures
Diazinon	Vatanparast *et al.*, 2013	rat Wistar	1 mg/kg/d sc DMSO	GD15-18 or PND1-4	No	Activity	PND60	Gestational: No effect in open field, M&F Postnatal: No effect in open field, M&F	No NOEL LOEL=1 mg/kg/d	1 M & 1 F per litter but sex not nested within litter in statistics F only affected with gestational exposure, both sexes affected with postnatal, looks like M more affected Large effect sizes Number of dams not mentioned Discrepancy in text on pup sample sizes
						Cognition	PND60-63	Gestational: Decreased latency to cross and increased time spent in dark side on retention trial, no effect acquisition in passive avoidance, F only Postnatal: Decreased latency to cross and increased time spent in dark side on retention trial, no effect acquisition in passive avoidance, M&F		
Diazinon	Win-Shwe *et al.*, 2013	mouse C3H/HeN	0.5, 5 mg/kg/d sc DMSO	PND8-11	No	**Cognition**	PND46-49, 81-84	PND46-49: Decreased novel object exploration and discrimination, 0.5 & 5 mg/kg/d PND81-84: Decreased novel object exploration and discrimination, 5 mg/kg/d only	No NOEL LOEL=0.5 mg/kg/d	M only tested Separate mice at two test times Litter allocation to dose group not described Assumes that ChE inhibition reported by Slotkin in rats would be same as in mice Larger sample size at later age
Dichlorvos	Lazarini *et al.*, 2004	rat Wistar	8 mg/kg po (dilution of technical product); vehicle from formulation	GD6-15	No	**Activity**	PND21, "adult"	PND21: Decreased locomotion open field, M only "Adult": Decreased locomotion and increased immobility, only M tested	No NOEL LOEL=8 mg/kg/d	Data analyzed as litter but sex not nested within litter in statistics No effect on physical and reflex preweaning development Only M tested as after PND21 Adult age not given
						Cognition	"Adult"	Decreased latency to cross on retention trial in passive avoidance		
Fenitrothion (sumithionR, 50% ai)	Lehotzky *et al.*, 1989	rat Lati	5, 10, 15 mg/kg/d po sunflower oil	GD7-15	No	**Neuromotor**	PND26, 36, 104	Decreased latency to fall off rotarod PND26, 104, not 36, 15 mg/kg/d	NOEL=5 mg/kg/d LOEL=10 mg/kg/d	Postnatal mortality at all doses (16-17.5%) No pup allocation described but had to have used some littermates Only M tested Statistics not described Measured startle, righting, contact placing on PND22 but no results given Shorter latency in cognitive task hard to interpret Nonsignificant decrease in activity at PND26
						Activity	PND26, 36, 104	Decreased activity in open field PND104, 15 mg/kg/d		
						Cognition	PND42, 104	Shorter escape latency in conditioned response during acquisition, 10 & 15 mg/kg/d		
						Social behavior	PND62	Increased time in social interaction, 10 & 15 mg/kg/d		

Methamidophos	deCastro *et al.*, 2000	rat Wistar	1 mg/kg/d po water	GD6-15	Pilot in nonpregnant F dosed for 10 d gives 17% plasma inhibition at 1 mg/kg/d	Activity	PND40	No effect in open field, sex no mentioned	NOEL=1 mg/kg/d	Used 2 pups/litter but no mention of sex, apparently used as independent observations No effect on preweaning swimming performance Decreased immobility time PND 14 only Open field measures with really high variability, not reliable
Methamidophos	Lima *et al.*, 2013	mouse Swiss	1 mg/kg/d sc DMSO	PND3-9	Pilot showed for 1 mg/kg/d: ~19% brain inhibition on PND10; ~36%, 46% brain inhibition 1, 4 hr after dosing on PND3; ~53, 61% brain inhibition 1, 4 hr after dosing on PND9; no brain inhibition in PND60 adults	Activity	PND61	No effect in open field, sex not mentioned	No NOEL LOEL=1 mg/kg/d	1 M & 1 F per litter No data for M & F separately or mention of statistical differences Dosing by litter High variability especially with passive avoidance
						Anxiety & Emotion	PND60-61	Increased immobility time in forced swim, sex not mentioned No effect on elevated plus maze, sex not mentioned		
						Cognition	PND63	No effect on passive avoidance, sex not mentioned		
Methyl parathion	Crowder *et al.*, 1980	rat Sprague Dawley	1 mg/kg/d po corn oil	GD7-15	No	**Activity**	PND23, 30, 44, 54, 65	Increased activity in open field, only PND23 and 54, sex not mentioned to criterion	No NOEL LOEL=1 mg/kg/d	Preweaning testing: possibly decreased wire cling time (not analyzed), no effect on righting, startle, placement response Increased postnatal mortality (30%) Littermates used Only 3 litters used Small sample size for maze testing Statistics not mentioned except for maze transfer test, just used t-test Sex not mentioned except for maze transfer test, data not given for M & F Methods & results cursory
						Cognition	>PND68	Slower transfer on 1st, 4th direction change in T-maze learning transfer, sex not mentioned		
Methyl parathion	Gupta *et al.*, 1985	rat Wistar-Furth F mated with F344 M	1 mg/kg/d in peanut butter, 1.5 mg/kg/d po peanut oil	GD6-20	Dams on GD19 show 20, 60% brain inhibition at 1, 1.5 mg/kg/d Pups show brain inhibition up to 50% on PND1, 7, 14, 21, 1 & 1.5 mg/kg/d; on PND28 only 1.5 mg/kg/d	**Cognition**	PND60	No effect on passive avoidance No effect on shuttle box avoidance Slower latency to bar press & increased days to asymptote on operant task (no schedule given), 1 mg/kg/d only, sex not mentioned	No NOEL LOEL=1 mg/kg/d but no effects at 1.5 mg/kg/d	High dose dams had cholinergic signs, increased resorptions Pups moved to foster mothers at 24 hr No effect on preweaning reflexive behaviors Pup allocation not described Statistics barely described M & F apparently tested but data for each not shown or mentioned Methods cursory No dose-response for behavior but there is dose-response for ChE inhibition
						Neuromotor	PND60	No effect on rotarod		
						Activity	PND60	Decreased activity, 1 mg/kg/d only, sex not mentioned		
						Anxiety & Emotion	PND60	Faster cage emergence, 1 mg/kg/d only, sex not mentioned		

						Sensory	PND120	No effect on acoustic startle response		Only 4/dose for operant testing
Methyl parathion	Johnson *et al.*, 2009	rat Sprague Dawley	Incrementing doses: low 0.2 mg/kg/d throughout; mid 0.2, 0.4, 0.6 mg/kg/d every 5-6 d; high 0.3, 0.6, 0.9 mg/kg/d every 5-6 d	PND1-21	Low dose showed 13-15% brain inhibition and high dose showed 63, 20, 18% brain inhibition on PND20, 30, 40; all doses recovered by PND50	**Cognition**	PND29-60	Increased working memory errors, mid and high dose, M only Increased reference memory errors, all doses, M only	No NOEL LOEL=0.2 mg/kg/d	Split-litter dose design Included litter as random effect in statistics No effect on preweaning measures of reflex development No effect in F
Oxydemeton methyl (metasystoxR, 91% ai)	Clemens *et al.*, 1990	rat CD	0.5, 1.5, 4.5 mg/kg/d water	GD6-15	Dams on GD16 show 30, 54, 72% plasma inhibition (RBC similar) & 22, 52, 68% brain inhibition at 0.5, 1.5, 4.5 mg/kg/d Dams on GD20 show 20, 39, 54% brain inhibition at 0.5, 1.5, 4.5 mg/kg/d, 40% RBC inhibition at 4.5 mg/kg/d, and no plasma inhibition Fetuses on GD20 show no brain inhibition	Cognition	PND25, 26, 35	No effect on M-maze	NOEL=4.5 mg/kg/d	High dose dams had tremors 1 M & 1 F per litter No data for M & F separately or mention of statistical differences Statistics barely described No effect on preweaning reflex or sensory tests
						Activity		No effect in open field		
Parathion	Al-Hachim & Fink, 1968	mouse CF1	3 mg/kg/d po Corn oil	3 dosing times: 1st, 2nd, or 3rd trimester	No	Cognition	PND30-37	No effect on conditioned avoidance learning	NOEL=3 mg/kg/d	Very similar experiment as other papers, maybe same study Pup allocation not clear but littermates probably used Inadequate statistics No mention of sex
Parathion	Al-Hachim & Fink, 1968	mouse CF1	3 mg/kg/d po Corn oil	3 dosing times: 1st, 2nd, or 3rd trimester	No	Activity	PND60-66	No effect in open field	NOEL=3 mg/kg/d	Very similar experiment as other papers, maybe same study Pup allocation not clear but littermates probably used Inadequate stats No mention of sex
Parathion	Levin *et al.*, 2010	rat Sprague Dawley	0.1, 0.2 mg/kg/d sc DMSO	PND1-4	Cites Slotkin *et al.* 2006: 0.1 mg/kg/d produced 5-15% brain inhibition on PND5, no data for 0.2 mg/kg/d	**Cognition**	PND420, 510, 570	PND420: Increased working memory errors in radial arm maze, 0.1 mg/kg/d, M only; increased reference memory errors, 0.1 & 0.2 mg/kg/d, M only PND510: Increased working memory errors in radial arm maze, 0.1 & 0.2 mg/kg/d, M only PND570: No effect in radial arm	No NOEL LOEL=0.1 mg/kg/d	Littermates of those used in Timofeeva 2008 Pups & dams redistributed daily 5% mortality high dose No effect in F Accepts p<0.1 as significant for interactions No dose-response for several measures

								maze		
Parathion	Stamper *et al.*, 1988	rat Long Evans	1.3, 1.9 mg/kg/d sc corn oil	PND5-20	35, 68% brn inh PND21; 26, 36% brn in PND28	Activity	PND24	No effect in open field	No NOEL LOEL=1.3 mg/kg/d	Split-litter dose design Pup allocation not clear but littermates probably used High dose produced cholinergic signs, says doses are 33 and 50% of LD50 in PND5 rat Decreased weight gain with both doses Preweaning, increased cliff avoidance latency, no effect righting, negative geotaxis, open field M only tested No post-hoc comparison of groups when significant, but looks like effects in both doses No dose-response in working memory errors
						Neuromotor	PND24	No effect on rotarod		
						Cognition	PND24, PND 35-37	Decreased alternation rate in T-maze, 1.3 & 1.9 mg/kg/d, only M tested Increased working memory errors, 1.3 & 1.9 mg/kg/d, only M tested		
Parathion	Timofeeva *et al.*, 2008	rat Sprague Dawley	0.1. 0.2 mg/kg/d sc DMSO	PND1-4	Cites Slotkin *et al.* 2006: 0.1 mg/kg/d produced 5-15% brain inhibition on PND5, no data for 0.2 mg/kg/d	Activity	PND58-61	No effect in figure-8 chambers	No NOEL LOEL=0.1 mg/kg/d	Pups & dams redistributed daily 5% mortality high dose Accepts p<0.1 as significant for interactions All radial arm maze effects only in low dose
						Cognition	PND35-45 PND112-182	No effect in T-maze spontaneous alternation Decreased working memory errors in radial arm maze, 0.1 mg/kg/d only, M&F		
						Anxiety & Emotion	PND50-53 PND64-72 PND81-94	Increased time in open arms in elevated plus maze, 0.2 mg/kg/d, M&F No effect on novelty suppressed feeding No effect on chocolate milk preference		
						Sensory	PND78-81	Decreased tactile startle, 0.2 mg/kg/d, M&F No effect on prepulse inhibition		

Appendix 2. Summary of Guideline DNT Studies Submitted to the Agency for OPs other than Chlorpyrifos.
(Only changes that were observed after exposure had ended (post weaning, adult) are listed. 'X' indicates no significant changes on tests for each domain.)

Chemicals	Cognition	Motor activity	Acoustic startle	Neuromotor (FOB)	Notes
Acephate	X	X	X	X	
Azinphos-methyl	X	X	X	X	
Coumaphos	X	X	X	X	
Diazinon	Biel maze: increase errors & latency high dose (~33.1 mg/g,/d, dam diet) M, PND24 & PND62; also mid dose (~3.4 mg/kg/d, dam diet) F, PND24	X	X	X	
Dichlorvos[1]	--	--	--	--	
Dicrotophos	X	X	X	X	
Dimethoate	X	X	X	X	
Disulfoton	X	X	X	X	
Ethoprop	M maze: increase trials to criterion high dose (~29.3 mg/kg/d, dam diet) M, PND60	X	X	X	
Fenamiphos	X	X	X	X	
Malathion	X	increased rearing in FOB open field mid dose (50 mg/kg/d to dams & pups), F, significant at PND45 only (maybe also PND60); no change automated motor activity	increased peak amplitude later blocks (perhaps habituation effect) all doses (5, 50, 150 mg/kg/d to dams & pups), F only, PND23; increased peak amplitude without prepulse low dose only, F only, PND60	altered gait mid & high dose (50, 150 mg/kg/d to dams & pups), M & F, PND60 but not earlier	
Methamidophos	~X	X	decreased peak amplitude early blocks mid & high dose (~1.65, 5.2 mg/kg/d dam diet), F only, significant at PND38, looks same but not significant at PND60	X	PA PND24: report says decreased latency but nothing significant & table is questionable; M maze PND60: report says increased trials to criterion (M) and increased (M) or decreased (F) errors, but nothing significant and table is questionable
Methyl parathion	X	X	X	X	

Naled	X	X	increased peak amplitude & decreased latency middle blocks low dose only (0.4 mg/kg/d to dams & pups), F only, PND60; report says decreased amplitude but not significant all blocks high dose (10 mg/kg/d to dams & pups), M only, PND23 & PND60	X	only swimming time in Y maze reported, varied significances, no mention of errors or other performance measures
Phorate	M maze: decrease number reaching criterion with relearning, low & mid dose (0.03 & 0.1 mg/kg/d to dams & pups), M only significant, PND30; not seen in second study with higher dose	X	decreased peak amplitude all blocks high dose (0.1 mg/kg/d to dams & pups), M only, PND60; not seen in second study with higher dose	X	combined two studies; one with low & mid dose, other with high dose; some data didn't agree
Profenofos	X	X	X	X	
Terbufos	X	X	X	X	
Tetrachlorvinphos	X	X	X	X	
Tribufos	X	X	X	X	
Trichlorfon	PA: decreased latency to enter on retention high dose (~205.1 mg/kg/d dam diet), M only, PND29; M maze: increased average errors second trial high dose, F only, PND60	decreased activity middle blocks (maybefaster habituation) mid dose (~76.2 mg/kg/d dam diet) only, F only, PND60	decreased peak amplitude all blocks high dose (~205.1 mg/kg/d dam diet), early blocks mid dose (~76.2 mg/kg/d dam diet), M & F, PND22; decreased amplitude middle blocks (not consistent) all doses (~23.3, 76.2, 205.1 mg/kg/d dam diet), F only, PND38; for M decreased amplitude apparent but not significant high dose PND38 & PND60	X	

[1] high pup mortality in all groups, including control, negated any valid neurotoxicity assessments of dichlorvos

Appendix 3. Low Quality Studies: Summary of Study Design Elements Impacting Study Quality Assignment

Study	Study Location	Cohort Name / Description of Study Population	Study Design	Exposure Assessment	Outcome Assessment	Confounder / Covariate Control	Statistical Analysis	Risk of (other) Bias
Acosta-Maldonado *et al*. (2009)	Chihuahua, Mexico	Singleton pregnancies	Cross-sectional, small pilot study – only 9 women exposed, participant selection and exclusion not detailed N=54 mothers (9 exposed, 45 comparison mothers)	Proxy indicator of exposure - Residence in agricultural community were pesticides had been applied; or home located < 5 km from a pesticide application zone; or cohabitating with worker exposed to pesticides or agricultural labor Also, AChE activity – Objective biomarker of exposure/altered function	Standardized but partially subjective assessment of placental maturity	Minimal. Adjustment for placental characteristics.	Appropriate multivariate analysis; Corrected hypothesis test results for multiple comparisons.	Selection bias unlikely; Residual confounding likely; substantial potential for differential misclassification of exposure.
Dawbrowski *et al*. (2003)	Lodz, Poland	Newborn children among Polish farmers	Case-control, large sample size N=389 Age: newborns	Prenatal pesticide exposure assessed via questionnaire (retrospective self-report). Site visit *after* delivery to evaluate pesticide exposure	Pregnancy outcomes assessed using birth records	Appropriate. Included maternal demographics, predictors of high-risk pregnancy (duration, maternal weight) and environmental toxicant exposure (ETS). No adjustment for SES indicators	Appropriate multivariate analysis.	Selection bias unlikely; Residual confounding likely small in magnitude; considerable potential for non-differential misclassification of exposure (unlikely to account for non-null findings). Minimal misclassification of outcome.
Cartier et al. (2016)	Brittany, France	PELAGIE study (French population; pregnant women and children)	Longitundinal birth cohort n = 231 Mother-infant pairs	Metabolite concentrations (total DAP, DM, and DE) of prenatal OP pesticide exposure quantified in two maternal spot urine samples provided at 16 and 26 weeks of gestation Maternal IQ and the family environment were also assessed using the Wechsler Adult Intelligence Scale and the HOME test	Wechsler Intelligence Scale administered to children to determine IQ	All models were adjusted for HOME score, breastfeeding duration, mothers' IQ, school, maternal education level, psychologist testing the child, and creatinine levels of mother and child	Appropriate: Linear regression and multivariate models	Urine collection methods and storage (urine samples collected at home and mailed back to laboratory); lack of frequent urine spot samling and due to the short half-life of urinary metabolites could have led to exposure misclassification
Fielder et al. (2015)	Bangkok, Thailand area	Thai population (Children aged 6 to 8, who were from two farming regions (aquaculture v. rice)	Cohort, small sample size n = 54 Age 6- 8 years	Child Exposure – Total DEAP, DAP, and DMAP metabolites measured in spot urine samples, collected at three, separate time points (every 6 months) Parental education was assessed via a vocabulary test; the home environment was scored via the HOME test	BARS examination administered to children to determine neurobehavorial performance.	All models were adjusted for age and the HOME scale	Appropriate: Multivariate linear regression model and mix model linear regression to determine interaction	Small sample size (n = 54); errors in exposure classification methods, specifically, urine collection methods and storage (urine samples collected at home); short half-life of urinary metabolites could have led to exposure misclassification
Grandjean *et al*.(2006)	Tabacundo, Ecuador	Healthy 2nd and 3rd grade children	Cross-sectional, small sample size N=72 Age <9 years	Prenatal occupational exposure assessed via questionnaire; Also recent child exposure biomarker assessment (DAP).	Objective anthropometric and other clinical outcomes; Numerous neurobehavioral outcomes evaluated using easy-to-administer screening instruments; Age appropriate. May be insensitive to subtle effects of OP pesticide exposure effects.	Appropriate. Homogenous population limited confounding by design; Included child demographics (age, sex, weight); SES indicators (maternal race, housing, running water, sewage), diet (meals/day), environmental toxicants (maternal alcohol and smoking) and medical history.	Appropriate multivariate analysis; Numerous hypotheses evaluated without correction for multiple comparisons.	Selection bias unlikely; Residual confounding likely small in magnitude; considerable potential for non-differential misclassification of exposure (likely to account for null findings). Potential misclassification of outcome.

Study	Study Location	Cohort Name / Description of Study Population	Study Design	Exposure Assessment	Outcome Assessment	Confounder / Covariate Control	Statistical Analysis	Risk of (other) Bias
Harari *et al.* (2010)	Tabacundo, Ecuador (Andean plateau north of Quito)	6- to 8-year-old children in the two lowest grades (called second and third) of one of two schools	Cross-sectional, small sample size n = 81 Age 6- 8 years	Child Current Exposure - DMP metabolites measured in a spot urine samples. A blood sample was analyzed for AChE. Maternal Exposure – Interview by skilled interviewers	Blood pressure and neurophysiologic measures - the instruments used for neurophsychologic measures were validated to avoid cross-cultural influences.	Appropriate: child's sex, age, BMI, number of daily meals (only in current exposure), stunting, hematocrit, school grade, having repeated one grade, maternal education level, family living in a traditional house, drinking water supply, and paternal education and employment	Appropriate: Standard parametric tests and logistic regression	Errors in exposure classification status since it was based on the maternal self-report, mothers likely being aware of the neurobehavioral status of their children, exposure assessment based on a spot urine sample
Kofman *et al.* (2006)	Israel (Negev region)	Bedouin population (Children aged 6 to 12 at the time of the study, who were victims of poisoning before age three)	Retrospective cohort study, small sample size N = 52 9-Exposed to OP; 17-Exposed to Kerosene/paint thinner 26-Controls Aged 6-12 years	OP poisoning was confirmed by low serum butyrylcholinesterase activity based on hospital records	Neuropsychological evaluation and structured interview of parents. Errors in assessment minimized as psychologists were qualified individuals, language and cultural differences taken into consideration, each child tested on same day and in same place as matched control.	Age, sex, background (cultural and demographic)	Difference in means	Errors is outcome classification likely since psychologists who administered the tests knew which children were exposed, small sample size
Koutroulakis *et al.* (2014)	Crete, Greece	Women with singleton pregnancies, permanent residents for at least two years, referral for amniocentesis to the Fetal-Maternal Unit, Department of Obstetrics and Gynecology, University Hospital of Heraklion	Prospective Cohort Study – large sample size, ethical issues n = 415 Age: newborns	Objective. DAP measurement in a single amniotic fluid samples collected at either 16th or 20th weeks of gestation – Novel biomarker. Questionnaire was also used.	Birth weight and head circumference. Unclear how the outcome information was obtained.	Neonatal sex, maternal age, agricultural activities, and gestational age at amniocentesis – Rationale for confounder selection not provided	Appropriate: multiple linear regression	Comparing exposure measurements in AF against known biomarker (e.g., OP metabolite levels in urine) for validation of AF not conducted. Smoking status of participants before/during pregnancy, high risk pregnancies, gestational diabetes, PON1 enzyme activity in the fetuses not considered.

Appendix 3. Low Quality Studies: Summary of Study Design Elements Impacting Study Quality Assignment (continued)

Study	Study Location	Cohort Name / Description of Study Population	Study Design	Exposure Assessment	Outcome Assessment	Confounder / Covariate Control	Statistical Analysis	Risk of (other) Bias
Lizardi *et al*. (2008)	Yuma County, Arizona, USA	Children's Pesticide Survey (CPS)	Cross-sectional, small sample size N=48 Age 7 years	Objective Biomarker of prenatal OP pesticide exposure (DAP) quantified in single child spot urine sample provided at the time of the cognitive assessment.	Cognitive assessment using battery of test instruments considered valid and reliable in similar populations. Spanish translation as necessary.	None	Correlation coefficients (type unspecified). Statistically significant confounders were not robust due to influential outliers	Selection bias unlikely; Residual confounding likely; considerable potential for non-differential misclassification of exposure Potential misclassification of outcome.
Lu *et al*. (2009)	Cota Brus, Costa Rica	4-10 yr. old children whose parents worked in organic coffee farm (La Amistad) and conventional coffee farms (Las Mellizas)	Cross-Sectional (pilot), low sample numbers N=35: 17 Organic farm 18 Conventional farms Age 4-10 years old	Good measure (urinary PNP, IMPY, TCPy), but no major differences between exposure groups	Good measure (CBARS) but different SES and demographic characteristics for exposure groups	Limited number (group, age, sex, handedness, grade)	Appropriate: Linear mixed effects for significant test outcomes from paired t-test analyses	Somewhat high - Convenience sample with different recruitment methods; exposure misclassification
Moreno-Banda *et al*. (2009)	Mexico (Villa Guerrero, Coatepec de Harinas, Tenancingo (Mexico); Cuernavaca, Cuautla, Jiutepec, Temixco, (Morelos)	Newborn children of floricultural workers and families	Cross-sectional, large sample size N=328 Age: newborns	Proxy indicator of prenatal occupational OP pesticide exposure (self-reported floricultural occupation)	Objectively measured birth outcomes assessed using birth certificate (partial). Self-reported by mother if birth certificate unavailable.	Appropriate, though minimal – history of adverse reproductive outcomes, infant sex, maternal smoking and alcohol use during pregnancy.	Appropriate multivariate analysis	Selection bias likely; Residual confounding likely small in magnitude; considerable potential for non-differential misclassification of exposure (likely accounts for null findings). Potential outcome misclassification (self-report in subset of participants).
Nevison (2014)	U.S. National population	Children with birth years 1970–2005 in 1) California Department of Developmental Services (CDDS) reports 2) Individuals with Disabilities Education Act (IDEA) reports	Ecological (time trend) N=NP (national database)	Non-specific, proxy OP exposure measure (lbs/yr)	Two large reporting DBs: CA Department of Developmental Services (CDDS), US Individuals with Disabilities Education Act (IDEA)	Limited: differences in autism definitions, changes in diagnostic criteria	Appropriate: ratio of age-resolved snapshot; tracking trend slopes; correlation coefficient between temporal trend and composite autism prevalence curve	Selection bias unlikely; do not know individual exposure

Appendix 3. Low Quality Studies: Summary of Study Design Elements Impacting Study Quality Assignment (continued)

Study	Study Location	Cohort Name / Description of Study Population	Study Design	Exposure Assessment	Outcome Assessment	Confounder / Covariate Control	Statistical Analysis	Risk of (other) Bias
Rohlman *et al*. (2005)	Oregon and North Carolina, USA	Latino children of immigrant parents living in Oregon or North Carolina	Cross-Sectional, small sample size N= 78 Age 48-71 months	Proxy indicator of chronic OP pesticide exposure - Residence in highly agricultural communities	Battery of neurocognitive development; Screening tools; Some instruments likely not appropriate for use in study population - Not all participants able to complete all evaluations. Poor administration. Tests administered twice; only performance on 2nd evaluation considered in analysis.	Self-reported covariate information collected via questionnaire. Adjustment for age, SES indicator (maternal education). No environmental toxicants.	One-sided hypothesis tests. Numerous hypotheses evaluated without correction for multiple comparisons.	Selection bias unlikely; Residual confounding likely; substantial potential for differential misclassification of exposure and outcome. Inadequate presentation of study results. Seemingly post-hoc evaluation of effect modification.
Samarawickrema *et al*. (2008)	Southern Sri Lanka	Pregnant women delivering at Embilipitiya Base Hospital	Cross-sectional Birth Cohort, small sample size N=41 end of two pesticide spraying seasons; N=25 at beginning of spraying season	Proxy indicator of prenatal OP pesticide exposure (delivery during pesticide spray season); Objective pesticide biomarkers assessed (OP pesticide residues), but detected in only two subject's specimens – not evaluated	Objective biomarkers of early biological effect outcomes - Maternal and fetal butyrylcholinesterase (BuChE) activity; antioxidant status; fetal oxidative stress; fetal DNA fragmentation	No adjustment for potential confounders (though comparison groups were considered to be relatively homogeneous).	Largely appropriate. Assumptions of some statistical tests likely violated.	Selection bias unlikely; Residual confounding likely; substantial potential for differential misclassification of exposure; Seemingly post-hoc evaluation of effect modification.
Savitz *et al*. (1997)	Ontario, Canada	Newborn children in the Ontario Farm Family Health Study;	Retrospective Birth Cohort, large sample size N= 1,898 couples; 3,984 Age: newborns	Proxy indicator of pre-conception paternal para-occupational OP pesticide exposure (self-reported male farm activities in 3-month period prior to conception.	Objectively measured birth outcomes assessed by maternal self-report	Appropriate. Included family/child demographics (sex, weight, maternal age, ethnicity); SES indicators (maternal and paternal education and occupation, per capita income) race, housing, running water, sewage), diet (meals/day), pregnancy risks (maternal caffeine, alcohol and smoking) and medical history.	Inappropriate multivariate regression analysis. Likely misspecification of true variance; No adjustment for multiple comparisons.	Selection bias probable; Residual confounding likely; substantial potential for differential misclassification of exposure and outcome.
Wickerham *et al*. (2012)	Zhejiang Province, China	Newborn children delivered at the Fuyang Maternal and Children's hospital	Cross-sectional, small pilot study n=116 Age: Full term infants	Objective biomarker of pesticide exposure (pesticide residues in cord blood) – parameterized as number of pesticide residues detected. Methods unlikely suitable for detecting low levels.	Birth weight assessed using birth records and maternal report	Appropriate. Assessed using questionnaire (maternal self-report) and medical records.	Appropriate multivariate analysis.	Selection bias unlikely; Residual confounding likely; substantial potential for differential misclassification of exposure. Some outcome misclassification unlikely .
Naksen et al (2015)	Fang district, Chiang Mai province, Thailand	Pregnant women delivering at Fang Hospital	Prospective Cohort, small pilot study n=52 Age: newborns	Objective biomarkers of pesticide exposure (DAPs). Also AChE, BChE, and PON1 genotype expression measurement. Maternal blood and urinary samples taken, plus cord blood. Questionnaire to assess other exposures and covariates.	Birth outcomes (Body weight and length, and head circumference) abstracted from medical records.	Appropriate. Assessed using questionnaire (maternal self-report) and medical records.	Some errors in statistical analysis were identified; e.g., for gestation age, log total DEAP at 32 weeks of pregnancy, a 0.7 beta was reported, but the confidence interval is reported as (-0.1, -1.4)]. No adjustment for multiple comparisons.	Inadequate presentation of study results. Selection bias possible due to loss to follow-up; Residual confounding likely, small in magnitude; potential for non-differential misclassification of exposure.

Appendix 4. Table of Systematic Review Analysis: Initial Search Research & Initial Exclusion Criteria.

Initial search yielded 299 studies. Removing duplicates (56), there were 243 articles, and 79 were determined to be epidemiological investigations of potential relevance. The 164 studies excluded from the analysis comprised 57 exposure only studies; 51 review articles; 33 reports of acute OP intoxication; 20 studies in non-human systems; and 3 were otherwise not relevant).

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
1	Abdallah, M. A. E., & Covaci, A. (2014). Organophosphate flame retardants in indoor dust from Egypt: implications for human exposure. [Journal article]. *Environmental Science & Technology, 48*, 4782-4789.	EXPO
2	Acosta-Maldonado, B., Sanchez-Ramirez, B., Reza-Lopez, S., & Levario-Carrillo, M. (2009). Effects of exposure to pesticides during pregnancy on placental maturity and weight of newborns: a cross-sectional pilot study in women from the Chihuahua State, Mexico. *Hum Exp Toxicol, 28*(8), 451-459.	EPI
3	Ali, P., Anwer, A., Bashir, B., Jabeen, R., Haroon, H., & Makki, K. (2012). Clinical pattern and outcome of organophosphorus poisoning. [Article]. *Journal of the Liaquat University of Medical and Health Sciences, 11*, 15-18.	ACR
4	Alizadeh, A. M., Hassanian-Moghaddam, H., Shadnia, S., Zamani, N., & Mehrpour, O. (2014). Simplified acute physiology score II/Acute physiology and chronic health evaluation ii and prediction of the mortality and later development of complications in poisoned patients admitted to intensive care unit. [Article]. *Basic and Clinical Pharmacology and Toxicology, 115*, 297-300.	ACR
5	Andersen, H. R., Debes, F., Wohlfahrt-Veje, C., Murata, K., & Grandjean, P. (2015). Occupational pesticide exposure in early pregnancy associated with sex-specific neurobehavioral deficits in the children at school age. *Neurotoxicology and Teratology, 47*, 1-9.	EPI
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7	Attfield, K. R., Hughes, M. D., Spengler, J. D., & Lu, C. S. (2014). Within- and between-child variation in repeated urinary pesticide metabolite measurements over a 1-year period. [Journal article]. *Environmental Health Perspectives, 122*, 201-206.	DUPLICATE
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19	Berton, T., Mayhoub, F., Chardon, K., Duca, R.-C., Lestremau, F., Bach, V., et al. (2014a). Development of an analytical strategy based on LC–MS/MS for the measurement of different classes of pesticides and theirs metabolites in meconium: Application and characterisation of foetal exposure in France. *Environmental Research, 132*, 311-320.	DUPLICATE
20	Berton, T., Mayhoub, F., Chardon, K., Duca, R.-C., Lestremau, F., Bach, V., et al. (2014b). Development of an analytical strategy based on LC–MS/MS for the measurement of different classes of pesticides and theirs metabolites in meconium: Application and characterisation of foetal exposure in France. *Environmental Research, 132*, 311-320.	DUPLICATE
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32	Bulgaroni, V., Lombardo, P., Rivero-Osimani, V., Vera, B., Dulgerian, L., Cerban, F., et al. (2013). Environmental pesticide exposure modulates cytokines, arginase and ornithine decarboxylase expression in human placenta. [Article]. *Reproductive Toxicology, 39*, 23-32.	TOX
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70	Eskenazi, B., Kogut, K., Huen, K., Harley, K. G., Bouchard, M., Bradman, A., et al. (2014a). Organophosphate pesticide exposure, PON1, and neurodevelopment in school-age children from the CHAMACOS study. *Environmental Research, 134*, 149-157.	EPI
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89	Furlong, M. A., Engel, S. M., Barr, D. B., & Wolff, M. S. (2014a). Prenatal exposure to organophosphate pesticides and reciprocal social behavior in childhood. *Environment International, 70*, 125-131.	DUPLICATE
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273	Van Thriel, C., Westerink, R. H. S., Beste, C., Bale, A. S., Lein, P. J., & Leist, M. (2012). Translating neurobehavioural endpoints of developmental neurotoxicity tests into in vitro assays and readouts. [Article]. *NeuroToxicology, 33*, 911-924.	REV

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
274	Van Wendel De Joode, B., Barraza, D., Ruepert, C., Mora, A. M., Cordoba, L., ÖBerg, M., et al. (2012). Indigenous children living nearby plantations with chlorpyrifos-treated bags have elevated 3,5,6-trichloro-2-pyridinol (TCPy) urinary concentrations. [article]. *Environmental research (New York, N.Y. : Print), 117*, 17-26.	DUPLICATE
275	Veale, D. J. H., Wium, C. A., & Müller, G. J. (2013). Toxicovigilance I: A survey of acute poisonings in South Africa based on Tygerberg Poison Information centre data. [Article]. *South African Medical Journal, 103*, 293-297.	ACR
276	Venerosi, A., Ricceri, L., Tait, S., & Calamandrei, G. (2012). Sex dimorphic behaviors as markers of neuroendocrine disruption by environmental chemicals: The case of chlorpyrifos. [Article]. *NeuroToxicology, 33*, 1420-1426.	TOX
277	Vera, B., Cruz, S. S., & Magnarelli, G. (2012). Plasma cholinesterase and carboxylesterase activities and nuclear and mitochondrial lipid composition of human placenta associated with maternal exposure to pesticides. [Article]. *Reproductive Toxicology, 34*(3), 402-407.	TOX
278	Vera, B., Santa Cruz, S., & Magnarelli, G. (2012). Plasma cholinesterase and carboxylesterase activities and nuclear and mitochondrial lipid composition of human placenta associated with maternal exposure to pesticides. [article]. *Reproductive toxicology (Elmsford, NY), 34*, 402-407.	DUPLICATE
279	Vogt, R., Bennett, D., Cassady, D., Frost, J., Ritz, B., & Hertz-Picciotto, I. (2012). Cancer and non-cancer health effects from food contaminant exposures for children and adults in California: A risk assessment. [Article]. *Environmental Health: A Global Access Science Source, 11*.	REV
280	Wang, P., Tian, Y., Wang, X. J., Gao, Y., Shi, R., Wang, G. Q., et al. (2011). Organophosphate pesticide exposure and perinatal outcomes in Shanghai, China. *Environ Int*.	DUPLICATE
281	Wang, P., Tian, Y., Wang, X.-J., Gao, Y., Shi, R., Wang, G.-Q., et al. (2012). Organophosphate pesticide exposure and perinatal outcomes in Shanghai, China. [Article]. *Environment International, 42*, 100-104.	EPI

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
282	Wason, S. C., Julien, R., Perry, M. J., Smith, T. J., & Levy, J. I. (2013). Modeling exposures to organophosphates and pyrethroids for children living in an urban low-income environment. [Article]. *Environmental Research, 124*, 13-22.	EXPO
283	Wason, S. C., Smith, T. J., Perry, M. J., & Levy, J. I. (2012). Using physiologically-based pharmacokinetic models to incorporate chemical and non-chemical stressors into cumulative risk assessment: A case study of pesticide exposures. [Article]. *International Journal of Environmental Research and Public Health, 9*, 1971-1983.	EXPO
284	Whyatt, R. M., Rauh, V., Barr, D. B., Camann, D. E., Andrews, H. F., Garfinkel, R., et al. (2004). Prenatal insecticide exposures and birth weight and length among an urban minority cohort. *Environ Health Perspect, 112*(10), 1125-1132.	EPI
285	Wickerham, E. L., Lozoff, B., Jie, S., Kaciroti, N., Yankai, X. I. A., & Meeker, J. D. (2012). Reduced birth weight in relation to pesticide mixtures detected in cord blood of full-term infants. [article]. *Environment international, 47*, 80-85.	EPI
286	Wickerham, E. L., Lozoff, B., Shao, J., Kaciroti, N., Xia, Y. K., & Meeker, J. D. (2012). Reduced birth weight in relation to pesticide mixtures detected in cord blood of full-term infants. [Article]. *Environment International, 47*, 80-85.	DUPLICATE
287	Williams, A. L., & DeSesso, J. M. (2014). Gestational/ Perinatal chlorpyrifos exposure is not associated with autistic-like behaviors in rodents. [Literature Review]. *Critical Reviews in Toxicology, 44*, 523-534.	REV
288	Wolff, M. S., Engel, S., Berkowitz, G., Teitelbaum, S., Siskind, J., Barr, D. B., et al. (2007). Prenatal pesticide and PCB exposures and birth outcomes. *Pediatr Res, 61*(2), 243-250.	EPI
289	Xiang, H., Nuckols, J. R., & Stallones, L. (2000). A geographic information assessment of birth weight and crop production patterns around mother's residence. *Environ Res, 82*(2), 160-167.	EPI

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
290	Ye, X., Pierik, F. H., Hauser, R., Duty, S., Angerer, J., Park, M. M., et al. (2008). Urinary metabolite concentrations of organophosphorous pesticides, bisphenol A, and phthalates among pregnant women in Rotterdam, the Netherlands: The Generation R study. [Research Support, N.I.H., Extramural]. *Environ Res, 108*(2), 260-267.	EXPO
291	Yolton, K., Cornelius, M., Ornoy, A., McGough, J., Makris, S., & Schantz, S. (2014). Exposure to neurotoxicants and the development of attention deficit hyperactivity disorder and its related behaviors in childhood. [Journal article]. *Neurotoxicology and Teratology, 44*, 30-45.	REV
292	Young, J. G., Eskenazi, B., Gladstone, E. A., Bradman, A., Pedersen, L., Johnson, C., et al. (2005). Association between in utero organophosphate pesticide exposure and abnormal reflexes in neonates. *Neurotoxicology, 26*(2), 199-209.	EPI
293	Yuan, Y., Chen, C., Zheng, C., Wang, X., Yang, G., Wang, Q., et al. (2014). Residue of chlorpyrifos and cypermethrin in vegetables and probabilistic exposure assessment for consumers in Zhejiang Province, China. [Article]. *Food Control, 36*, 63-68.	EXPO
294	Yusà, V., Coscollà, C., & Millet, M. (2014). New screening approach for risk assessment of pesticides in ambient air. [Article]. *Atmospheric Environment, 96*, 322-330.	REV
295	Zhang, X., Wallace, A. D., Du, P., Lin, S., Baccarelli, A. A., Jiang, H., et al. (2012). Genome-wide study of DNA methylation alterations in response to diazinon exposure in vitro. [Article]. *Environmental Toxicology and Pharmacology, 34*, 959-968.	TOX
296	Zhang, Y., Han, S., Liang, D., Shi, X., Wang, F., Liu, W., et al. (2014). Prenatal exposure to organophosphate pesticides and neurobehavioral development of neonates: A birth cohort study in Shenyang, China. [Article]. *PLoS ONE, 9*.	EPI
297	Zhang, Y., Song, H., Liang, D., Shi, X., Wang, F., Liu, W., et al. (2014). Prenatal exposure to organophosphate pesticides and neurobehavioral development of neonates: a birth cohort study in Shenyang, China. [Journal article]. *PLoS ONE, 9*, e88491.	DUPLICATE

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
298	Zhao, Q., Gadagbui, B., & Dourson, M. (2005). Lower birth weight as a critical effect of chlorpyrifos: A comparison of human and animal data. *Regul Toxicol Pharmacol, 42*(1), 55-63.	REV
299	Zhou, S., Rosenthal, D. G., Sherman, S., Zelikoff, J., Gordon, T., & Weitzman, M. (2014). Physical, Behavioral, and Cognitive Effects of Prenatal Tobacco and Postnatal Secondhand Smoke Exposure. *Current Problems in Pediatric and Adolescent Health Care, 44*, 219-241.	REV

Appendix 5. Table of Systematic Review Analysis: Second Tier Exclusion Criteria.

Among the 79 potentially relevant epidemiologic studies, 41 were excluded; 17 articles were previously reviewed in 2012; 16 were epidemiological methods papers including exposure validation studies without an original epidemiological risk estimate; and 8 were otherwise not relevant for various reasons. Among the 40 remaining studies, 2 were additionally excluded (one was a duplicate study published a second time; the other did not make a measure of an OP pesticide. Therefore, 38 articles are included in the 2015 literature review.

Number	Citations (Of 79: 17 - part of 3 cohorts, previously reviewed 24-excluded (NOT OP, NOT ND, NOT EPI) 38-include (9 no direct OP measure)	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English	Include? Outcome==pediatric ND/NB/motor control/morphology/motor control; Exposure==OP (biomarker, questionnaire, env media); epi study (cohort/case control/cross sectional (not ecologic))	Include? Rationale
1	Burdorf, A., Brand, T., Jaddoe, V. W., Hofman, A., Mackenbach, J. P., & Steegers, E. A. (2011). The effects of work-related maternal risk factors on time to pregnancy, preterm birth and birth weight: the Generation R Study. *Occup Environ Med, 68*(3), 197-204.	EPI	NO	NO OP; ALL PESTICIDE ONLY
2	Xiang, H., Nuckols, J. R., & Stallones, L. (2000). A geographic information assessment of birth weight and crop production patterns around mother's residence. *Environ Res, 82*(2), 160-167.	EPI	NO	No pesticide measure
3	Ferreira, J. D., Couto, A. C. z., Pombo-de-Oliveira, M. S., & Koifman, S. (2013). In utero pesticide exposure and leukemia in Brazilian children < 2 years of age. [Note]. *Environmental Health Perspectives, 121*, 269-275.	EPI	NO	NOT ND
4	How, V., Hashim, Z., Ismail, P., Said, S. M., Dzolkhifli, O., & Shamsul Bahri, M. T. (2014). Exploring cancer development in adulthood: cholinesterase depression and genotoxic effect from chronic exposure to organophosphate pesticides among rural farm children. [Journal article]. *Journal of Agromedicine, 19*, 35-43.	EPI	NO	not ND
5	Huen, K., Harley, K., Beckman, K., Eskenazi, B., & Holland, N. (2013). Associations of PON1 and Genetic Ancestry with Obesity in Early Childhood. [Article]. *PLoS ONE, 8*.	EPI	NO	not ND

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Number	Citations (Of 79: 17 - part of 3 cohorts, previously reviewed 24-excluded (NOT OP, NOT ND, NOT EPI) 38-include (9 no direct OP measure)	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English	Include? Outcome==pediatric ND/NB/motor control/morphology/motor control; Exposure==OP (biomarker, questionnaire, env media); epi study (cohort/case control/cross sectional (not ecologic))	Include? Rationale
6	Jones, K., Everard, M., & Harding, A. H. (2014). Investigation of gastrointestinal effects of organophosphate and carbamate pesticide residues on young children. [Journal article]. *International Journal of Hygiene and Environmental Health, 217*, 392-398.	EPI	NO	not ND
7	Khan, K., Ismail, A. A., Abdel Rasoul, G., Bonner, M. R., Lasarev, M. R., Hendy, O., et al. (2014). Longitudinal assessment of chlorpyrifos exposure and self-reported neurological symptoms in adolescent pesticide applicators. [Article]. *BMJ Open, 4*.	EPI	NO	not ND
8	Michalakis, M., Tzatzarakis, M. N., Kovatsi, L., Alegakis, A. K., Tsakalof, A. K., Heretis, I., et al. (2014). Hypospadias in offspring is associated with chronic exposure of parents to organophosphate and organochlorine pesticides. [Article]. *Toxicology Letters, 230*, 139-145.	EPI	NO	not ND
9	Rohlman, D. S., Ismail, A. A., Abdel-Rasoul, G., Lasarev, M., Hendy, O., & Olson, J. R. (2014). Characterizing exposures and neurobehavioral performance in Egyptian adolescent pesticide applicators. [Article]. *Metabolic Brain Disease, 29*, 845-855.	EPI	NO	not ND
10	Rojas, M., Agreda, O., & Infante, S. (2008). A preliminary statistical study of whether pesticide use could be related to birth defects in a rural area of Venezuela. *Rev Salud Publica (Bogota), 10*(1), 85-93.	EPI	NO	not ND
11	Simescu, M., Igna, C. P., Nicolaescu, E., Ion, I., Ion, A. C., Caragheorgheopol, A., et al. (2014). MULTIPLE PESTICIDES EXPOSURE OF GREENHOUSE WORKERS AND THYROID PARAMETERS. [article]. *International journal of sustainable development and planning (Print), 9*, 15-28.	EPI	NO	not ND

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Number	Citations (Of 79: 17 - part of 3 cohorts, previously reviewed 24-excluded (NOT OP, NOT ND, NOT EPI) 38-include (9 no direct OP measure)	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English	Include? Outcome==pediatric ND/NB/motor control/morphology/motor control; Exposure==OP (biomarker, questionnaire, env media); epi study (cohort/case control/cross sectional (not ecologic))	Include? Rationale
12	Cecchi, A., Rovedatti, M. G., Sabino, G., & Magnarelli, G. G. (2012a). Environmental exposure to organophosphate pesticides: Assessment of endocrine disruption and hepatotoxicity in pregnant women. [Article]. *Ecotoxicology and Environmental Safety, 80*, 280-287.	EPI	NO	not ND directly; maternal ED/thyroid change->adverse fetal (unmeasured)
13	Rohlman, D. S., Lasarev, M., Anger, W. K., Scherer, J., Stupfel, J., & McCauley, L. (2007a). Neurobehavioral performance of adult and adolescent agricultural workers. *Neurotoxicology, 28*(2), 374-380.	EPI	NO	not ND, not OP
14	Carmichael, S. L., Yang, W., Roberts, E. M., Kegley, S. E., Wolff, C., Guo, L., et al. (2013). Hypospadias and residential proximity to pesticide applications. [Article]. *Pediatrics, 132*, e1216-e1226.	EPI	NO	not ND; measured all pesticides, some OP
15	Carmichael, S. L., Yang, W., Roberts, E., Kegley, S. E., Padula, A. M., English, P. B., et al. (2014). Residential agricultural pesticide exposures and risk of selected congenital heart defects among offspring in the San Joaquin Valley of California. [Article]. *Environmental Research, 135*, 133-138.	EPI	NO	not ND; measured all pesticides, some OP
16	Eskenazi, B., Chevrier, J., Rauch, S. A., Kogut, K., Harley, K. G., Johnson, C., et al. (2013). In Utero and Childhood Polybrominated Diphenyl Ether (PBDE) Exposures and Neurodevelopment in the CHAMACOS Study. [Article]. *Environmental Health Perspectives, 121*(2), 257-262.	EPI	NO	not OP
17	Kezios, K. L., Liu, X. H., Cirillo, P. M., Cohn, B. A., Kalantzi, O. I., Wang, Y. Z., et al. (2013). Dichlorodiphenyltrichloroethane (DDT), DDT metabolites and pregnancy outcomes. [Article]. *Reproductive Toxicology, 35*, 156-164.	EPI	NO	not OP

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Number	Citations (Of 79: 17 - part of 3 cohorts, previously reviewed 24-excluded (NOT OP, NOT ND, NOT EPI) 38-include (9 no direct OP measure)	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English	Include? Outcome==pediatric ND/NB/motor control/morphology/motor control; Exposure==OP (biomarker, questionnaire, env media); epi study (cohort/case control/cross sectional (not ecologic))	Include? Rationale
18	Kicinski, M., Vrijens, J., Vermier, G., Hond, E. D., Schoeters, G., Nelen, V., et al. (2015). Neurobehavioral function and low-level metal exposure in adolescents. *International Journal of Hygiene and Environmental Health, 218*, 139-146.	EPI	NO	not OP
19	Ostrea, E. M., Reyes, A., Villanueva-Uy, E., Pacifico, R., Benitez, B., Ramos, E., et al. (2011). Fetal exposure to propoxur and abnormal child neurodevelopment at 2 years of age. *NeuroToxicology*.	EPI	NO	not OP
20	Snijder, C. A., Heederik, D., Pierik, F. H., Hofman, A., Jaddoe, V. W., Koch, H. M., et al. (2013). Fetal growth and prenatal exposure to bisphenol A: The generation R study. [Article]. *Environmental Health Perspectives, 121*, 393-396.	EPI	NO	not OP
21	Tan, J., Loganath, A., Chong, Y. S., & Obbard, J. P. (2009). Exposure to persistent organic pollutants in utero and related maternal characteristics on birth outcomes: A multivariate data analysis approach. *Chemosphere, 74*(3), 428-433.	EPI	NO	not OP
22	Torres-Sanchez, L., Rothenberg, S. J., Schnaas, L., Cebrian, M. E., Osorio, E., Del Carmen Hernandez, M., et al. (2007). In utero p,p'-DDE exposure and infant neurodevelopment: A perinatal cohort in Mexico. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Environ Health Perspect, 115*(3), 435-439.	EPI	NO	not OP
23	Torres-Sanchez, L., Schnaas, L., Cebrian, M. E., Hernandez Mdel, C., Valencia, E. O., Garcia Hernandez, R. M., et al. (2009). Prenatal dichlorodiphenyldichloroethylene (DDE) exposure and neurodevelopment: a follow-up from 12 to 30 months of age. [Research Support, Non-U.S. Gov't]. *NeuroToxicology, 30*(6), 1162-1165.	EPI	NO	not OP

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Number	Citations (Of 79: 17 - part of 3 cohorts, previously reviewed 24-excluded (NOT OP, NOT ND, NOT EPI) 38-include (9 no direct OP measure)	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English	Include? Outcome==pediatric ND/NB/motor control/morphology/motor control; Exposure==OP (biomarker, questionnaire, env media); epi study (cohort/case control/cross sectional (not ecologic))	Include? Rationale
24	Acosta-Maldonado, B., Sanchez-Ramirez, B., Reza-Lopez, S., & Levario-Carrillo, M. (2009). Effects of exposure to pesticides during pregnancy on placental maturity and weight of newborns: a cross-sectional pilot study in women from the Chihuahua State, Mexico. *Hum Exp Toxicol, 28*(8), 451-459.	EPI	YES	
25	Andersen, H. R., Debes, F., Wohlfahrt-Veje, C., Murata, K., & Grandjean, P. (2015). Occupational pesticide exposure in early pregnancy associated with sex-specific neurobehavioral deficits in the children at school age. *Neurotoxicology and Teratology, 47*, 1-9.	EPI	YES	
26	Barr, D. B., Ananth, C. V., Yan, X., Lashley, S., Smulian, J. C., Ledoux, T. A., et al. (2010). Pesticide concentrations in maternal and umbilical cord sera and their relation to birth outcomes in a population of pregnant women and newborns in New Jersey. *Sci Total Environ, 408*(4), 790-795.	EPI	YES	
27	Berkowitz, G. S., Wetmur, J. G., Birman-Deych, E., Obel, J., Lapinski, R. H., Godbold, J. H., et al. (2004). In utero pesticide exposure, maternal paraoxonase activity, and head circumference. *Environ Health Perspect, 112*(3), 388-391.	EPI	YES-3COHORTS	
28	Bouchard, M. F., Bellinger, D. C., Wright, R. O., & Weisskopf, M. G. (2010). Attention-deficit/hyperactivity disorder and urinary metabolites of organophosphate pesticides. [Research Support, N.I.H., Extramural]. *Pediatrics, 125*(6), e1270-1277.	EPI	YES	
29	Bouchard, M. F., Chevrier, J., Harley, K. G., Kogut, K., Vedar, M., Calderon, N., et al. (2011). Prenatal exposure to organophosphate pesticides and IQ in 7-year-old children. *Environ Health Perspect, 119*(8), 1189-1195.	EPI	YES-3COHORTS	

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Number	Citations (Of 79: 17 - part of 3 cohorts, previously reviewed 24-excluded (NOT OP, NOT ND, NOT EPI) 38-include (9 no direct OP measure)	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English	Include? Outcome==pediatric ND/NB/motor control/morphology/motor control; Exposure==OP (biomarker, questionnaire, env media); epi study (cohort/case control/cross sectional (not ecologic))	Include? Rationale
30	Dabrowski, S., Hanke, W., Polanska, K., Makowiec-Dabrowska, T., & Sobala, W. (2003). Pesticide exposure and birthweight: an epidemiological study in Central Poland. *Int J Occup Med Environ Health, 16*(1), 31-39.	EPI	YES	
31	Ding, G. D., Wang, P., Tian, Y., Zhang, J., Gao, Y., Wang, X. J., et al. (2012). Organophosphate Pesticide Exposure and Neurodevelopment in Young Shanghai Children. [Article]. *Environmental Science & Technology, 46*(5), 2911-2917.	EPI	YES	
32	Engel, S. M., Berkowitz, G. S., Barr, D. B., Teitelbaum, S. L., Siskind, J., Meisel, S. J., et al. (2007). Prenatal organophosphate metabolite and organochlorine levels and performance on the Brazelton Neonatal Behavioral Assessment Scale in a multiethnic pregnancy cohort. *Am J Epidemiol, 165*(12), 1397-1404.	EPI	YES-3COHORTS	
33	Engel, S. M., Wetmur, J., Chen, J., Zhu, C., Barr, D. B., Canfield, R. L., et al. (2011). Prenatal exposure to organophosphates, paraoxonase 1, and cognitive development in childhood. *Environ Health Perspect, 119*(8), 1182-1188.	EPI	YES-3COHORTS	
34	Eskenazi, B., Harley, K., Bradman, A., Weltzien, E., Jewell, N. P., Barr, D. B., et al. (2004). Association of in utero organophosphate pesticide exposure and fetal growth and length of gestation in an agricultural population. *Environ Health Perspect, 112*(10), 1116-1124.	EPI	YES-3COHORTS	
35	Eskenazi, B., Huen, K., Marks, A., Harley, K. G., Bradman, A., Barr, D. B., et al. (2010). PON1 and neurodevelopment in children from the CHAMACOS study exposed to organophosphate pesticides in utero. *Environ Health Perspect, 118*(12), 1775-1781.	EPI	YES-3COHORTS	

ED_002896_00064133-00167

Number	Citations (Of 79: 17 - part of 3 cohorts, previously reviewed 24-excluded (NOT OP, NOT ND, NOT EPI) 38-include (9 no direct OP measure)	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English	Include? Outcome==pediatric ND/NB/motor control/morphology/motor control; Exposure==OP (biomarker, questionnaire, env media); epi study (cohort/case control/cross sectional (not ecologic))	Include? Rationale
36	Eskenazi, B., Kogut, K., Huen, K., Harley, K. G., Bouchard, M., Bradman, A., et al. (2014a). Organophosphate pesticide exposure, PON1, and neurodevelopment in school-age children from the CHAMACOS study. *Environmental Research, 134*, 149-157.	EPI	YES-3COHORTS	
37	Eskenazi, B., Marks, A. R., Bradman, A., Harley, K., Barr, D. B., Johnson, C., et al. (2007). Organophosphate pesticide exposure and neurodevelopment in young Mexican-American children. *Environ Health Perspect, 115*(5), 792-798.	EPI	YES-3COHORTS	
38	Fortenberry, G. Z., Meeker, J. D., Sanchez, B. N., Barr, D. B., Panuwet, P., Bellinger, D., et al. (2014). Urinary 3,5,6-trichloro-2-pyridinol (TCPY) in pregnant women from Mexico City: distribution, temporal variability, and relationship with child attention and hyperactivity. *Int J Hyg Environ Health, 217*(2-3), 405-412.	EPI	YES	
39	Fortenberry, G. Z., Meeker, J. D., Sánchez, B. N., Bellinger, D., Peterson, K., Schnaas, L., et al. (2014). Paraoxonase I polymorphisms and attention/hyperactivity in school-age children from Mexico City, Mexico. [Article]. *Environmental Research, 132*, 342-349.	EPI	YES	
40	Furlong, M. A., Engel, S. M., Barr, D. B., & Wolff, M. S. (2014). Prenatal exposure to organophosphate pesticides and reciprocal social behavior in childhood. *Environ Int, 70*, 125-131.	EPI	YES	
41	Grandjean, P., Harari, R., Barr, D. B., & Debes, F. (2006). Pesticide exposure and stunting as independent predictors of neurobehavioral deficits in Ecuadorian school children. [Research Support, Non-U.S. Gov't]. *Pediatrics, 117*(3), e546-556.	EPI	YES	

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Number	Citations (Of 79: 17 - part of 3 cohorts, previously reviewed 24-excluded (NOT OP, NOT ND, NOT EPI) 38-include (9 no direct OP measure)	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English	Include? Outcome==pediatric ND/NB/motor control/morphology/motor control; Exposure==OP (biomarker, questionnaire, env media); epi study (cohort/case control/cross sectional (not ecologic))	Include? Rationale
42	Guodong, D., Pei, W., Ying, T., Jun, Z., Yu, G., Xiaojin, W., et al. (2012). Organophosphate pesticide exposure and neurodevelopment in young Shanghai children. *Environ Sci Technol*.	EPI	YES	
43	Handal, A. J., Harlow, S. D., Breilh, J., & Lozoff, B. (2008). Occupational exposure to pesticides during pregnancy and neurobehavioral development of infants and toddlers. *Epidemiology, 19*(6), 851-859.	EPI	YES	
44	Handal, A. J., Lozoff, B., Breilh, J., & Harlow, S. D. (2007a). Effect of community of residence on neurobehavioral development in infants and young children in a flower-growing region of Ecuador. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Environ Health Perspect, 115*(1), 128-133.	EPI	YES	
45	Handal, A. J., Lozoff, B., Breilh, J., & Harlow, S. D. (2007b). Neurobehavioral development in children with potential exposure to pesticides. *Epidemiology, 18*(3), 312-320.	EPI	YES	
46	Harari, R., Julvez, J., Murata, K., Barr, D., Bellinger, D. C., Debes, F., et al. (2010). Neurobehavioral deficits and increased blood pressure in school-age children prenatally exposed to pesticides. *Environ Health Perspect, 118*(6), 890-896.	EPI	YES	
47	Harley, K. G., Huen, K., Schall, R. A., Holland, N. T., Bradman, A., Barr, D. B., et al. (2011). Association of organophosphate pesticide exposure and paraoxonase with birth outcome in Mexican-American women. *PLoS One, 6*(8), e23923.	EPI	YES-3COHORTS	

Number	Citations (Of 79: 17 - part of 3 cohorts, previously reviewed 24-excluded (NOT OP, NOT ND, NOT EPI) 38-include (9 no direct OP measure)	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English	Include? Outcome==pediatric ND/NB/motor control/morphology/motor control; Exposure==OP (biomarker, questionnaire, env media); epi study (cohort/case control/cross sectional (not ecologic))	Include? Rationale
48	Horton, M. K., Kahn, L. G., Perera, F., Barr, D. B., & Rauh, V. (2012). Does the home environment and the sex of the child modify the adverse effects of prenatal exposure to chlorpyrifos on child working memory? *Neurotoxicol Teratol, 34*(5), 534-541.	EPI	YES-3COHORTS	
49	Kofman, O., Berger, A., Massarwa, A., Friedman, A., & Jaffar, A. A. (2006). Motor inhibition and learning impairments in school-aged children following exposure to organophosphate pesticides in infancy. *Pediatr Res, 60*(1), 88-92.	EPI	YES	NO: LONG TERM EFFECTS OF ONE TIME POISONING
50	Koutroulakis, D., Sifakis, S., Tzatzarakis, M. N., Alegakis, A. K., Theodoropoulou, E., Kavvalakis, M. P., et al. (2014). Dialkyl phosphates in amniotic fluid as a biomarker of fetal exposure to organophosphates in Crete, Greece; association with fetal growth. [Article]. *Reproductive Toxicology, 46*, 98-105.	EPI	YES	
51	Kristensen, P., Irgens, L. M., Andersen, A., Bye, A. S., & Sundheim, L. (1997). Gestational age, birth weight, and perinatal death among births to Norwegian farmers, 1967-1991. *Am J Epidemiol, 146*(4), 329-338.	EPI	YES	
52	Lizardi, P. S., O'Rourke, M. K., & Morris, R. J. (2008). The effects of organophosphate pesticide exposure on Hispanic children's cognitive and behavioral functioning. *J Pediatr Psychol, 33*(1), 91-101.	EPI	YES	
53	Llop, S., Julvez, J., Fernandez-Somoano, A., Santa Marina, L., Vizcaino, E., Iñiguez, C., et al. (2013). Prenatal and postnatal insecticide use and infant neuropsychological development in a multicenter birth cohort study. *Environment International, 59*, 175-182.	EPI	YES	

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54	Lovasi, G. S., Quinn, J. W., Rauh, V. A., Perera, F. P., Andrews, H. F., Garfinkel, R., et al. (2011). Chlorpyrifos exposure and urban residential environment characteristics as determinants of early childhood neurodevelopment. *Am J Public Health, 101*(1), 63-70.	EPI	YES-3COHORTS	
55	Lu, C., Essig, C., Root, C., Rohlman, D. S., McDonald, T., & Sulzbacher, S. (2009). Assessing the association between pesticide exposure and cognitive development in rural Costa Rican children living in organic and conventional coffee farms. *Int J Adolesc Med Health, 21*(4), 609-621.	EPI	YES	
56	Marks, A. R., Harley, K., Bradman, A., Kogut, K., Barr, D. B., Johnson, C., et al. (2010). Organophosphate pesticide exposure and attention in young Mexican-American children: the CHAMACOS study. *Environ Health Perspect, 118*(12), 1768-1774.	EPI	YES-3COHORTS	
57	Moreno-Banda, G., Blanco-Munoz, J., Lacasana, M., Rothenberg, S. J., Aguilar-Garduno, C., Gamboa, R., et al. (2009). Maternal exposure to floricultural work during pregnancy, PON1 Q192R polymorphisms and the risk of low birth weight. *Sci Total Environ, 407*(21), 5478-5485.	EPI	YES	
58	Nevison, C. D. (2014). A comparison of temporal trends in United States autism prevalence to trends in suspected environmental factors. [Article]. *Environmental Health: A Global Access Science Source, 13*.	EPI	YES	NO: this is a review; ecol comparison
59	Oulhote, Y., & Bouchard, M. F. (2013). Urinary metabolites of organophosphate and pyrethroid pesticides and behavioral problems in Canadian children. [Article]. *Environmental Health Perspectives, 121*, 1378-1384.	EPI	YES	

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Number	Citations (Of 79: 17 - part of 3 cohorts, previously reviewed 24-excluded (NOT OP, NOT ND, NOT EPI) 38-include (9 no direct OP measure)	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English	Include? Outcome==pediatric ND/NB/motor control/morphology/motor control; Exposure==OP (biomarker, questionnaire, env media); epi study (cohort/case control/cross sectional (not ecologic))	Include? Rationale
60	Petit, C., Chevrier, C., Durand, G., Monfort, C., Rouget, F., Garlantezec, R., et al. (2010). Impact on fetal growth of prenatal exposure to pesticides due to agricultural activities: A prospective cohort study in Brittany, France. [Research Support, Non-U.S. Gov't]. *Environ Health, 9*, 71.	EPI	YES	
61	Quiros-Alcala, L., Alkon, A. D., Boyce, W. T., Lippert, S., Davis, N. V., Bradman, A., et al. (2011). Maternal prenatal and child organophosphate pesticide exposures and children's autonomic function. *Neurotoxicology, 32*(5), 646-655.	EPI	YES	
62	Rauch, S. A., Braun, J. M., Barr, D. B., Calafat, A. M., Khoury, J., Montesano, M. A., et al. (2012). Associations of prenatal exposure to organophosphate pesticide metabolites with gestational age and birth weight. [Article]. *Environmental Health Perspectives, 120*, 1055-1060.	EPI	YES	
63	Rauh, V. A., Garfinkel, R., Perera, F. P., Andrews, H. F., Hoepner, L., Barr, D. B., et al. (2006). Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children. *Pediatrics, 118*(6), e1845-1859.	EPI	YES-3COHORTS	
64	Rauh, V. A., Perera, F. P., Horton, M. K., Whyatt, R. M., Bansal, R., Hao, X., et al. (2012). Brain anomalies in children exposed prenatally to a common organophosphate pesticide. *Proc Natl Acad Sci U S A, 109*(20), 7871-7876.	EPI	YES-3COHORTS	
65	Rauh, V., Arunajadai, S., Horton, M., Perera, F., Hoepner, L., Barr, D. B., et al. (2011). Seven-year neurodevelopmental scores and prenatal exposure to chlorpyrifos, a common agricultural pesticide. *Environ Health Perspect, 119*(8), 1196-1201.	EPI	YES-3COHORTS	

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Number	Citations (Of 79: 17 - part of 3 cohorts, previously reviewed 24-excluded (NOT OP, NOT ND, NOT EPI) 38-include (9 no direct OP measure)	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English	Include? Outcome==pediatric ND/NB/motor control/morphology/motor control; Exposure==OP (biomarker, questionnaire, env media); epi study (cohort/case control/cross sectional (not ecologic))	Include? Rationale
66	Rohlman, D. S., Arcury, T. A., Quandt, S. A., Lasarev, M., Rothlein, J., Travers, R., et al. (2005). Neurobehavioral performance in preschool children from agricultural and non-agricultural communities in Oregon and North Carolina. *NeuroToxicology, 26*(4 Spec. Iss.), 589-598.	EPI	YES	
67	Ruckart, P. Z., Kakolewski, K., Bove, F. J., & Kaye, W. E. (2004). Long-term neurobehavioral health effects of methyl parathion exposure in children in Mississippi and Ohio. [Comparative Study]. *Environ Health Perspect, 112*(1), 46-51.	EPI	YES	
68	Samarawickrema, N., Pathmeswaran, A., Wickremasinghe, R., Peiris-John, R., Karunaratna, M., Buckley, N., et al. (2008). Fetal effects of environmental exposure of pregnant women to organophosphorus compounds in a rural farming community in Sri Lanka. *Clin Toxicol (Phila), 46*(6), 489-495.	EPI	YES	
69	Savitz, D. A., Arbuckle, T., Kaczor, D., & Curtis, K. M. (1997). Male pesticide exposure and pregnancy outcome. *Am J Epidemiol, 146*(12), 1025-1036.	EPI	YES	
70	Shelton, J. F., Geraghty, E. M., Tancredi, D. J., Delwiche, L. D., Schmidt, R. J., Ritz, B., et al. (2014). Neurodevelopmental disorders and prenatal residential proximity to agricultural pesticides: The charge study. [Article]. *Environmental Health Perspectives, 122*, 1103-1109.	EPI	YES	
71	Suarez-Lopez, J. R., Himes, J. H., Jacobs Jr, D. R., Alexander, B. H., & Gunnar, M. R. (2013). Acetylcholinesterase activity and neurodevelopment in boys and girls. [Article]. *Pediatrics, 132*, e1649-e1658.	EPI	YES	

Number	Citations (Of 79: 17 - part of 3 cohorts, previously reviewed 24-excluded (NOT OP, NOT ND, NOT EPI) 38-include (9 no direct OP measure)	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English	Include? Outcome==pediatric ND/NB/motor control/morphology/motor control; Exposure==OP (biomarker, questionnaire, env media); epi study (cohort/case control/cross sectional (not ecologic))	Include? Rationale
72	Suarez-Lopez, J. R., Jacobs Jr, D. R., Himes, J. H., & Alexander, B. H. (2013). Acetylcholinesterase activity, cohabitation with floricultural workers, and blood pressure in Ecuadorian children. [Article]. *Environmental Health Perspectives, 121*, 619-624.	EPI	YES	
73	Suarez-Lopez, J. R., Jacobs, D. R., Himes, J. H., Alexander, B. H., Lazovich, D., & Gunnar, M. (2012). Lower acetylcholinesterase activity among children living with flower plantation workers. [Article]. *Environmental Research, 114*, 53-59.	EPI	YES	
74	Wang, P., Tian, Y., Wang, X.-J., Gao, Y., Shi, R., Wang, G.-Q., et al. (2012). Organophosphate pesticide exposure and perinatal outcomes in Shanghai, China. [Article]. *Environment International, 42*, 100-104.	EPI	YES	
75	Whyatt, R. M., Rauh, V., Barr, D. B., Camann, D. E., Andrews, H. F., Garfinkel, R., et al. (2004). Prenatal insecticide exposures and birth weight and length among an urban minority cohort. *Environ Health Perspect, 112*(10), 1125-1132.	EPI	YES-3COHORTS	
76	Wickerham, E. L., Lozoff, B., Jie, S., Kaciroti, N., Yankai, X. I. A., & Meeker, J. D. (2012). Reduced birth weight in relation to pesticide mixtures detected in cord blood of full-term infants. [article]. *Environment international, 47*, 80-85.	EPI	YES	
77	Wolff, M. S., Engel, S., Berkowitz, G., Teitelbaum, S., Siskind, J., Barr, D. B., et al. (2007). Prenatal pesticide and PCB exposures and birth outcomes. *Pediatr Res, 61*(2), 243-250.	EPI	YES	
78	Young, J. G., Eskenazi, B., Gladstone, E. A., Bradman, A., Pedersen, L., Johnson, C., et al. (2005). Association between in utero organophosphate pesticide exposure and abnormal reflexes in neonates. *Neurotoxicology, 26*(2), 199-209.	EPI	YES-3COHORTS	

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79	Zhang, Y., Han, S., Liang, D., Shi, X., Wang, F., Liu, W., et al. (2014). Prenatal exposure to organophosphate pesticides and neurobehavioral development of neonates: A birth cohort study in Shenyang, China. [Article]. *PLoS ONE, 9*.	EPI	YES	

Appendix 6. Plausible hypotheses on MOA/AOP for neurodevelopmental outcomes (Extracted from Section 4.4.3. RHHRA for chlorpyrifos)

Numerous studies on the possible mechanistic aspects of neurodevelopmental effects have been published. The results have led some research groups to propose that changes in brain connectivity and/or neurochemistry may underlie the long-term *in vivo* neurobehavioral changes observed into adulthood. While multiple biologically plausible hypotheses are being pursued by researchers, no one pathway has sufficient data to be considered more credible than the others. The SAP concurred with the Agency in 2008 and 2012 about the lack of definable key events in a MOA/AOP leading to neurobehavioral effects. The Agency has considered the new literature since the 2012 SAP related to mechanistic hypotheses as described below (Appendix 11), and note that such a MOA/AOP still cannot be established.

- *Acetylcholinesterase (AChE) as a morphogen*: The classically understood role of AChE is the rapid hydrolysis of acetylcholine at synapses in the brain and at neuromuscular junctions, thereby regulating cholinergic neurotransmission. Consistent with this role, AChE is predominant at cholinergic synapses at neurons and in muscle, and inhibition of its catalytic activity results in the signs and symptoms of cholinergic overstimulation. Several lines of evidence suggest that AChE can also serve as a morphogen, influencing the growth of cells during neurodevelopment distinct from its role as an esterase. Alterations in the expression or structure of the AChE protein can disrupt various aspects of neuronal differentiation and growth, as has recently been shown in vitro (using NG108-15 cell line) following exposure to another OP, paraoxon (Campanha et al., 2014). While perturbation of the morphogenic activity of AChE is a plausible adverse outcome pathway for chlorpyrifos, a number of questions remain, including effective concentrations compared to those that inhibit catalytic activity of AChE. There is, however, no direct evidence showing that disruption of the morphogenic function of AChE can alter axon or dendritic growth *in vivo*. While limited *in vivo* studies using zebrafish indicate that chlorpyrifos or its metabolite chlorpyrifos oxon can disrupt axonal growth (Yang et al. 2011), it has not been demonstrated that this effect is due to alteration of the morphogenic function of AChE versus other potential mechanisms.

- *Cholinergic system:* There are several lines of evidence showing that signaling through cholinergic receptors is involved in neurodevelopment. Activation of muscarinic and/or nicotinic cholinergic receptors can regulate neural progenitor cell proliferation and differentiation (Resende & Adhikari, 2009), and *in vivo* studies demonstrate that cholinergic signaling is likely involved in brain morphogenesis (Hohmann & Berger-Sweeney, 1998). While ChE inhibitors can affect cholinergic signaling by inhibition of the catalytic activity of AChE and subsequent increase in acetylcholine, some inhibitors, including chlorpyrifos and chlorpyrifos oxon, can also directly interact with cholinergic receptors. Thus, direct interaction with cholinergic receptors by chlorpyrifos represents a potential adverse outcome pathway for disruption of neurodevelopment distinct from AChE/ChE inhibition. Some OPs have been shown to directly interact with cholinergic muscarinic receptors at relatively low concentrations. The muscarinic receptors are

members of the G-protein receptor family and five subtypes (m1-m5) have been identified. Ward et al. (1993) examined the relationship between ChE inhibition and direct binding to muscarinic receptors for a series of OPs and their active oxon metabolites. The results indicated a strong correlation between AChE activity of OPs, including chlorpyrifos and chlorpyrifos oxon, and the ability to compete for CD binding sites (m2 receptors) in rat brain homogenates. Binding affinities of the oxons were in the nanomolar range, at or below concentrations that inhibited AChE (Huff et al, 1994); specifically, chlorpyrifos oxon had a binding affinity of 22 nM in rat striatum and 2 nM in rat cortex (Huff, et al., 1994; Ward & Mundy, 1996). In total, these studies suggest that direct interactions with muscarinic receptors, and especially the m2 subtype, represent an alternative site of action for OPs including chlorpyrifos and chlorpyrifos oxon, with the oxon forms having high affinity. Together, the studies cited above outline a plausible adverse outcome pathway for chlorpyrifos and chlorpyrifos oxon to affect brain development via actions at the m2 subtype of muscarinic receptors. However, while there are studies showing that chlorpyrifos oxon can affect neurite outgrowth *in vitro* and decrease cell proliferation and differentiation both *in vitro* (Jameson et al , 2006; Qiao et al, , 2001; Song, et al., 1998) and *in vivo* (Dam et al, , 1998; Qiao, et al., 2003), there is no experimental evidence that these effects are a result of direct actions on the m2 receptor.

- *Endocannabinoid system:* Several lines of research have suggested that disruption of the endocannabinoid (EC) system due to chlorpyrifos exposure could play a role in its acute and/or long-term toxicity, and could also be extended to potential developmental toxicity. The EC system modulates neurotransmission as well as playing a morphogenic role during development of the nervous system. Chemicals (*e.g.*, drugs of abuse) which act on this system, produce long-term neurodevelopmental disorders in animal models and human studies. Chlorpyrifos also interacts with this system, both *in vitro* and *in vivo*. By this hypothesis, the EC system represents a possible adverse outcome pathway for developmental effects of chlorpyrifos. There is a body of studies on the interaction of OPs with relevant enzymes but only two studies have examined the effects of chlorpyrifos on the EC system in developing animals. Carr et al. (2011, 2013) has dosed preweanling rats for 5 or 7 days (1-5 mg/kg/day, p.o.), and showed that endocannabinoid-related enzymes were inhibited in rat brain tissue taken 4 to 48 hours after the last dose. Interestingly, fatty acid amid hydrolase (FAAH) showed a greater degree and more persistent inhibition compared to AChE inhibition measured in the same rats. A more recent publication (Carr et al., 2014) repeated these findings using a lower dose (0.5 mg/kg/d for 7 days), still showing significant FAAH inhibition but with no measurable AChE inhibition. This suggests a greater sensitivity of the EC system, at least in terms of the hydrolase compared to AChE activity, in the pups. However, there were no other ages tested, no downstream or correlative measure of changes in EC system function, and no subsequent neurodevelopmental effects that could be linked to the action. Additional studies along these lines are needed.

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- *Reactive Oxygen Species:* The production of reactive oxygen species (ROS) and resulting cellular damage has been proposed as a mechanism for a wide variety of neurotoxicants. Due to lower levels of protective enzymes and antioxidants, and relatively low numbers of glia relative to the adult, the developing brain may be particularly sensitive to neural cell damage caused by oxidative stress. In addition, recent work suggests that ROS can act as second messengers. Relatively small changes in the oxidative status of the cell (redox potential) can lead to changes in redox sensitive signaling pathways that regulate cell physiology. In the nervous system, redox signaling is involved in the regulation of neurodevelopmental processes including neural stem cell proliferation and differentiation (Le Belle et al., 2011; Vieira et al, 2011). A number of studies suggest that chlorpyrifos and chlorpyrifos oxon can induce oxidative stress in various neural cell types. Thus, generation of reactive oxygen species and/or alteration of cellular redox potential by chlorpyrifos represent a possible initiating event leading to developmental neurotoxicity. Data from both *in vitro* studies with neuronal cells (including neural precursors) and *in vivo* studies in developing brain demonstrate that chlorpyrifos can induce oxidative stress. The *in vitro* data suggests that this effect may not be due to AChE inhibition, since the parent compound chlorpyrifos is either equipotent or more potent than the oxon (for example, Crumpton et al., 2000). There was, however, no concurrent analysis of AChE inhibition in most of these studies. Several known developmental neurotoxicants have been shown to disrupt neural precursor cell proliferation *in vitro* through a common pathway that is initiated by increasing the oxidative state of the cell (Li et al, 2007), and the antioxidant vitamin E protected PC12 cells from the anti-proliferative effect of chlorpyrifos (Slotkin et al, 2007). Thus, the *in vitro* data suggest that chlorpyrifos can affect a critical neurodevelopmental process, at least in part, via generation of ROS. Though limited, *in vivo* studies show both direct evidence (lipid peroxidation) and indirect evidence (alteration in the expression of oxidative stress response genes) of oxidative stress in the developing brain after exposure to chlorpyrifos. Recent evidence suggests that oxidative stress can alter neurodevelopment *in vitro* and *in vivo* by the dysregulation of signaling pathways controlling neuroprogenitor cell function (Le Belle, et al., 2011; Vieira, et al., 2011). It has been demonstrated *in vivo* that antioxidant treatment can attenuate the induction of oxidative stress produced by chlorpyrifos in adult rats (Singh and Panwar, 2014), but there are as yet no such studies addressing its developmental neurotoxicity. Thus, there is the potential for initiation of an AOP via induction of oxidative stress, but supportive studies in developing animals have not been reported.

- *Serotonergic system:* Beyond its classical neurotransmitter actions, serotonin has other roles during development. In their review, Thompson and Stanwood (2009) described serotonin as a pleiotropic molecule, meaning that it can produce multiple, diverse effects, regulating different functions at different times during development. The serotonergic system is integral in many developmental processes including, but not limited to, neurogenesis, migration, and differentiation, synaptogenesis, and cardiac development before assuming its more well-known function as a neurotransmitter in the adult nervous system (reviewed in Frederick & Stanwood, 2009). Serotonin also

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plays crucial roles in thalamocortical patterning (reviewed in (Frederick & Stanwood, 2009). As serotonin is present extremely early in development, it is thought that it modulates cellular function even before neurogenesis. Later in development, serotonin is temporarily taken up by so-called transient serotonergic neurons mainly involved in sensory processing, and is involved in activity-dependent patterning of the brain. Later in development, serotonin has also been shown to modulate differentiation in the brain. There are numerous studies of the effects of perinatal chlorpyrifos administration on the patency of the serotonergic system coming from both Duke University and Istituto Superiore di Sanita in Italy; however, there have been no additional reports since the 2012 SAP. Endpoints in various brain regions include serotonin levels, serotonin turnover, serotonin receptor levels, serotonin reuptake receptor levels, serotonin elicited second messenger activity, gene expression of serotonin receptor and metabolism related genes, serotonin related behavioral assessments, and behavior after serotonergic drug challenge. All the data indicate that there are acute, as well as permanent, effects of neonatal chlorpyrifos treatment on the maturation of the serotonergic nervous system. The effects are often gender-specific, region-specific and dose-related.

There is ample evidence that chlorpyrifos exposure during development causes permanent changes in the serotonergic nervous system; there are, however, few papers that assessed concurrently the ChE inhibition (either brain or blood) in those same animals. In some cases, although ChE activity was not assessed concurrently, a dosing regimen was used that had been characterized previously with regard to ChE activity. It does appear, however, that most of the studies on the effects of chlorpyrifos on the serotonergic nervous system were conducted with doses of chlorpyrifos that likely produced inhibition of ChE activity.

As many steps in this chlorpyrifos AOP are possible and plausible, and in laboratory animals the serotonergic nervous system is sufficiently sensitive to low doses of chlorpyrifos during development to alter its function, it is plausible that exposure to chlorpyrifos during development could alter brain development and the function of the serotonergic nervous system. Although chlorpyrifos effects on the serotonergic nervous system in laboratory animals likely is initiated within 24 hours (Slotkin & Seidler, 2007), the actual initiating event of this potential adverse outcome pathway is unknown.

- *Tubulin, Microtubule Associated Proteins and Axonal Transport:* Microtubules, one component of the dynamic cytoskeletal scaffolding within each cell, are composed of heterodimers of α- and β-tubulin, as well as microtubule associated proteins. The microtubule associated proteins appear to have three main functions: (1) to stabilize the microtubules; (2) to aid in tubulin dissociation and (3) to act as motor proteins moving substances forward and backward along the microtubules (Avila et al, , 1994; Pellegrini & Budman, 2005; Sánchez et al, 2000). Not only does the microtubule cytoskeleton determine neuronal morphology (Matus, 1988, 1990; Sánchez, et al., 2000), but the dynamic reorganization of the microtubules and microtubule associated proteins within

a cell may also coordinate neurite extension/retraction, as well as growth cone advancement. In addition to these integral roles in brain structure and growth, microtubules and the microtubule associated motor proteins kinesin (Hirokawa & Noda, 2008) and dynein (Vallee et al, 2004) also provide a "railway" for transport of materials throughout the cell, *i.e.*, axonal transport (Fukushima et al, 2009), another process which is integral to the health of the central and peripheral nervous system, playing a pivotal role in neuronal network formation and synapse maturation (Hirokawa & Takemura, 2004). The construction of an adverse outcome pathway using chlorpyrifos-induced effects on tubulin and microtubule associated proteins is still in its infancy. While it is thought that tubulin, microtubule associated proteins and axonal transport are integral to nervous system development and maintenance, there is no experimental evidence that perturbations of these endpoints by chlorpyrifos during development has neurotoxic outcomes.

Overall, a definitive mode of action or adverse outcome pathway leading to effects on the developing brain cannot yet be established because of insufficient data establishing the causal linkages among different levels of biological organization to adversity. For example, while there is *in vitro* evidence relating binding of chlorpyrifos or the chlorpyrifos oxon to AChE and the subsequent decrease in neurite outgrowth at the cellular level, the relationship between neurite outgrowth and neurodevelopmental consequences has not been established. As described in the NRC report, "Toxicity Testing in the 21st Century" (NRC, 2007), to develop an adverse outcome pathway not only is it necessary to establish plausible relationships among the key events, but quantitative relationships also need to be established. In other words, how much of a change in one key event is needed to result in an adverse effect at the next level of biological organization? Thus, certain exposures to a chemical may impact normal physiological responses in a way that may not necessarily be adverse, and thus, the AOP concept requires an understanding of adaptive/homeostatic capacity of biological systems and their limits, relative to concentration and duration of exposure.

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Message

From: Guilaran, Yu-Ting [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=A698774E93E34A2B9181D4B3032B8A32-YTGUILAR]
Sent: 3/6/2018 4:09:52 PM
To: Perry, Tracy [Perry.Tracy@epa.gov]
CC: Sherman, Kelly [Sherman.Kelly@epa.gov]
Subject: RE: ESA Follow-up items
Attachments: draft.FRN.NMFS.12.29.2017.BiOp.3.OPs.ytg.docx

Tracy
Thanks for drafting the FRN, attached please find my edits, mainly rearrangements, albeit there is still some duplications. Hopefully I did not mess up any of the changes you've made based on the comments Nancy Bect sent to us yesterday, so please double check. If you are good with this version, we'd run this by OGC and Rick/Marietta simultaneously. Thanks.

Regards,

Yu-Ting Guilaran, P.E.
Director

Pesticide Re-evaluation Division (PRD)
Office of Pesticide Programs
Office of Chemical Safety and Pollution Prevention
(tel) 703 308 0052
(fax)703 308 8005
Mail code 7508P
Room number PY S9623

From: Perry, Tracy
Sent: Monday, March 05, 2018 6:25 PM
To: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>
Cc: Sherman, Kelly <Sherman.Kelly@epa.gov>
Subject: RE: ESA Follow-up items

Yu-Ting,

Attached is a draft FRN for the NMFS BiOp (I included the edits that Nancy sent in her recent email). I pulled some registration review text from the FRN used to announce the draft BE for these three OPs, b/c it seems strange not to mention registration review since we are consulting on listed species as part of the re-evaluation of these chemicals: https://www.federalregister.gov/documents/2016/04/11/2016-08279/chlorpyrifos-diazinon-and-malathion-registration-review-draft-biological-evaluations-notice-of

I included most of the text from the draft Note to Reader in the FRN, but rearranged it to fit the FRN format. I did not include one part of the Note to Reader in the FRN b/c it didn't seem to fit or be necessary (I've marked it in the second attachment).

Please let me know if you have any questions.

Tracy

From: Guilaran, Yu-Ting
Sent: Monday, March 05, 2018 2:13 PM
To: Perry, Tracy <Perry.Tracy@epa.gov>
Cc: Sherman, Kelly <Sherman.Kelly@epa.gov>
Subject: RE: USE THIS VERSION: ESA Follow-up items

Great, thanks.

Regards,

Yu-Ting Guilaran, P.E.
Director

Pesticide Re-evaluation Division (PRD)
Office of Pesticide Programs
Office of Chemical Safety and Pollution Prevention
(tel) 703 308 0052
(fax)703 308 8005
Mail code 7508P
Room number PY S9623

From: Perry, Tracy
Sent: Monday, March 05, 2018 1:06 PM
To: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>
Cc: Sherman, Kelly <Sherman.Kelly@epa.gov>
Subject: RE: USE THIS VERSION: ESA Follow-up items

I can send you a draft FRN before I leave today.

From: Guilaran, Yu-Ting
Sent: Monday, March 05, 2018 12:12 PM
To: Perry, Tracy <Perry.Tracy@epa.gov>
Cc: Sherman, Kelly <Sherman.Kelly@epa.gov>
Subject: RE: USE THIS VERSION: ESA Follow-up items

Please let me know if you can get me a draft FRN today. I have a general with Rick tomorrow morning and would like to close the loop on this, thanks.

Regards,

Yu-Ting Guilaran, P.E.
Director

Pesticide Re-evaluation Division (PRD)
Office of Pesticide Programs
Office of Chemical Safety and Pollution Prevention
(tel) 703 308 0052
(fax)703 308 8005
Mail code 7508P
Room number PY S9623

From: Perry, Tracy
Sent: Monday, March 05, 2018 10:46 AM
To: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>
Cc: Sherman, Kelly <Sherman.Kelly@epa.gov>
Subject: RE: USE THIS VERSION: ESA Follow-up items

Thanks!

From: Guilaran, Yu-Ting
Sent: Friday, March 02, 2018 10:40 AM
To: Perry, Tracy <Perry.Tracy@epa.gov>
Cc: Sherman, Kelly <Sherman.Kelly@epa.gov>
Subject: Fwd: USE THIS VERSION: ESA Follow-up items

Just keeping you in the loop Tracy. Thanks

Sent from my iPhone

Begin forwarded message:

> **From:** "Keigwin, Richard" <Keigwin.Richard@epa.gov>
> **Date:** March 2, 2018 at 7:39:58 AM EST
> **To:** "Bertrand, Charlotte" <Bertrand.Charlotte@epa.gov>, "Beck, Nancy" <Beck.Nancy@epa.gov>, "Wise, Louise" <Wise.Louise@epa.gov>
> **Cc:** "Keller, Kaitlin" <keller.kaitlin@epa.gov>, "Dinkins, Darlene" <Dinkins.Darlene@epa.gov>, "Echeverria, Marietta" <Echeverria.Marietta@epa.gov>, "Guilaran, Yu-Ting" <Guilaran.Yu-Ting@epa.gov>, "Miller, Wynne" <Miller.Wynne@epa.gov>
> **Subject: USE THIS VERSION: ESA Follow-up items**
>
> Attached are two documents: the narrative that we propose to include in the FR notice opening a public comment period on the December 2017 NMFS BiOp and a timeline that we think is achievable (albeit still ambitious) to implement any needed risk mitigation. Both documents have been reviewed by PTSLO. We will have a draft FR Notice ready to share next week, but would appreciate any feedback on the attached narrative so that we can incorporate those comments into the first draft of the FR Notice.

Message

From: Guilaran, Yu-Ting [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=A698774E93E34A2B9181D4B3032B8A32-YTGUILAR]
Sent: 3/9/2018 9:06:28 PM
To: Perry, Tracy [Perry.Tracy@epa.gov]
Subject: RE: Action: please revise/review NOAA GC/OPR comments on the EPA docket announcement
Attachments: final draft.FRN.NMFS.3.09.2018.BiOp.OPs.tracked.ytg.docx

Thank you Tracy! Here is the revised version.

Regards,

Yu-Ting Guilaran, P.E.
Director

Pesticide Re-evaluation Division (PRD)
Office of Pesticide Programs
Office of Chemical Safety and Pollution Prevention
(tel) 703 308 0052
(fax)703 308 8005
Mail code 7508P
Room number PY S9623

From: Perry, Tracy
Sent: Friday, March 09, 2018 3:42 PM
To: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>
Subject: RE: Action: please revise/review NOAA GC/OPR comments on the EPA docket announcement

The first attachment is clean, the second has tracked changes.

From: Guilaran, Yu-Ting
Sent: Friday, March 09, 2018 10:59 AM
To: Perry, Tracy <Perry.Tracy@epa.gov>; Dyner, Mark <dyner.mark@epa.gov>; Perlis, Robert <Perlis.Robert@epa.gov>
Cc: Echeverria, Marietta <Echeverria.Marietta@epa.gov>; Sherman, Kelly <Sherman.Kelly@epa.gov>
Subject: Action: please revise/review NOAA GC/OPR comments on the EPA docket announcement

Tracy

Please see attached, can you take a look and I will set up some time this afternoon for us to go through them. I will include Marietta and Bob as well since Mark is out today. Thanks.

Regards,

Yu-Ting Guilaran, P.E.
Director

Pesticide Re-evaluation Division (PRD)
Office of Pesticide Programs
Office of Chemical Safety and Pollution Prevention
(tel) 703 308 0052
(fax)703 308 8005

Mail code 7508P
Room number PY S9623

From: Beck, Nancy
Sent: Friday, March 09, 2018 10:52 AM
To: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>; Keigwin, Richard <Keigwin.Richard@epa.gov>
Cc: Bertrand, Charlotte <Bertrand.Charlotte@epa.gov>; Keller, Kaitlin <keller.kaitlin@epa.gov>; Echeverria, Marietta <Echeverria.Marietta@epa.gov>
Subject: RE: Here are the NOAA GC/OPR comments on the EPA docket announvement

NOAA combined comments into one set. And then I've done some editing to modify some of their suggestions (some have been rejected).
I think you can ignore all the comments as they are addressed through edits, but I have one comment where I think checking with OGC would be good.
Lets make sure they do a final scrub but this can be done while its at RCS—lets not hold it up.

If something is wrong or doesn't make sense please don't incorporate!!

Please let me know if you have questions.
Thanks!
Nancy

Nancy B. Beck, Ph.D., DABT
Deputy Assistant Administrator, OCSPP
P: 202-564-1273
Personal Phone / Ex. 6
beck.nancy@epa.gov

From: Guilaran, Yu-Ting
Sent: Thursday, March 8, 2018 3:49 PM
To: Beck, Nancy <Beck.Nancy@epa.gov>; Keigwin, Richard <Keigwin.Richard@epa.gov>
Cc: Bertrand, Charlotte <Bertrand.Charlotte@epa.gov>; Keller, Kaitlin <keller.kaitlin@epa.gov>; Echeverria, Marietta <Echeverria.Marietta@epa.gov>
Subject: RE: Here are the NOAA GC/OPR comments on the EPA docket announvement

Yes, I am relying the way OPP?PRD gets FRN encoded (see guidance below). I'm sure it's easier if the changes come from OCSPP IO. We can certainly send the current document forward to RCS for encoding if you feel it'll save time. I hope this helps, sorry about any confusion I've caused.

FRN resource guide for PRD CRMs:

- RCS will encode and return hardcopies to drafters for corrections/modifications/routing for signature; minor changes can be handwritten on hardcopy
    - If extensive changes are needed after encoding, drafter may wish to request Word version, make changes, and have the document encoded again
    - Be aware that there is a possibility for late-breaking changes to the Notice if a case is added or subtracted; changes could result in changes to the typesetting request, too.

Regards,

Yu-Ting Guilaran, P.E.
Director

Pesticide Re-evaluation Division (PRD)
Office of Pesticide Programs
Office of Chemical Safety and Pollution Prevention
(tel) 703 308 0052
(fax)703 308 8005
Mail code 7508P
Room number PY S9623

From: Beck, Nancy
Sent: Thursday, March 08, 2018 3:11 PM
To: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>; Keigwin, Richard <Keigwin.Richard@epa.gov>
Cc: Bertrand, Charlotte <Bertrand.Charlotte@epa.gov>; Keller, Kaitlin <keller.kaitlin@epa.gov>; Echeverria, Marietta <Echeverria.Marietta@epa.gov>
Subject: RE: Here are the NOAA GC/OPR comments on the EPA docket announvement

But RCS always has an electronic version as well that we can edit. Am I still confused?

I'm trying to get clarity from NOAA as the 2 versions they sent are not similar so its unclear what they really want..

Nancy B. Beck, Ph.D., DABT
Deputy Assistant Administrator, OCSPP
P: 202-564-1273
Personal Phone / Ex. 6
beck.nancy@epa.gov

From: Guilaran, Yu-Ting
Sent: Thursday, March 8, 2018 3:09 PM
To: Beck, Nancy <Beck.Nancy@epa.gov>; Keigwin, Richard <Keigwin.Richard@epa.gov>
Cc: Bertrand, Charlotte <Bertrand.Charlotte@epa.gov>; Keller, Kaitlin <keller.kaitlin@epa.gov>; Echeverria, Marietta <Echeverria.Marietta@epa.gov>
Subject: RE: Here are the NOAA GC/OPR comments on the EPA docket announvement

While it is possible to make changes after encoding, it is a hard-copy, redline-strikeout by hand so it is generally reserved for minor edits/corrections. The better way is to firm up the text before sending.

Regards,

Yu-Ting Guilaran, P.E.
Director

Pesticide Re-evaluation Division (PRD)
Office of Pesticide Programs
Office of Chemical Safety and Pollution Prevention
(tel) 703 308 0052
(fax)703 308 8005
Mail code 7508P
Room number PY S9623

From: Beck, Nancy
Sent: Thursday, March 08, 2018 3:07 PM

To: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>; Keigwin, Richard <Keigwin.Richard@epa.gov>
Cc: Bertrand, Charlotte <Bertrand.Charlotte@epa.gov>; Keller, Kaitlin <keller.kaitlin@epa.gov>; Echeverria, Marietta <Echeverria.Marietta@epa.gov>
Subject: RE: Here are the NOAA GC/OPR comments on the EPA docket announvement

Isn't it easy to make some edits after its encoded or is there something I'm not understanding?

Thanks.

Nancy B. Beck, Ph.D., DABT
Deputy Assistant Administrator, OCSPP
P: 202-564-1273
Personal Phone / Ex. 6
beck.nancy@epa.gov

From: Guilaran, Yu-Ting
Sent: Thursday, March 8, 2018 3:06 PM
To: Keigwin, Richard <Keigwin.Richard@epa.gov>; Beck, Nancy <Beck.Nancy@epa.gov>
Cc: Bertrand, Charlotte <Bertrand.Charlotte@epa.gov>; Keller, Kaitlin <keller.kaitlin@epa.gov>; Echeverria, Marietta <Echeverria.Marietta@epa.gov>
Subject: RE: Here are the NOAA GC/OPR comments on the EPA docket announvement

FYI for everyone that we just completed all the transfer to the FRN format and were ready to send for encoding but will wait for further instructions. Thanks.

Regards,

Yu-Ting Guilaran, P.E.
Director

Pesticide Re-evaluation Division (PRD)
Office of Pesticide Programs
Office of Chemical Safety and Pollution Prevention
(tel) 703 308 0052
(fax)703 308 8005
Mail code 7508P
Room number PY S9623

From: Keigwin, Richard
Sent: Thursday, March 08, 2018 2:35 PM
To: Beck, Nancy <Beck.Nancy@epa.gov>
Cc: Bertrand, Charlotte <Bertrand.Charlotte@epa.gov>; Keller, Kaitlin <keller.kaitlin@epa.gov>; Echeverria, Marietta <Echeverria.Marietta@epa.gov>; Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>
Subject: FW: Here are the NOAA GC/OPR comments on the EPA docket announvement

We just received these comments from NOAA/NMFS. Please advise regarding how you would like us to proceed.

From: Cathy Tortorici - NOAA Federal [mailto:cathy.tortorici@noaa.gov]
Sent: Thursday, March 08, 2018 2:33 PM
To: Keigwin, Richard <Keigwin.Richard@epa.gov>; Echeverria, Marietta <Echeverria.Marietta@epa.gov>; Guilaran, Yu-

Ting <Guilaran.Yu-Ting@epa.gov>
Subject: Here are the NOAA GC/OPR comments on the EPA docket announvement

Dear all -

Our people at NOAA did not realize that they were supposed to send these to you. So my front office asked me to send these comments to you all. These are comments from my Office of Protected Resources and NOAA General Counsel. as well as comments coming from NOAA.

If you can please forward these comments to Nancy Beck I would appreciate it since you said she was leading this document review process.

I would be happy to talk to whomever you want at EPA about these comments.

Thanks so much -

Cathy T.

--
Cathy Tortorici
Chief, ESA Interagency Cooperation Division
Office of Protected Resources
NOAA's National Marine Fisheries Service
1315 East-West Highway
Silver Spring, MD 20910
(w) 301.427.8495
(c) 301.602.2193
cathy.tortorici@noaa.gov

Message

From: Guilaran, Yu-Ting [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=A698774E93E34A2B9181D4B3032B8A32-YTGUILAR]
Sent: 1/4/2018 5:58:02 PM
To: Pease, Anita [Pease.Anita@epa.gov]
Subject: FW: For your review: draft desk statement on NMFS' BiOp

Importance: High

Regards,

Yu-Ting Guilaran, P.E.
Director

Pesticide Re-evaluation Division (PRD)
Office of Pesticide Programs
Office of Chemical Safety and Pollution Prevention
(tel) 703 308 0052
(fax)703 308 8005
Mail code 7508P
Room number PY S9623

From: Echeverria, Marietta
Sent: Tuesday, January 02, 2018 3:48 PM
To: Keigwin, Richard <Keigwin.Richard@epa.gov>
Cc: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>; Smith, Charles <Smith.Charles@epa.gov>; Anderson, Brian <Anderson.Brian@epa.gov>; Mosby, Jackie <Mosby.Jackie@epa.gov>; Han, Kaythi <Han.Kaythi@epa.gov>; Overstreet, Anne <overstreet.anne@epa.gov>
Subject: FW: For your review: draft desk statement on NMFS' BiOp
Importance: High

Rick,

As we discussed, we are getting requests through OGC for making the final NMFS BiOp available to interested parties. We discussed three options to respond to these requests with option 1 being our recommendation:

Deliberative Process / Ex. 5

Thanks,
Marietta

From: Han, Kaythi
Sent: Tuesday, January 02, 2018 3:09 PM
To: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>; Smith, Charles <Smith.Charles@epa.gov>; Echeverria, Marietta <Echeverria.Marietta@epa.gov>; Nesci, Kimberly <Nesci.Kimberly@epa.gov>
Cc: Sherman, Kelly <Sherman.Kelly@epa.gov>; Perry, Tracy <Perry.Tracy@epa.gov>; Anderson, Brian <Anderson.Brian@epa.gov>; Corbin, Mark <Corbin.Mark@epa.gov>; Overstreet, Anne <overstreet.anne@epa.gov>; Herndon, George <Herndon.George@epa.gov>; Mosby, Jackie <Mosby.Jackie@epa.gov>
Subject: For your review: draft desk statement on NMFS' BiOp
Importance: High

Yu-Ting, Billy, Marietta and Kimberly,

Please review the draft desk statement below on the recently received Biological Opinion from NMFS. Brian, Tracy and Kelly have reviewed and approved this version. We will need management to provide feedback on whether we want to house the BiOp in our docket, which is something that we have done in the past for other BiOps.

Thanks,
Kaythi

Draft Desk Statement:

Deliberative Process / Ex. 5

From: Mosby, Jackie
Sent: Friday, December 29, 2017 12:02 PM
To: Overstreet, Anne <overstreet.anne@epa.gov>; Lantz, Tracy <Lantz.Tracy@epa.gov>; Overbey, Dian <Overbey.Dian@epa.gov>; Borges-Silva, Quentin <Borges-Silva.Quentin@epa.gov>; OPP FEAD CSB <OPP_FEAD_CSB@epa.gov>
Subject: FW: NCAP v. NMFS

Please let me know who is in from CSB -- we need to start working on the NMFS desk statement. See email below. Thanks, Jackie

Jacqueline E. Mosby, MPH
Director, Field & External Affairs Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
Phone: 703-308-2226, Cell: 202-999-9784, Fax: 703-305-6244
1200 Pennsylvania Avenue, N.W.

Mailstop: 7506P
Washington, DC 20460
Mosby.jackie@epa.gov

From: Keigwin, Richard
Sent: Friday, December 29, 2017 11:17 AM
To: Bertrand, Charlotte <Bertrand.Charlotte@epa.gov>
Cc: Echeverria, Marietta <Echeverria.Marietta@epa.gov>; Hughes, Hayley <hughes.hayley@epa.gov>; Mosby, Jackie <Mosby.Jackie@epa.gov>; Herndon, George <Herndon.George@epa.gov>; Overstreet, Anne <overstreet.anne@epa.gov>
Subject: Re: NCAP v. NMFS

Thanks Charlotte. I'm looping in FEAD leadership so that we can start working on the desk statement.

There may be an opportunity to reinitiate consultation after the issuance of the NMFS BiOp. We can begin discussing this possibility with OGC next week.

Rick Keigwin
Director, Office of Pesticide Programs
U.S. Environmental Protection Agency
Phone: 703-305-7090
Website: www.epa.gov/pesticides
Sent from my iPhone

On Dec 29, 2017, at 10:38 AM, Bertrand, Charlotte <Bertrand.Charlotte@epa.gov> wrote:

> Resending to include Haley
>
> Sent from my iPhone
>
> On Dec 29, 2017, at 7:27 AM, Bertrand, Charlotte <Bertrand.Charlotte@epa.gov> wrote:
>
> > Hi - want to let you know that Eric and I reached out to CEQ, left a message for Chris. Will let you know if we hear anything back. I think we should start preparing a desk statement. I assume Deliberative Process / Ex. 5 Deliberative Process / Ex. 5 Deliberative Process / Ex. 5? Hope you both are doing well and had a happy holiday! Charlotte
> >
> > Sent from my iPhone
> >
> > On Dec 28, 2017, at 6:26 AM, Echeverria, Marietta <Echeverria.Marietta@epa.gov> wrote:
> >
> > > Yes, I informed Cathy that it is our understanding that coordination at the senior leadership is expected prior to transmittal. She was going to take that back.
> > >
> > > Sent from my iPhone

On Dec 27, 2017, at 7:18 PM, Keigwin, Richard <Keigwin.Richard@epa.gov> wrote:

Attorney Client / Ex. 5

From: Dyner, Mark
Sent: Wednesday, December 27, 2017 3:13 PM
To: Keigwin, Richard <Keigwin.Richard@epa.gov>; Echeverria, Marietta <Echeverria.Marietta@epa.gov>; Baptist, Erik <Baptist.Erik@epa.gov>; Bertrand, Charlotte <Bertrand.Charlotte@epa.gov>; Beck, Nancy <Beck.Nancy@epa.gov>; Anderson, Brian <Anderson.Brian@epa.gov>
Cc: Mclean, Kevin <Mclean.Kevin@epa.gov>; Perlis, Robert <Perlis.Robert@epa.gov>; Knorr, Michele <knorr.michele@epa.gov>
Subject: NCAP v. NMFS

Attorney Client / Ex. 5

Mark

Mark Dyner
Office of General Counsel
(202) 564-1754

Message

From: Guilaran, Yu-Ting [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=A698774E93E34A2B9181D4B3032B8A32-YTGUILAR]
Sent: 1/25/2018 3:17:40 PM
To: OPP PRD Managers [OPP_PRD_Managers@epa.gov]
Subject: FW: California Notice 2018-05: Final Decision Concerning Reevaluation of Diazinon Pesticides

Regards,

Yu-Ting Guilaran, P.E.
Director

Pesticide Re-evaluation Division (PRD)
Office of Pesticide Programs
Office of Chemical Safety and Pollution Prevention
(tel) 703 308 0052
(fax)703 308 8005
Mail code 7508P
Room number PY S9623

From: Keigwin, Richard
Sent: Wednesday, January 24, 2018 9:28 PM
To: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>; Echeverria, Marietta <Echeverria.Marietta@epa.gov>
Subject: Fwd: California Notice 2018-05: Final Decision Concerning Reevaluation of Diazinon Pesticides

Rick Keigwin
Director, Office of Pesticide Programs
U.S. Environmental Protection Agency
Phone: 703-305-7090
Website: www.epa.gov/pesticides
Sent from my iPhone

Begin forwarded message:

> **From:** "CANote@CDPR" <canote@cdpr.ca.gov>
> **Date:** January 24, 2018 at 6:53:28 PM EST
> **To:** "'canote@pestreg.cdpr.ca.gov'" <canote@pestreg.cdpr.ca.gov>
> **Subject: California Notice 2018-05: Final Decision Concerning Reevaluation of Diazinon Pesticides**
>
> This document and other California Notices to Stakeholders can also be viewed at <http://cdpr.ca.gov/docs/registration/canot/camenu.htm>.

FINAL DECISION CONCERNING REEVALUATION OF DIAZINON PESTICIDES

The Director of the Department of Pesticide Regulation (DPR) files this notice with the Secretary of the California Natural Resources Agency for posting pursuant to Title 3, California Code of Regulations (CCR). Title 3 CCR requires the Secretary of the California Natural Resources Agency and DPR to post this notice for thirty (30) days for public inspection.

REEVALUATION

In February 2003, DPR commenced reevaluation of agricultural products containing the active ingredient diazinon and intended for use as dormant sprays (California Notice 2003-02). In June 2010, DPR expanded the reevaluation to include in-season uses, as well as dormant season applications. This reevaluation involves two registrants and four pesticide products. A list of products included in the reevaluation is available on DPR's Web site at <http://cdpr.ca.gov/docs/registration/reevaluation/chemicals/diazinon.htm>.

BASIS OF REEVALUATION

DPR placed diazinon into reevaluation based on monitoring studies conducted between 1991 and 2001 by the U.S. Geological Survey, Dow AgroSciences, Central Valley Reginal Water Quality Control Board (CVRWQCB), State Water Resource Control Board (SWRCB), and DPR. These studies demonstrate the presence of diazinon in the Sacramento and San Joaquin Valleys at levels that exceed the water quality criteria (WQC) established by the Department of Fish and Wildlife (DFW) (formerly known as the Department of Fish and Game), especially during the dormant spray season. DPR placed all agricultural use diazinon products into reevaluation to determine the source of surface water contamination and to examine potential mitigation measures.

Under this reevaluation, diazinon registrants were required to: (1) identify the processes by which diazinon dormant spray products are contributing to detections in surface water at levels that exceed WQC; and, (2) identify mitigation strategies that reduce or eliminate diazinon residues in surface water. With the 2010 reevaluation scope expansion to include in-season sprays, DPR required diazinon registrants to: (1) collect and evaluate all relevant (2005-2009) surface water monitoring data; and, (2) establish crop-specific mitigation measures based upon results of submitted monitoring data.

During the course of this reevaluation, DPR implemented various mitigation measures and continues to work to prevent aquatic toxicity from diazinon residues in the Sacramento and San Joaquin rivers. DPR continued to monitor the efforts of U.S. Environmental Protection Agency (U.S. EPA), SWRCB, and CVRWQCB for possible opportunities to collaborate on mitigation.

REEVALUATION SUMMARY

In July 2005, DPR approved monitoring protocols submitted by the primary manufacturer intended to evaluate the effectiveness of the proposed mitigation strategies. In September 2006, the final studies were submitted, but did not address whether diazinon registrants intended to use the information to develop and implement additional mitigation measures. Meanwhile, DPR began working on possible mitigation measures. In July 2006, DPR adopted dormant spray regulations that placed further restrictions on the use of diazinon products. These regulations require the operator of the property to follow certain practices, prohibits certain applications, and

requires written recommendation from a pest control adviser before an application. By December 2006, all dormant spray diazinon product labels were amended.

In January 2007, U.S. EPA cancelled certain agricultural uses of diazinon in order to reduce risks to agricultural workers and the environment. In February 2007, DPR received a report prepared by University of California, Davis (UCD) titled, *Residues of the 2006 TMDL Monitoring of Pesticides in California's Central Valley Waterways, January–March 2006*. This study found diazinon concentrations measured during the 2006 dormant spray season were still exceeding WQC. DPR forwarded the UCD study to diazinon registrants and requested the development and implementation of further mitigation measures to reduce or eliminate diazinon residues in surface water. In February 2008, the primary manufacturer submitted two reports titled, *Analysis of Diazinon Environmental Monitoring Data from the Sacramento/Feather River Watersheds: 2001–2007* and *Project Report: Landguard OP-A as a Best Management Practice in Dormant Season Use, December 2007*. In October 2008, the primary manufacturer submitted another report titled, *Analysis of Diazinon Environmental Monitoring Data from the San Joaquin River Watershed: 2001–2007*.

In October 2009, DPR analysis of monitoring data from 2003-2008 found diazinon was detected in 637 out of 2,635 samples collected from water bodies located in the Central Valley, Central Coast, and southeastern California. As a result, on June 22, 2010, the Director expanded the reevaluation to include in-season uses, as well as dormant season applications and required additional data of diazinon registrants to better assess surface water runoff and exceedances. In March 2011, the primary manufacturer submitted a combined monitoring report for both dormant and in-season monitoring titled, *Summary of Diazinon Water Column Monitoring Data for Nine California Regions: 2005-2010*, which DPR found to be acceptable. In September 2011, DPR completed an analysis memo titled, *Analysis of Diazinon Agricultural Use in Regions of Frequent Surface Water Detections*.

Since its peak in 1993/1994, agricultural use of diazinon has been declining. Agricultural use in 2014 was only 5.4% of peak use in 1994. Likewise, there has been a general downward trend in water quality threshold exceedance frequencies. DPR has determined the current level of aquatic risk is *de minimis*. In June 2017, DPR completed an analysis of diazinon monitoring and use data titled, *A Review of Diazinon Use Contamination in Surface Waters, and Regulatory Actions in California across Water Years 1992-2014*, which was published in the journal of *Environmental Monitoring and Assessment*. DPR scientists also evaluated surface water monitoring results for 2015-2017. There have been no exceedances over the lethal concentration (LC_{50}) values for crustaceans, insects, or fish.

FINAL REEVALUATION DECISION

DPR has completed its evaluation of California diazinon use and surface water monitoring data and concluded that the use of diazinon in recent years has decreased to a level that does not pose a risk to aquatic organisms. DPR has determined no additional mitigation measures are necessary at this point. Therefore, the reevaluation is concluded.

For information on DPR's Reevaluation Program, please visit DPR's Web site at <http://cdpr.ca.gov/docs/registration/reevaluation/reevals.htm> or contact Ms. Denise Alder at <Denise.Alder@cdpr.ca.gov> or by telephone at 916-324-3522.

* *

PLEASE DO NOT REPLY DIRECTLY TO THIS MESSAGE. If you would like to contact a DPR representative about the California Registration Notice to Registrants , please send an e-mail
message to: canote@cdpr.ca.gov. Include your name, your e-mail address, a daytime phone number where you can be reached, and a brief description of the information you are interested in, and a representative will contact you.

FLEX YOUR POWER! For simple ways to reduce energy demand and costs, see <www.cdpr.ca.gov>.

* *

Action Information Sheet

SAN: ####; FRL: 9975-59

Action Title: Chlorpyrifos, Diazinon, and Malathion; National Marine Fisheries Service Biological Opinion Issued Under the Endangered Species Act; Notice of Availability

Docket No.: EPA-HQ-OPP-2018-0141

Purpose: This **Federal Register** notice announces the availability of and requests comment on the final Biological Opinion (BiOp) issued under the Endangered Species Act (ESA) by the National Marine Fisheries Service (NMFS), regarding the potential effects of chlorpyrifos, malathion, and diazinon on federally listed threatened or endangered species (listed species) and their designated critical habitats.

Background: The ESA requires Federal agencies to ensure that their actions are not likely to jeopardize the continued existence of species listed as threatened or endangered under the ESA, or destroy or adversely modify the designated critical habitat of such species. The registration of a pesticide under the Federal Insecticide, Fungicide, and Rodenticide Act constitutes an EPA "action" under the ESA. If EPA determines a pesticide may affect a listed species or its designated critical habitat, EPA must initiate consultation with the U.S. Fish and Wildlife Service (FWS) or NMFS (the Service or Services), as appropriate. In response to a Federal agency initiating formal consultation, the Service(s) develops a BiOp in which it provides its opinion on whether the "action" is likely to jeopardize the continued existence of a listed species and/or is likely to destroy or adversely modify designated critical habitat and, if so, describes reasonable and prudent alternatives (RPAs) to avoid the determination. The BiOp will also address whether the action will result in incidental take of listed species and, if so, provide a statement specifying the amount of any permitted incidental take and setting forth reasonable and prudent measures (RPMs) necessary or appropriate to minimize the impact of such take.

Consistent with EPA's responsibility under the ESA, on January 18, 2017, EPA released national-level endangered species Biological Evaluations (BEs) for chlorpyrifos, diazinon, and malathion to assess risks to listed species from registered uses of these pesticides. These BEs were completed in accordance with the joint Interim Approaches developed to implement the recommendations of the April 2013 National Academy of Sciences (NAS) report, *Assessing Risks to Endangered and Threatened Species from Pesticides*. The NAS report outlined recommendations on specific scientific and technical issues related to the development of pesticide risk assessments that EPA and the FWS and NMFS must conduct to meet their obligations under the ESA. In November 2013, the Services, EPA, and the U.S. Department of Agriculture released a white paper containing a summary of their joint Interim Approaches for assessing risks to listed species from pesticides. Details of the joint Interim Approaches are contained in the November 1, 2013 white paper *Interim Approaches for National-Level Pesticide Endangered Species Act Assessments Based on the Recommendations of the National Academy of Sciences April 2013 Report.* The methods developed as part of the joint Interim Approaches

will continue to be vetted before EPA utilizes these methods broadly to meet its ESA obligations.

On December 29, 2017, in response to a court-ordered deadline in the case of *Nw. Coal. for Alternatives to Pesticides, et al. v. NMFS*, Stipulation and Order, Dkt. 50, No. 07-1791-RSL (D. Wash. May 21, 2014), NMFS transmitted to EPA its final BiOp regarding the effects of the registration review under section 3 of FIFRA of these pesticides on listed species. The BiOp addressed the effects of these three pesticides on 77 listed species and 50 designated critical habitats and, in sum, 38 different species would likely be jeopardized with extinction and 37 critical habitat units would be destroyed or adversely modified. NMFS had sought from the court, but was not provided, additional time to complete the BiOp. On January 8, 2018, EPA confirmed receipt of the BiOp and informed NMFS of EPA's intention to reinitiate informal consultation on the BiOp so that the consultation on the pesticides could be informed by (1) input from stakeholders, (2) further interagency discussion and agreement on the jeopardy determination interim methods, and (3) additional data and analysis, including consideration of the best scientific and commercial data available on use and usage information. On February 21, 2018, EPA sent NMFS a letter requesting informal consultation on the same action. EPA will use the information and analysis received and developed in the course of the informal consultation to inform whether formal reinitiation of consultation on the BiOp is appropriate.

Stakeholder Involvement: Stakeholder input will be sought via written comment on the attached notice.

Key Issues/Internal Review: The documents were developed with participation from the program office and the Office of General Counsel.

Deadlines: As soon as possible. This action solicits critical public input on NMFS' BiOp, which should have been completed prior to the finalization of the BiOp in December, 2017.

Timing: Publication in the **Federal Register** should occur ASAP.

Contact: Tracy Perry, OPP, (703) 308-0128

Message

From: Guilaran, Yu-Ting [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=A698774E93E34A2B9181D4B3032B8A32-YTGUILAR]
Sent: 3/15/2018 2:52:19 PM
To: Chun, Melissa [Chun.Melissa@epa.gov]
CC: Perry, Tracy [perry.tracy@epa.gov]
Subject: FW: Signature Ready: Chlorpyrifos, Diazinon, and Malathion; Notice of Availability 18P-0053 9975-59
Attachments: 18P-0053.prd.docx

Importance: High

Hi Melissa
Attached is the revised version with the change to the subtitle "C". The hard copy will reflect this change. Thanks.

Regards,

Yu-Ting Guilaran, P.E.
Director

Pesticide Re-evaluation Division (PRD)
Office of Pesticide Programs
Office of Chemical Safety and Pollution Prevention
(tel) 703 308 0052
(fax)703 308 8005
Mail code 7508P
Room number PY S9623

From: Hofmann, Angela
Sent: Wednesday, March 14, 2018 3:20 PM
To: OCSPP RCS-FRDocuments <OCSPP_RCS-FRDocuments@epa.gov>; Perry, Tracy <Perry.Tracy@epa.gov>
Cc: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>; Artis, Sandra-S <artis.sandra-s@epa.gov>; Keigwin, Richard <Keigwin.Richard@epa.gov>; Beck, Nancy <Beck.Nancy@epa.gov>; Chun, Melissa <Chun.Melissa@epa.gov>
Subject: Signature Ready: Chlorpyrifos, Diazinon, and Malathion; Notice of Availability 18P-0053 9975-59
Importance: High

Hi Tracy,

I have completed the RCS Typeset Review, using the mark-up from Melissa. Attached is the typeset FR documents for your signature package. Please follow the instructions in this Email to avoid delays and complications.

What am I providing you?

1) FR Document – The final signature ready copy in Word.

<u>Important Note:</u>
You must print a SINGLE-SIDED copy of the FR document to use as the original for inclusion in the signature package that will be given to the official who must sign the FR document. The Office of the Federal Register (OFR) requires the original signature to be on a single-sided FR document. If the original signature is on a double-sided document, the OFR will NOT accept it and you will have to get your manager to sign a single-sided version. The other documents can be double sided.

<u>Process for Correcting the FR Document</u>:

I've attached the word version, in the event of needed changes. **Email the corrected version BEFORE you get the document signed. That way paper and electronic version match. Also, when you print your document for signature, please make sure it's in Times New Roman, 12 pt. and single sided. Make sure the signature page is on a separate page.**

2) **Concurrence Sheet -** Include this sheet in your signature package to document the reviewer's concurrences for your records. You do NOT need to return this to us – it is for YOUR records.

3) **Typesetting Request Form –**

   PLEASE make sure when you fill out the typesetting request form, in the large empty block, in the left top corner, you insert, my initials (SA) and 18T-0041.

   Follow the attached guidance document that was sent on June 16, 2015 that provides detailed instructions for staff and managers in OCSPP that must obtain the required publication funding commitment for an FR document. In addition to explaining how the form should be completed, this guidance document describes the typical process used to get the necessary funding commitment.

   Remember that you MUST complete the TSRF and get your funding commitment BEFORE the FR document is signed. You MUST submit a completed and funded TSRF to the RCS when you submit the signed FR Document to RCS for publication processing. (see attached pdfs)

4) **Docket Verification and Certification Form -** Use the electronically fillable and signature enabled pdf form that is available through FDMS. The completed form will be used to satisfy the "proof of docket" requirement because it shows that the docket manager has verified that the docket you cited has indeed been established for your action, and that you have or are in the process of submitting all of the relevant documentation that must be in the docket. You will need to provide completed form to us when you submit the signed FR Document for publication processing (see below).

5) **Action Information Sheet –** Required information from OP for all documents going to the OFR. Please fill out the information and return the form or else your document will not be processed for publication by OP.

AFTER signature, deliver the following to RCS in WJC East Rm 3139:

1) **FR Document – The original signed & dated FR Document.**

   Remember: The original must be SINGLE-SIDED!

2) **Typesetting Request Form – The original signed, funded & dated form.**

   Remember: Include documentation of the funding commitment.

3) **Docket Verification and Certification Form – The signed & dated form.**

4) **Action Information Sheet – send back the paper copy!**

If you have questions, let me know.

THANKS,

Angela Hofmann

OCSPP/RCS
202-564-0258

From: OCSPP RCS-FRDocuments
Sent: Wednesday, March 14, 2018 9:11 AM
To: Perry, Tracy <Perry.Tracy@epa.gov>
Cc: Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>; Hofmann, Angela <Hofmann.Angela@epa.gov>; Artis, Sandra-S <artis.sandra-s@epa.gov>
Subject: Chlorpyrifos, Diazinon, and Malathion; Notice of Availability 18P-0053 9975-59
Importance: High

This Email confirms that your FR document has been received by RCS. The initial screening is complete. Your document has been entered into our system, forwarded to the Director to assign an RCS Reviewer to do the typeset review, and it is assigned the following tracking numbers:

RCS 18P-0053 FRL 9975-59

Once the RCS Director assigns this document, you will receive a follow-up Email from the Director that identifies the RCS Reviewer. That Email will also contain other important information about the processing of your document.

Use the numbers identified above in any subsequent inquiry or communications about this document.

Minnie Lanier
Document Management Specialist
Regulatory Coordination Staff
Office of Chemical Safety and Pollution Prevention
EPA East-West Connecting Wing
Rm. 3385A
RCS Office Telephone: 202-566-1580

From: Perry, Tracy
Sent: Friday, March 09, 2018 4:21 PM
To: OCSPP RCS-FRDocuments <OCSPP_RCS-FRDocuments@epa.gov>
Cc: Hofmann, Angela <Hofmann.Angela@epa.gov>; Guilaran, Yu-Ting <Guilaran.Yu-Ting@epa.gov>
Subject: FRN for encoding--OCSPP IO priority
Importance: High

Hello,

We believe the publication of the attached FRN is a priority action for Nancy Beck.

Tracy L. Perry
Senior Regulatory Advisor
Risk Management and Implementation Branch III
Pesticide Re-evaluation Division

Office of Pesticide Programs
U.S. Environmental Protection Agency
(703) 308-0128

BILLING CODE 6560-50-P

ENVIRONMENTAL PROTECTION AGENCY

[EPA-HQ-OPP-2018-0141; FRL-9975-59]

Chlorpyrifos, Diazinon, and Malathion; National Marine Fisheries Service Biological Opinion Issued Under the Endangered Species Act; Notice of Availability

AGENCY: Environmental Protection Agency (EPA).

ACTION: Notice.

SUMMARY: The Environmental Protection Agency (EPA) is seeking comment on the final Biological Opinion (BiOp) issued under the Endangered Species Act (ESA) by the National Marine Fisheries Service (NMFS), regarding the potential effects of chlorpyrifos, malathion, and diazinon on federally listed threatened or endangered species (listed species) and their designated critical habitats.

DATES: Comments must be received on or before [*insert date [60] days after date of publication in the* **Federal Register**].

ADDRESSES: Submit your comments, identified by docket identification (ID) number EPA-HQ-OPP-2018-0141, by one of the following methods:

• *Federal eRulemaking Portal*: *http://www.regulations.gov*. Follow the online instructions for submitting comments. Do not submit electronically any information you consider to be Confidential Business Information (CBI) or other information whose disclosure is restricted by statute.

• *Mail*: OPP Docket, Environmental Protection Agency Docket Center (EPA/DC), (28221T), 1200 Pennsylvania Ave., NW., Washington, DC 20460-0001.

• *Hand Delivery*: To make special arrangements for hand delivery or delivery of

boxed information, please follow the instructions at *http://www.epa.gov/dockets/contacts.html*.

Additional instructions on commenting or visiting the docket, along with more information about dockets generally, is available at *http://www.epa.gov/dockets*.

FOR FURTHER INFORMATION CONTACT: Tracy Perry, Pesticide Re-Evaluation Division (7508P), Office of Pesticide Programs, Environmental Protection Agency, 1200 Pennsylvania Ave., NW., Washington, DC 20460-0001; telephone number: (703) 308-0128; email address: Perry.Tracy@epa.gov.

SUPPLEMENTARY INFORMATION:

I. General Information

A. Does this Action Apply to Me?

This action is directed to the public in general, and may be of interest to a wide range of stakeholders including environmental, farm worker, and agricultural advocates; the chemical industry; pesticide users; and members of the public interested in the sale, distribution, or use of pesticides and/or the potential impacts of pesticide use on listed species and designated critical habitat. Since others also may be interested, the Agency has not attempted to describe all the specific entities that may be affected by this action.

B. What Should I Consider as I Prepare My Comments for EPA?

1. *Submitting CBI.* Do not submit this information to EPA through regulations.gov or email. Clearly mark the part or all of the information that you claim to be CBI. For CBI information in a disk or CD-ROM that you mail to EPA, mark the outside of the disk or CD-ROM as CBI and then identify electronically within the disk or CD-ROM the specific information that is claimed as CBI. In addition to one complete version of the comment that

includes information claimed as CBI, a copy of the comment that does not contain the information claimed as CBI must be submitted for inclusion in the public docket. Information so marked will not be disclosed except in accordance with procedures set forth in 40 CFR part 2.

2. *Tips for preparing your comments*. When preparing and submitting your comments, see the commenting tips at *http://www.epa.gov/dockets/comments.html*.

C. How Can I Get Copies of this Document and Other Related Information?

A copy of the *Biological Opinion on Pesticides: Chlorpyrifos, Diazinon, and Malathion* is available in the docket under docket identification (ID) number EPA-HQ-OPP-2018-0141.

II. What Action is the Agency Taking?

A. Authority

The ESA requires Federal agencies, such as EPA, to ensure that their actions are not likely to jeopardize the continued existence of species listed as threatened or endangered under the ESA, or destroy or adversely modify the designated critical habitat of such species. The registration of a pesticide under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) constitutes an EPA "action" under the ESA. If EPA determines a pesticide may affect a listed species or its designated critical habitat, EPA must initiate consultation with the U.S. Fish and Wildlife Service (FWS) or NMFS (the Service or Services), as appropriate. In response to a Federal agency initiating formal consultation, the Service(s) develops a BiOp in which it provides its opinion on whether the "action" is likely to jeopardize the continued existence of a listed species and/or is likely to destroy or adversely modify designated critical habitat and, if so, describes reasonable and prudent alternatives (RPAs) to avoid the

determination. The BiOp will also address whether the action will result in incidental take of listed species and, if so, provide a statement specifying the amount of any permitted incidental take and setting forth reasonable and prudent measures (RPMs) necessary or appropriate to minimize the impact of such take.

B. Background

Consistent with EPA's responsibility under the ESA, on January 18, 2017, EPA released national-level endangered species Biological Evaluations (BEs) for chlorpyrifos, diazinon, and malathion to assess risks to listed species from registered uses of these pesticides. These BEs were completed in accordance with the joint Interim Approaches developed to implement the recommendations of the April 2013 National Academy of Sciences (NAS) report, *Assessing Risks to Endangered and Threatened Species from Pesticides*. The NAS report outlined recommendations on specific scientific and technical issues related to the development of pesticide risk assessments that EPA and the FWS and NMFS must conduct to meet their obligations under the ESA. In November 2013, the Services, EPA, and the U.S. Department of Agriculture (USDA) released a white paper containing a summary of their joint Interim Approaches for assessing risks to listed species from pesticides. Details of the joint Interim Approaches are contained in the November 1, 2013 white paper *Interim Approaches for National-Level Pesticide Endangered Species Act Assessments Based on the Recommendations of the National Academy of Sciences April 2013 Report*. The methods developed as part of the joint Interim Approaches will continue to be vetted before EPA utilizes these methods broadly to meet its ESA obligations. Additional information on endangered species risk assessment and the NAS report recommendations are available at **[HYPERLINK "https://www.epa.gov/endangered-species/implementing-nas-report-**

recommendations-ecological-risk-assessment-endangered-and"].

On December 29, 2017, in response to a court-ordered deadline in the case of *Nw. Coal. for Alternatives to Pesticides, et al. v. NMFS*, Stipulation and Order, Dkt. 50, No. 07-1791-RSL (D. Wash. May 21, 2014), NMFS transmitted to EPA its final BiOp regarding the effects of the registration review under section 3 of FIFRA of these pesticides on listed species. The BiOp addressed the effects of these three pesticides on 77 listed species and 50 designated critical habitats and, in sum, 38 different species would likely be jeopardized with extinction and 37 critical habitat units would be destroyed or adversely modified. NMFS had sought from the court, but was not provided, additional time to complete the BiOp. On January 8, 2018, EPA confirmed receipt of the BiOp and informed NMFS of EPA's intention to reinitiate informal consultation on the BiOp so that the consultation on the pesticides could be informed by (1) input from stakeholders, (2) further interagency discussion and agreement on the jeopardy determination interim methods, and (3) additional data and analysis, including consideration of the best scientific and commercial data available on use and usage information. On February 21, 2018, EPA sent NMFS a letter requesting informal consultation on the same action. EPA will use the information and analysis received and developed in the course of the informal consultation to inform whether formal reinitiation of consultation on the BiOp is appropriate.

C. Public Involvement ~~to Date~~Process

As a result of the U.S. District Court Western District of Washington at Seattle's failure to extend NMFS's court-ordered deadline, NMFS issued the final BiOp without having received input from the public and applicants (pesticide registrants), which is at odds with EPA's 2013 public stakeholder process for ESA consultations – an open and transparent

process supported by the Services, EPA, and USDA. As explained in the 2013 public stakeholder document, stakeholder input is critical to the development and evaluation of any measures EPA may implement to address risks to listed species and designated critical habitat. Accordingly, EPA is seeking comment on the BiOp to receive stakeholder and public input prior to either reinitiating consultation on the BiOp or implementing the measures of BiOp. EPA will evaluate the input received in determining how it will proceed with respect to the final BiOp.

D. Public Comments Sought

The BiOp for chlorpyrifos, diazinon, and malathion is being included in the docket (EPA-HQ-OPP-2018-0141) to seek input on NMFS's jeopardy findings, RPMs and RPAs, and to solicit additional use and usage information. Specifically:

1. Comments on the scientific approaches and data sources used to support the BiOp and reach determinations for the listed species and critical habitat.

2. Comments on the RPAs and RPMs. Can they be reasonably implemented? If not, describe why not. Are there different measures that may provide equivalent protection to the ones in the BiOp but result in less impact to pesticide users?

3. Comments on national- and state-level use and usage data and information. In particular, EPA is seeking usage data and information for non-agricultural use sites (e.g., nurseries, managed forests, pasture, rights-of-way, golf courses, and wide-area mosquito control). If possible, provide sources of data and information that should be considered.

Authority: 7 U.S.C. 136 *et seq.*

Dated: _____

_____

Yu-Ting Guilaran,

Director, Pesticide Re-Evaluation Division, Office of Pesticide Programs.

Appointment

From: Jewell, Shannon [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=1862c405cf704ee692d5f61faff12688-Jewell, Sha]
Sent: 9/3/2019 4:27:30 PM
To: Keigwin, Richard [Keigwin.Richard@epa.gov]; Miller, Wynne [Miller.Wynne@epa.gov]; Messina, Edward [Messina.Edward@epa.gov]; Lowit, Anna [Lowit.Anna@epa.gov]; Dawson, Jeffrey [Dawson.Jeff@epa.gov]; OPP Division Directors [OPP_Division_Directors@epa.gov]; Buckley, Katherine [Buckley.Katherine@epa.gov]; Arceo, Julian [arceo.julian@epa.gov]; Craig, Evisabel [Craig.Evisabel@epa.gov]; Wilbur, Donald [Wilbur.Donald@epa.gov]
CC: Mendelsohn, Mike [Mendelsohn.Mike@epa.gov]; Wozniak, Chris [wozniak.chris@epa.gov]; Borges, Shannon [Borges.Shannon@epa.gov]; Overstreet, Anne [overstreet.anne@epa.gov]; Hebert, John [Hebert.John@epa.gov]

Subject: Hold for EFSA Visit
Attachments: Agenda_EFSA_EPAOPP_06092019_FINAL.docx; 2019 EFSA visit presentation.pptx; Brussels Mission Cable May 2019.docx; Follow Up Actions List from EPA Wheeler visit to the EU May 2019.docx; Working Arrangements Document from May 3_scanned copy.pdf
Location: PYS-4370/80

Start: 9/6/2019 1:00:00 PM
End: 9/6/2019 4:30:00 PM
Show Time As: Busy

9/4: Attaching slides "2019 EFSA visit presentation" that Evisabel will present.
Additionally, Katherine Buckley, Europe and Eurasia Senior Program Manager from OITA, who will attend Friday's meeting, wanted to make sure that the OPP group is aware of current cooperative activities between OPP and the European Commission's Directorate-General for the Environment (DG ENV). Katherine sent the 3rd , 4th and 5th attached docs to this end, which center on the Administrator's visit to Brussels, and meeting with DG ENV and Directorate-General for Health and Food Safety (DG SANTE), in May of 2019. In short, both DG ENV and EFSA are expressing interest in increased cooperation and data exchange.

The full Brussels meeting reporting cable is attached, as is the Follow Up Actions List, but here are OPP related excerpts:

Excerpt from US Mission to the EU Reporting Cable on EPA Administrator Mission to Brussels in May 2019:
EU Interested in Cooperating on Pesticides

4. (SBU) The EPA Administrator and Director-General for Health and Food Safety Anne Bucher met on May 2 to discuss U.S. and EU pesticide policies, particularly the differences between hazard-based versus risk-based assessment programs; Maximum Residue Limits (MRLs) and import tolerances; and the regulatory status of certain pesticides, including clothianidin, glyphosate, and chlorpyrifos. EPA raised concerns with the hazard-based criteria used by the EU for pesticide assessments. DG SANTE officials responded that the EU evaluation program is generally risk-based, with the exception of narrowly defined hazard-criteria for certain categories: carcinogenic, mutagenic, and endocrine disruptors (EDs). (Comment: The hazard criteria cover more than these three categories, so are broader than DG SANTE expressed. End Comment.) When establishing MRLs, DG SANTE said it uses a risk-based approach, and EU member states can apply for import tolerances for banned substances. This process usually takes about two years. (Comment: The USG is unaware of any banned substance going through the full import tolerance request procedure and cannot verify this timing. End Comment). DG SANTE stressed that its risk-based and hazard-based criteria evaluations are both compliant with international trade rules and are science-based. They were keen that no misleading statements about the EU pesticide evaluation program be shared at international fora such as the WTO SPS Committee. (Comment: DG SANTE skirted the points that WTO SPS rules are risk-based and that the United States is one of a dozen countries raising the EU's hazard-based approach at the WTO SPS Committee. End Comment)

5. (SBU) Both sides also discussed the impact of media reports on consumer confidence in pesticides and agreed to work together to ensure consumers know the foods they consume are safe. DG SANTE asked about the recent U.S. decision on glyphosate and the potential impact on U.S. court cases. EPA said it just released an interim review decision for glyphosate, reiterating its determination that it is not carcinogenic, but could not say how this decision would impact appeal cases. EPA offered to host the next technical bilateral exchange later this year to follow up from the previous collaboration in April.

OPP-related items from the Follow Up Actions List:
- Seeking opportunities to increase the sharing of information (e.g. evaluations, data gaps etc.) and possible relaunch of the "rolling work plan" (ECHA, ENV)
- Exchange of best practices to reduce animal testing.
- ENV to share information on measures related to methylene chloride and legislation adopted at National level.
- ENV to visit EPA at technical level in June

8/23: Replacing agenda with an updated version in which topic 2(a) is now specific to BMD in risk assessment, rather than chemical risk assessment in general. Dana V. and Anna Lowit are looking into this update.
8/5: Firming up meeting time and adding the final agenda. Please note: EFSA Officials really wanted to know who would be attending. I told them I could give them names of those who had accepted the meeting, but could not assure them that those specific officials would be there, and may send deputies, etc. So if you see your name on the agenda but you won't actually attend, it is okay.

Hi group,

The European Food Safety Authority (EFSA) Executive Director, Bernhard Url spent his first term looking at EU issues. Now, during his second term, he wants to focus on international issues and relationships. He is particularly focused on getting formalized agreements in place; see the paragraph highlighted below on this, as well as a list of topics of interest. Risk Assessments are top of the list and during a call with Shannon Jewell about scheduling the meeting, Jeff Moon and Antonia Corini of EFSA, focused almost exclusively on the topic of RAs.

EFSA welcomes your input and topics for the meeting. Shannon can relay them, so feel free to send anything you have to her.

Related emails:
From: CORINI Antonia <Antonia.CORINI@ext.efsa.europa.eu> **On Behalf Of** international.cooperation
Sent: Thursday, June 13, 2019 6:25 AM
To: Jewell, Shannon <jewell.shannon@epa.gov>
Cc: international.cooperation <international.cooperation@efsa.europa.eu>; MOON Jeffrey <Jeffrey.MOON@efsa.europa.eu>
Subject: RE: High Level meeting EFSA Executive Director, September

Dear Shannon,

Thank you very much for your time and availability yesterday. We very much appreciate your help in organizing the bilateral meeting between EPA and EFSA, on 6 September 2019. As discussed, please find below a short description of EFSA and its remit and some of the topics we are keen to further explore and believe might be of common interest. We understand that further exploration is also ongoing for a possible inter-agency meeting on cross-agency topics such as methodology, which would be very interesting and valuable. The EFSA delegation for the USA and Canada visit will consist of our Executive Director, Bernhard Url, the Directors of our two science departments, our Chief scientist and the External Engagement Team Leader.

Please let us know if this information would be sufficient to help you with further follow-up steps. In any case, don't hesitate to contact us if you would need further clarification.

Thank you very much in advance
Looking forward to a fruitful collaboration

Best Regards,
Antonia

EFSA is an independent European agency funded by the European Union. The agency was legally established by the EU under the General Food Law (Regulation (EC) No 178/2002). It is responsible for risk assessment and also has a duty to communicate its scientific findings to the public. Risk management is carried out by the European Commission and the Member States authorities - not by EFSA.

EFSA produces scientific opinions and provides scientific advice that form the basis for European policies and legislation. Its remit covers:

- *Food and feed safety*
- *Nutrition*
- *Animal health and welfare*
- *Plant protection*
- *Plant health*
- *Environmental risk assessment, where needed to evaluate the possible impact of the food chain on the biodiversity of plant and animal habitats.*

A set of key values guides all of EFSA's activities. These are:

- *Scientific excellence*
- *Independence*
- *Openness*
- *Innovation*
- *Cooperation*

Scientific cooperation is central to EFSA's scientific work. EFSA works closely with partners and stakeholders not only across Europe but also around the world, sharing scientific expertise, data and knowledge. The main purpose of EFSA's visit to the USA is to consolidate the ongoing cooperation, engage in discussions of mutual interest to establish a convergence of approach and to identify new areas for cooperation as a means for preparedness.
At present there is no cooperation agreement in place although there were discussions, with EPA, on this in 2013. This could be a topic to discuss further.
To recall some of the past interactions between EFSA and EPA, since 2016, EFSA takes part in the international group "Accelerating the Pace of Chemical Risk Assessment (APCRA)" and participated to the workshop held at EPA (ORD) in 2016. Also, our colleague Didier Verloo (HoU in the Assessment and Methodological Support Unit) visited EPA in March 2018 and your colleague Leonid Kopylev spent period in EFSA (September-October 2018). There have been also other exchanges (e.g. in November 2018: joint webinar on EFSA/ EPA's experiences on Systematic Review and the use of corresponding AI tools). Also EPA is involved in the ILMERAC as well as EFSA.

Possible topics of discussion in the areas of plant and animal health, chemical and microbiological risk assessment could be:

- *Methodology development for risk assessment:*
  - *environmental risk assessment tools*
  - *RA of cumulative exposure,*
  - *chemical risk assessment,*
  - *environmental impact of dsRNA products,*
  - *AI and automatization of systematic reviews for evidence-based decision making*
- *New technologies*
  - *gene drive application,*

- synthetic biology,
- whole genome sequencing,
- gene editing,
- nanotechnology

- *Data access, management and usage*
  - information sharing,
  - environmental effects of pesticide use on feed on AMR,
  - food composition;
  - data structure and data models
- *Risk communication and stakeholder engagement*

The above list could be amended following your feedback and indication of possible other themes of discussion.

Dr. Antonia Corini, PhD
External Engagement Team
ENCO UNIT
Communication, Engagement & Cooperation Department

Via Carlo Magno 1A
43126 Parma (Italy)
www.efsa.europa.eu
twitter.com/EFSA_EU
youtube.com/EFSAchannel

Message

From: Sims, Diann [Sims.Diann@epa.gov]
Sent: 1/24/2018 5:40:46 PM
To: Pease, Anita [Pease.Anita@epa.gov]
Subject: RE: ESA Usage Data Deliverables Table

Thanks Anita,
I spoke with Brian earlier and we will adjust our delivery schedule a bit to give EFED a bit more lead time.
Nice tag line below, btw ☺

From: Pease, Anita
Sent: Wednesday, January 24, 2018 12:39 PM
To: Sims, Diann <Sims.Diann@epa.gov>
Cc: Miller, Wynne <Miller.Wynne@epa.gov>; Anderson, Brian <Anderson.Brian@epa.gov>
Subject: RE: ESA Usage Data Deliverables Table

Hi Diane. Sounds like we are on the same page. FYI Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Deliberative Process / Ex. 5

Thanks,

Anita Pease
Acting Director
Antimicrobials Division (AD)
Office of Pesticide Programs
U.S. Environmental Protection Agency

703-305-0392
pease.anita@epa.gov

From: Sims, Diann
Sent: Wednesday, January 24, 2018 11:09 AM
To: Pease, Anita <Pease.Anita@epa.gov>
Subject: FW: ESA Usage Data Deliverables Table

Hi Anita,
Hope all is well in AD. Wynne brought to my attention that Rick is using the dates below in his correspondence concerning the completion of the B.E.s. I want to make sure that there is no miscommunication on our part.
We do plan to deliver the methomyl to EFED on schedule (a bit of a push as we don't actually go to PRP until Jan 31st; I don't anticipate major changes as we have already met with EFED on the draft.
Let me know if we need to offer further clarifications.

From: Sims, Diann
Sent: Tuesday, December 5, 2017 5:34 PM
To: Pease, Anita <Pease.Anita@epa.gov>
Cc: Paisley-Jones, Claire <Paisley-Jones.Claire@epa.gov>; Atwood, Donald <Atwood.Donald@epa.gov>; Miller, Wynne <Miller.Wynne@epa.gov>
Subject: ESA Usage Data Deliverables Table

Anita,
Here is a table with our planned milestones for completed the usage data for the Services. We plan to update this table periodically. Let me know if you have questions or suggestions.

Chemical	Analyst	Status
Methomyl	Atwood	In Progress
Carbaryl	Paisley-Jones	In Progress
Chlorpyrifos	Paisley-Jones	Pending
Malathion	Atwood	Pending

Deliberative Process / Ex. 5

Message

From: EPA Pesticides Programs [oppt.epa@public.govdelivery.com]
Sent: 3/23/2018 5:21:11 PM
To: Pease, Anita [Pease.Anita@epa.gov]
Subject: Pesticide Program Update: EPA Opens Comment Period on NMFS's Assessment on Chlorpyrifos, Diazinon, and Malathion

EPA Pesticide Program Updates

From EPA's Office of Pesticide Programs

www.epa.gov/pesticides

March 23, 2018

In This Update:

EPA Opens Comment Period on NMFS's Assessment on Chlorpyrifos, Diazinon, and Malathion

EPA is seeking comment on the National Marine Fisheries Service's (NMFS's) Biological Opinion (BiOp) for the pesticides chlorpyrifos, diazinon, and malathion. EPA is asking for input from stakeholders on NMFS's jeopardy findings, Reasonable and Prudent Measures (RPMs), and Reasonable and Prudent Alternatives (RPAs), and is soliciting additional information, including comment on:

1. The scientific approaches and data sources used to support the BiOp and reach determinations for the listed species and critical habitat.
2. The RPAs and RPMs. Can they reasonably be implemented? If not, why not? Are there different measures that may provide equivalent protection to the ones in the BiOp but result in less impact on pesticide users?
3. National- and state-level use and usage data and information, in particular, information for non-agricultural use sites (e.g., nurseries, managed forests, pasture, rights-of-way, golf courses, and wide-area mosquito control). If possible, provide sources for data that should be considered.

EPA encourages input on the RPMs and RPAs from pesticide users, registrants, public interest organizations, other interested parties, and state, tribal, and local governments. This request for input is necessary because, as a result of the U.S. District Court Western District of Washington's failure to extend NMFS's court-ordered deadline, NMFS had to issue the final BiOp in December 2017 without having received input from the public and applicants (pesticide registrants). This was at odds with EPA's 2013 public stakeholder process for ESA consultations – an open and transparent process supported by the Services, EPA, and USDA.

This input will be considered prior to either reinitiating consultation on the BiOp or implementing the measures of BiOp. EPA will evaluate the comments received in determining how to proceed with respect to the final BiOp, whether the alternatives or measures can reasonably be implemented, and

whether there are different measures that may provide equivalent protection but result in less impact on pesticide users.

The Biological Opinion is included in docket EPA-HQ-OPP-2018-0141 at Regulations.gov. EPA will forward to NMFS for its consideration all public comments we receive on this Biological Opinion.

Submit your comments, identified by the docket identification (ID) number EPA-HQ-OPP-2018-0141 and the pesticides to which the Biological Opinion pertains, by one of the following methods:

- **Federal eRulemaking Portal:** Regulations.gov. Follow the online instructions for submitting comments.
- **Mail:** Office of Pesticide Programs (OPP) Docket, Environmental Protection Agency, Mail Code 28221T, 1200 Pennsylvania Ave. NW., Washington, DC 20460.
- **Delivery:** EPA Docket Center (EPA/DC), EPA West, Room 3334, 1301 Constitution Ave. NW., Washington, DC 20460. Such deliveries are only accepted during the Docket's normal hours of operation, and special arrangements should be made for deliveries of boxed information. Hours of operation are 8:30 a.m. to 4:30 p.m., Monday through Friday, excluding legal holidays. The telephone number is (202) 566-1744.

EPA distributes its Pesticide Program Updates to external stakeholders and citizens who have expressed an interest in the agency's pesticide program activities and decisions. This update service is part of EPA's continuing effort to improve public access to federal pesticide information.

For general questions about pesticides and pesticide poisoning prevention, contact the National Pesticide Information Center (NPIC), by email at npic@ace.orst.edu or, by visiting http://npic.orst.edu.

For information about ongoing activities in the Office of Pesticide Programs, visit our homepage at: https://www.epa.gov/pesticides.

You can unsubscribe or update your subscriptions or e-mail address at any time on your Subscriber Preferences Page. All you will need is your e-mail address. If you have any questions or problems, please e-mail subscriberhelp.govdelivery.com for assistance.

This service is provided to you at no charge by the U.S. Environmental Protection Agency.

This email was sent to pease.anita@epa.gov using GovDelivery Communications Cloud on behalf of: U.S. EPA Office of Chemical Safety and Pollution Prevention · 707 17th St, Suite 4000 · Denver, CO 80202 · 1-800-439-1420

Message

From: Jimmy Liu [liu@fien.com]
Sent: 3/23/2018 3:24:35 PM
To: liu@fien.com
CC: Perry, Tracy [Perry.Tracy@epa.gov]
Subject: Update - Chlorpyrifos, Diazinon, and Malathion - Assessing Pesticide Risks to Endangered Species - EPA is seeking comments on the final Biological Opinion issued under the Endangered Species Act by the National Marine Fisheries Service (NMFS), regarding..

Update - Chlorpyrifos, Diazinon, and Malathion - Assessing Pesticide Risks to Endangered Species - EPA is seeking public comments on the "... final Biological Opinion issued under the Endangered Species Act (ESA) by the National Marine Fisheries Service (NMFS), regarding the potential effects of chlorpyrifos, malathion, and diazinon on federally listed threatened or endangered species ... and their designated critical habitats ..."

Document Title: * The title of the March 23, 2018 EPA Federal Register Notice is "Chlorpyrifos, Diazinon, and Malathion; National Marine Fisheries Service Biological Opinion Issued Under the Endangered Species Act; Notice of Availability"
* The title of the June 6, 2016 EPA Federal Register Notice is "Stakeholder Workshop To Discuss Interim Scientific Methods Used in Draft Biological Evaluations; Notice of Public Meeting"
* The title of the April 22, 2016 Pesticide News Story is "Save the Date & Register for Technical Webinar on Recently Released Endangered Species Assessments"
* The title of the April 11, 2016 EPA Federal Register Notice is "Chlorpyrifos, Diazinon, and Malathion Registration Review; Draft Biological Evaluations; Notice of Availability"
* The title of the April 6, 2016 EPA Pesticide News Story is "EPA Releases Draft Biological Evaluations of Three Chemicals' Impacts on Endangered Species"
* The title of the December 11, 2015 EPA Pesticide News Story is "Supporting Documents for Endangered Species Biological Evaluations Now Available"
* The title of the March 26, 2015 EPA Federal Register Notice is "Environmental Protection Agency; Notice of Public Meeting"
* The title of the December 15, 2014 EPA Pesticide News Story is "Status Report to Congress on Endangered Species Act Implementation in Pesticide Regulation"
* The title of the September 17, 2014 and April 2, 2014 EPA Federal Register Notices is "Office of Pesticide Programs; Notice of Public Meeting"
* The title of the November 1, 2013 Pesticide News Story is "Assessing Pesticide Risks to Endangered Species - Workshop on Implementing NAS Recommendations"

Organization: The March 23, 2018 Federal Register Notice was signed on March 15, 2018 by Yu-Ting Guilaran who is the Director of the Pesticide Re-evaluation Division of the Office of Pesticide Programs, a unit of the EPA Office of Chemical Safety and Pollution Prevention

The June 6, 2016 and April 11, 2016 Federal Register Notices were signed on May 31, 2016 and March 28, 2016, respectively, by Michael Goodis who is the Acting Director of the Pesticide Re-evaluation Division of the Office of Pesticide Programs, a unit of the EPA Office of Chemical Safety and Pollution Prevention

The April 22, 2016, April 6, 2016, December 11, 2015 and December 15, 2014 Pesticide News Stories were issued by the EPA Office of Pesticide Programs

The March 26, 2015 and September 17, 2014 Federal Register Notices were signed on March 17, 2015 and September 11, 2014, respectively, by Richard P. Keigwin, Jr. who is the Director of the Pesticide Re-evaluation

Division of the Office of and Pesticide Programs, a unit of the EPA Office of Chemical Safety and Pollution Prevention

The April 2, 2014 Federal Register Notice was signed on March 25, 2014 by Michael Goodis who is the Acting Director of the Pesticide Re-evaluation Division of the Office of Pesticide Programs, a unit of the EPA Office of Chemical Safety and Pollution Prevention

Source: * March 23, 2018, June 6, 2016, April 11, 2016, March 26, 2015, September 17, 2014 and April 2, 2014 EPA Federal Register Notices
* April 22, 2016, April 6, 2016, December 11, 2015, December 15, 2014 and November 1, 2013 EPA Pesticide News Stories

Comments Due By: May 22, 2018

Web site: The March 23, 2018 EPA Federal Register Notice is posted at
http://www.gpo.gov/fdsys/pkg/FR-2018-03-23/html/2018-06026.htm

A copy of the Biological Opinion on Pesticides: Chlorpyrifos, Diazinon, and Malathion will be made available at
http://www.regulations.gov/searchResults?rpp=25&po=0&s=EPA-HQ-OPP-2018-0141&fp=true&ns=true

The biological evaluations for each pesticide are posted at:
Chlorpyrifos: http://www.epa.gov/endangered-species/biological-evaluation-chapters-chlorpyrifos-esa-assessment
Diazinon: http://www.epa.gov/endangered-species/biological-evaluation-chapters-diazinon-esa-assessment
Malathion: http://www.epa.gov/endangered-species/biological-evaluation-chapters-malathion-esa-assessment

Additional information on endangered species risk assessment and the NAS report recommendations are available at
http://www.epa.gov/endangered-species/implementing-nas-report-recommendations-ecological-risk-assessment-endangered-and

The June 6, 2016 EPA Federal Register Notice is posted at
http://www.gpo.gov/fdsys/pkg/FR-2016-06-06/html/2016-13301.htm

The 2013 NAS report is available at
http://www.nap.edu/catalog.php?record_id=18344

The April 22, 2016 EPA Pesticide News Story is posted at
http://www.epa.gov/pesticides/save-date-register-technical-webinar-recently-released-endangered-species-assessments

The April 11, 2016 EPA Federal Register Notice is posted at
http://www.gpo.gov/fdsys/pkg/FR-2016-04-11/html/2016-08279.htm

The April 6, 2016 EPA Pesticide News Story is posted at
http://www.epa.gov/pesticides/epa-releases-draft-biological-evaluations-three-chemicals-impacts-endangered-species

EPA Instructions for Commenting on the Draft Biological Evaluations for Chlorpyrifos, Diazinon, and Malathion are posted at
http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2008-0850-0855

The December 11, 2015 EPA Pesticide News Story is posted at
http://www.epa.gov/pesticides/supporting-documents-endangered-species-biological-evaluations-now-available

The background documents on Provisional Models are posted at
http://www.epa.gov/endangered-species/provisional-models-endangered-species-pesticide-assessments

The March 26, 2015 EPA Federal Register Notice is posted at
http://www.gpo.gov/fdsys/pkg/FR-2015-03-26/html/2015-06931.htm

The December 15, 2014 EPA Pesticide News Story is posted at
http://www.epa.gov/oppfead1/cb/csb_page/updates/2014/esa-reporttocongress.html

The November 2014 "Interim Report to Congress on Endangered Species Act Implementation in Pesticide Evaluation Programs" is posted at
http://www.epa.gov/oppfead1/endanger/2014/esa-reporttocongress.pdf

The September 17, 2014 EPA Federal Register Notice is posted at
http://www.gpo.gov/fdsys/pkg/FR-2014-09-17/html/2014-22162.htm

The May 28, 2014 EPA "Response to Growers for ESA Transparency (GET) Petition" is posted at
http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2010-0854-0040

The April 2, 2014 EPA Federal Register Notice is posted at
http://www.gpo.gov/fdsys/pkg/FR-2014-04-02/html/2014-07102.htm

The November 1, 2013 Pesticide News Story is posted at
http://www.epa.gov/oppfead1/cb/csb_page/updates/2013/nas-workshop.html

Materials from the November 2013 stakeholder workshop are available at
http://www.epa.gov/oppfead1/endanger/2013/nas.html

Additional information about Assessing Pesticides under the Endangered Species Act is available at
http://www.epa.gov/endangered-species/assessing-pesticides-under-endangered-species-act

The previous FIEN message related to this topic is posted at
http://www.fien.com/articleDisplay.php?id=37245

Contact: Questions may be directed to Tracy Perry who is with the Pesticide Re-evaluation Division of the EPA Office of Pesticide Programs in Arlington, Virginia at 703 308 0128; e-mail: Perry.Tracy@EPA.gov

Summary: The following information is taken from the March 23, 2018 EPA Federal Register Notice:

B. Background

Consistent with EPA's responsibility under the ESA, on January 18, 2017, EPA released national-level endangered species Biological Evaluations (BEs) for chlorpyrifos, diazinon, and malathion to assess risks to listed species from registered uses of these pesticides. These BEs were completed in accordance with the joint

Interim Approaches developed to implement the recommendations of the April 2013 National Academy of Sciences (NAS) report, Assessing Risks to Endangered and Threatened Species from Pesticides. The NAS report outlined recommendations on specific scientific and technical issues related to the development of pesticide risk assessments that EPA and the FWS and NMFS must conduct to meet their obligations under the ESA. In November 2013, the Services, EPA, and the U.S. Department of Agriculture (USDA) released a white paper containing a summary of their joint Interim Approaches for assessing risks to listed species from pesticides. Details of the joint Interim Approaches are contained in the November 1, 2013 white paper Interim Approaches for National-Level Pesticide Endangered Species Act Assessments Based on the Recommendations of the National Academy of Sciences April 2013 Report. The methods developed as part of the joint Interim Approaches will continue to be vetted before EPA utilizes these methods broadly to meet its ESA obligations. Additional information on endangered species risk assessment and the NAS report recommendations are available at http://www.epa.gov/endangered-species/implementing-nas-report-recommendations-ecological-risk-assessment-endangered-and .

On December 29, 2017, in response to a court-ordered deadline in the case of Nw. Coal. for Alternatives to Pesticides, et al. v. NMFS, Stipulation and Order, Dkt. 50, No. 07-1791-RSL (D. Wash. May 21, 2014), NMFS transmitted to EPA its final BiOp regarding the effects of the registration review under section 3 of FIFRA of these pesticides on listed species. The BiOp addressed the effects of these three pesticides on 77 listed species and 50 designated critical habitats and, in sum, 38 different species would likely be jeopardized with extinction and 37 critical habitat units would be destroyed or adversely modified. NMFS had sought from the court, but was not provided, additional time to complete the BiOp. On January 8, 2018, EPA confirmed receipt of the BiOp and informed NMFS of EPA's intention to reinitiate informal consultation on the BiOp so that the consultation on the pesticides could be informed by (1) input from stakeholders, (2) further interagency discussion and agreement on the jeopardy determination interim methods, and (3) additional data and analysis, including consideration of the best scientific and commercial data available on use and usage information. On February 21, 2018, EPA sent NMFS a letter requesting informal consultation on the same action. EPA will use the information and analysis received and developed in the course of the informal consultation to inform whether formal reinitiation of consultation on the BiOp is appropriate.

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Go to the EPA Notice for additional information and details.

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This article (#42867) was distributed by e-mail on March 23, 2018 to those whose names are on the FIEN, LLC Subject Matter Distribution Lists for Crop Protection; Endangered Species; Fisheries; Risk Assessment and Communication

-end-

The above information was sent to you by Jimmy Liu of the Food Industry Environmental Network, LLC --- http://www.fien.com --- mobile: 240 476 5958 --- e-mail: liu@fien.com
* Messages prefaced with [SFM] are FIEN Shortened Messages which are distinguished from FIEN Traditional Messages as described at http://www.fien.com/SFM.php
* The Searchable Index of Previously Distributed FIEN Messages is available at http://www.fien.com/login/logins.php?RELOAD=/search_article.php
* Please take the time to respond to our Feedback Survey at http://www.fien.com/survey.php

Message

From: Pease, Anita [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=DBBEF4B4951144499885D4CDF88D46D0-ANITA PEASE]
Sent: 3/25/2019 4:20:15 PM
To: OPP AD Managers [OPP_AD_Managers@epa.gov]
Subject: FW: Environmental Modeling Public Meeting (EMPM) agenda and abstracts

Please feel free to share with staff that may be interested in attending.

Anita Pease
Acting Director, Antimicrobials Division
Office of Pesticide Programs
U.S. Environmental Protection Agency

703-305-0392
pease.anita@epa.gov

From: Echeverria, Marietta
Sent: Monday, March 25, 2019 11:29 AM
To: OPP Deputy & Associate Directors <OPP_Deputy_&_Associate_Directors@epa.gov>; OPP Division Directors <OPP_Division_Directors@epa.gov>
Cc: Shelby, Andrew <Shelby.Andrew@epa.gov>; Lazarus, Rebecca <lazarus.rebecca@epa.gov>; Sankula, Sujatha <Sankula.Sujatha@epa.gov>
Subject: Environmental Modeling Public Meeting (EMPM) agenda and abstracts

FYI – EFED is hosting the Environmental Modeling Public Meeting this Wednesday on the 1st floor. Please feel free to share with your staff that may be interested in any of the presentations.

Environmental Modeling Public Meeting (EMPM) Agenda - March 27, 2019

Topic: Environmental Modeling of Vapor-Phase Pesticides

Time (am/pm)	Presentation Title	Presenter(s)
9:00 - 9:15	Welcome and Introductions Opening Remarks	Andrew Shelby and Rebecca Lazarus (US EPA) Brian Anderson, Assoc. Director, EFED, US EPA
	Updates	
9:15 - 9:25	Honeybee Toxicity Study Frequently Asked Questions (FAQs) and Tier I Data Retrospective Analysis	Frank Farruggia (US EPA)
9:25 - 9:40	Updates to the Pesticide Water Calculator	Dirk Young (US EPA)
9:40 - 9:55	New Scenarios for the Pesticide Water Calculator	Nelson Thurman (US EPA)
9:55 - 10:25	Quantitative use of surface water monitoring data	Rochelle Bohaty (US EPA)
10:25 - 10:40	**Break**	
	Morning Presentations	
10:40 - 11:05	Estimating Risk to Non-Target Plants and Animals from Semi-volatile Pesticides	Chuck Peck (US EPA)

Time (am/pm)	Presentation Title	Presenter(s)
11:05 - 11:30	Vapor vs Spray Drift Deposition and Meteorological Factors Influencing the Vapor Deposition Gradient	Jody Stryker (Stone Environmental)
11:30 - 11:55	PERFUM Turns 15: A New Version to Meet New Needs	Rick Reiss (Exponent)
11:55 - 1:00	**Lunch**	
	Afternoon Presentations	
1:00 - 1:25	Development of the SOil Fumigant Exposure Assessment (SOFEA) model	John Buonagurio (Exponent)
1:25 - 1:50	Estimating Exposure to Fumigant and Non-Fumigant Pesticide Concentrations in Ambient Air using SOFEA and MCABLE	Jeff Driver (Risksciences.net, LLC)
1:50 - 2:15	SOFEA Model Validation and Comparison with ISCST3 versus AERMOD	Ian Van Wesenbeeck (Illahe Environmental)
2:15 - 2:30	**Break**	
2:30 - 2:55	Predicting Pesticide Volatilization from Agricultural Fields with the PLoVo Model	Kimberly Hageman (Utah State University)
2:55 - 3:20	Preliminary evaluation of SEAWAVE-QEX and recommendations	Wenlin Chen (Syngenta)
3:20 - 3:45	Using SAM for Assessing Concentrations in Surface Water	Russell Jones (Bayer)
3:45 - 4:10	Higher tier modeling endpoints derivation from lab and field studies based on EFSA opinion on aged sorption	Prasesh Sharma (Corteva)
4:10 - 4:20	Closing Remarks	Andrew Shelby and Rebecca Lazarus (US EPA)

Estimating Risk to Non-Target Plants and Animals from Semi-volatile Pesticides

Chuck Peck, Faruque Khan, Kris Garber, USEPA

The U.S. EPA Office of Pesticide Programs conducts ecological risk assessments of pesticides for animals and plants, which may be exposed via off-site transport to non-target areas. These transport pathways include drift, runoff and volatilization. This presentation will focus on how OPP evaluates risk of semi-volatile pesticides via the volatilization and drift transport routes. Chemical-specific fate data are obtained from laboratory studies (*e.g.*, wind tunnel, humidome, etc.) and field studies (*e.g.*, spray drift field deposition, field volatility, etc.). These data are used to parameterize volatilization and deposition models (*e.g.*, AERSCREEN/AERMOD, PERFUM, and AgDRIFT/AGDISP) that estimate exposure in non-target areas. Additionally, a new type of non-guideline study, combining the measurement techniques of a traditional field volatility study with those of a vegetative vigor study (*i.e.*, measurements of plant height and growth), has been conducted to explore offsite effects to non-target plants. Exposure estimates are compared to available toxicity data to evaluate risk to off-field, non-target plants and animals from the application of semi-volatile pesticides to treated fields.

Vapor vs Spray Drift Deposition and Meteorological Factors Influencing the Vapor Deposition Gradient

Jody Stryker, Stone Environmental; Lauren Padilla, Stone Environmental; John Brausch, BASF

The off-field spray drift and vapor drift of volatile and semi-volatile pesticides are two important atmospheric transport processes they may result in ecological and human exposure. Accurately attributing off-field transport to the respective sources is important for guiding appropriate mitigation strategies. However, there is no definitive method for determining whether off-target

exposure occurred as the result of spray drift versus vapor phase deposition. For this presentation, we first compare vapor and spray drift deposition with distance curves based on a reference emission rate for a hypothetical semi-volatile compound. The objective is to illustrate where vapor and spray drift deposition may overlap and assess similarities in the gradient with distance that would be expected from each source under similar environmental conditions. By linking theoretical assumptions about off-target effects to the deposition profiles, we evaluate whether the pathways of spray drift verses vapor drift might be uniquely identified from observations of effects in distant fields. Additionally, in order to better understand the potential for off-target exposure from the vapor drift pathway, we aim to determine the driving influences on vapor phase pesticide transport and deposition. We use a sensitivity analysis to evaluate weather factors that lead to the steepest and flattest vapor deposition profiles. The conclusion of this work is a summarization of environmental conditions that would result in the potential for off-target plant effects with and without an observed spatial gradient. Through this effort, we also highlight key environmental parameters to control in field studies that seek to quantify vapor-phase deposition.

PERFUM Turns 15: A New Version to Meet New Needs

Rick Reiss and John Buonagurio, Exponent

The Probabilistic Exposure and Risk model for FUMigants (PERFUM) was originally built in 2004 to provide a probabilistic methodology for estimating air concentrations and buffer zones following fumigant applications. It was favorably reviewed at a FIFRA Scientific Advisory Panel (SAP) meeting in 2004 and has been used by EPA for most fumigant risk assessment since then. The model was been steadily expanded over the years to include new features such as the ability to model fumigant applications in structures and improved algorithms for estimating probabilistic distributions. Over the last few years, Version 3 of the model has been built. Version 3 is a major upgrade and will be reviewed in this presentation. It is designed for use for fumigants and other volatile or semi-volatile pesticides, and for use in human health risk assessment or ecological risk assessment, including for non-target plants. Foremost among the changes, a Graphical User Interface (GUI) has been built, which will allow users to more easily set up model runs and analyze the results. Also, the original version relied on the EPA ISCST3 dispersion model. The new version includes ISCST3, AERMOD (EPA's replacement for ISCST3), and CALPUFF (a sophisticated puff model). The user can select which model to use. Other new capabilities include the ability to model dry and wet deposition, greater flexibility in specifying key parameters, and a new batch processing system for performing multiple runs.

Development of the SOil Fumigant Exposure Assessment (SOFEA) model

J. Buonagurio, S.A. Cryer., R. Reiss, J.Driver, Van Wesenbeeck, I.J., O. de Cirugeda Helle, P.L. Havens

SOFEA development began in 1998, to address the need to model chemical concentrations in ambient air in basins spanning several California (CA) townships for the purpose of assessing long-term human inhalation exposures and potential human health risks associated with use of the soil fumigant 1,3-dichloropropene (1,3-D). The SOFEA model began as an Excel interface to facilitate input parameter generation for the ISCST3 air dispersion model, including area source parameters, weather data files, receptor locations, etc.. The SOFEA interface also stored large output data files and calculated annual average and other moving average concentrations. Initially, SOFEA was developed as a prospective model, intended to

probabilistically model ambient air concentration distributions resulting from hypothetical 1,3-D product use scenarios in CA. SOFEA generated input parameter probability density functions (PDFs) for properties such as field size, application rate, date and depth and placed treated fields according to either historical or projected 1,3-D use by township and section. SOFEA (v.1.0) was reviewed and accepted by an EPA Scientific Advisory Panel (SAP) in 2004, and has been used extensively in CA to set safe limits on 1,3-D use. SOFEA was also modified to run in 'retrospective' mode so that it could be used to simulate observed concentrations at receptors, when parameterized with actual spatial and temporal product use data. In November, 2005, the EPA formally adopted AERMOD, in lieu of ISCST3, as the official EPA regulatory air dispersion model. AERMOD is an advanced plume model that incorporates scientific advancements in the treatment of planetary boundary layer (PBL) theory, understanding of atmospheric turbulence and dispersion, and handling of terrain interactions. AERMOD was incorporated into SOFEA in 2016, at the request of the EPA. The use of AERMOD in SOFEA improved predictions of the 1,3-D air monitoring data, primarily due to the improved prediction of mixing heights. In 2017 DAS retained Exponent to upgrade SOFEA to modern software engineering standards, develop a GUI interface to manage model inputs and outputs, comprehensive diagnostic tools for analyzing weather data, interactive exploration of flux profiles, receptor generation for any field geometry, and high-performance post-processing of results. A Beta version of the model (v4.2.1) is currently being tested and evaluated. When completed, SOFEA will be made publicly available via Exponent, LLC.

Estimating Exposure to Fumigant and Non-Fumigant Pesticide Concentrations in Ambient Air using SOFEA and MCABLE.

J. Driver, R. Reiss S.A. Cryer., I.J. Van Wesenbeeck, , J. Buonagurio, O. de Cirugeda Helle, P.L. Havens

Bystander exposure to atmospheric contaminants is dependent on their proximity to their source over time, temporal human activity patterns and related exposure factors (e.g., inhalation rate), and the contaminant source strength which is dependent on the volume of product applied, the area treated, timing and the emission rate (flux) of the product after application. Product use information for chlorpyrifos and 1,3-D was obtained from the CA PUR database for sites in CA, and from growers and professional applicators for sites outside of CA, and was used to parameterize the mass of product applied, the field area treated, and the date of application. The flux profiles for chlorpyrifos and 13D were obtained from field volatility studies. SOFEA was used to simulate 24 hour concentrations of each pesticide over a receptor grid covering a 9 township area in over a two year period. Weather data was obtained from the nearest CIMIS station and processed using the AERMET pre-processor prior to use in SOFEA. SOFEA v4.2.1 was used to simulate daily ambient air concentrations resulting from multiple 'real world' pesticide applications in each use region for two consecutive years. The agricultural regions modeled for the soil fumigant 1,3-D include high use areas in California, North Carolina, Washington, and Florida representing diverse agronomic use scenarios for 1,3-D in the USA. Chlorpyrifos concentrations were simulated in the highest use townships in Kern County, CA. The distributions of short, medium and long-term concentrations may also be used in conjunction with human life-stage-specific exposure factors, including residential mobility, time-activity patterns, and age-specific inhalation rates and body weights, to assess potential bystander temporal exposures to 1,3-D or chlorpyrifos in ambient air for specified geographic areas.

SOFEA Model Validation and Comparison with ISCST3 versus AERMOD.

Van Wesenbeeck, I.J., S.A. Cryer., O. de Cirugeda Helle, R. Reiss, J. Driver, J. Buonagurio, P.L. Havens

SOFEA v4.2.1 (*i.e.* SOFEA+AERMOD) was validated using the Merced monitoring dataset which included 14 months of continuously measured 1,3-D concentrations at 9 locations in a high use area for 1,3-D. The monitoring study also recorded the application date, field size, and mass of product applied for each of 478 1,3-D applications made in the study area. That study identified mixing height (MH) as one of the most sensitive variables, and showed that ISCST3 and its associated meteorological pre-processer significantly over estimated the MH during stable air (calm) conditions and as a result, under estimation of the concentration in air. The validation study also showed that the mixing heights could be adjusted, using wind speed and temperature parameters in the weather file, in order to improve the model fit to the monitoring data. SOFEA v4.2.1 model results were also compared to the 1,3-D concentrations from the Merced validation dataset, and improved the fit to the measured data, by changing the model under-estimation of the global annual average concentration across all receptors at the 50th percentile, from a 400% under-estimation to a 10% over-estimation. This analysis showed that SOFEA, when using AERMOD in lieu of ISCST3 as the air dispersion model, improved the prediction of observed 1,3-D concentrations, and obviated the need for adjustment of the MHs in the processed weather file. This improvement is a result of the refined algorithms in AERMOD for predicting MH during calm conditions, based on updated understanding of Planetary Boundary Layer (PBL) dynamics, the use of the Monin-Obukhov length scale (L), and the calculation of a convective and mechanical MH, the latter which is used only for stable conditions (when $L > 0$). SOFEA v4.2.1 over-estimated observed concentrations above the 95th percentile by approximately 2-fold, while model predictions were within 10% of observed at the 50th percentile. The results show that SOFEA (with AERMOD) improves model performance during calm periods, and provides realistic yet conservative ambient air concentrations for use in exposure and risk assessments. A 'quasi-validation' of SOFEA v4.2.1 was also conducted for a non-fumigant pesticide (chlorpyrifos) by parameterizing SOFEA with chlorpyrifos use data from the PUR database, and comparing simulated chlorpyrifos concentrations in ambient air to measured concentrations in the ARB monitoring network. The benefits and limitations of using SOFEA for modeling non-fumigant pesticide exposures is discussed.

Predicting Pesticide Volatilization from Agricultural Fields with the PLoVo Model

Associate Professor Kimberly Hageman, Department of Chemistry and Biochemistry, Utah State University

Pesticide volatilization from agricultural fields can have a significant impact on non-target, sensitive ecosystems, and can result in a loss of revenue for farmers. We developed and validated the Pesticide Loss via Volatilization (PLoVo) model, based on multiphase partitioning, to predict pesticide volatilization loss from a planted agricultural field. The original version of this model predicted volatilization from agricultural soils only[1,2] whereas the current version also includes volatilization from plants. The model allows the user to adjust the chemical-physical properties of the pesticide, leaf parameters, soil type, and climatic conditions. Plant-air ($K_{plant\text{-}air}$) partition coefficients are used to describe the plant-surface chemistry interactions with pesticides. The model was validated by comparing modelled volatilization rates to measured ones reported in the literature. We then used the model to investigate questions about whether pesticides tend to volatilize more from plants or soil, and how plant species and growth stage affect the amount of pesticide lost to the atmosphere. Results are displayed on chemical space diagrams for sets of hypothetical $K_{plant\text{-}air}$, $K_{soil\text{-}air}$ and $K_{water\text{-}air}$ combinations under different scenarios. I will also discuss two recent field studies designed to further test and validate the model. The PLoVo model is a

promising new tool to predict and understand trends in pesticide volatilization from agricultural fields.

1. References:

An improved screening tool for predicting volatilization of pesticides applied to soils. Davie-Martin, C.L., Hageman, K.J., Chin, Y.-P. (2013) *Environmental Science & Technology*, 47, 868-876.

Influence of temperature, relative humidity, and soil properties on the soil-air partitioning of semivolatile pesticides: Laboratory measurements and predictive models. Davie-Martin, C.L., Hageman, K.J., et al. (2015) *Environmental Science & Technology*, *49*, 10431-10439

Preliminary evaluation of SEAWAVE-QEX and recommendations

Wenlin Chen[1], Jeremy Aldworth[2], Paul Mosquin[2], Daniel Perkins[3] [1]Syngenta Crop Protection, LLC. [2]RTI International, RTP, NC, USA [3]Waterborne Environmental, Inc.

Water quality monitoring data, specifically for pesticides, can represent best-available exposure profiles relevant to drinking water and/or ecological risk assessments; however, there are challenges in synthesizing these data toward making conclusions about the nature of the potential range of exposure. Some of these challenges include: data collection frequency, monitoring period duration, interpreting exposure profiles from one location to another, and monitoring system scale. To address these challenges, US EPA has recently proposed to use SEAWAVE-QEX (a time-series regression model) to characterize potential exposure profiles based on monitoring data. Other statistical approaches such as Universal Kriging (UK) using stream flow as covariate has also been published (Mosquin et al., 2017). Additionally, process-based numerical modeling approaches such as PRZM-SWAT can offer a different perspective on synthesis and interpretation of monitoring data. PRZM-SWAT is a spatially-distributed hydrologic and chemical transport numerical model that combines upland chemical and hydrologic processes from PRZM and stream flow and chemical transport processes from SWAT. A preliminary comparative evaluation among the three different modeling approaches (SEAWAVE-QEX, UK, and PRZM-SWAT) was conducted to evaluate strengths and weaknesses of representing, synthesizing, and conclusions from monitoring data. This comparison was developed from pesticide measurements of intensively-monitored (daily/near daily) HUC12 headwater watersheds in the Midwest. The predictive quality and limitations of these models was assessed against observed, daily concentration measurements as well as hypothetical data collection frequency and timing (derived from sub-sampling the daily data sets). Recommendations of using these models and next steps for testing model performance on different chemistries are discussed.

Using SAM for Assessing Concentrations in Surface Water

R. L. Jones, J. Chen, W. Chen, D. Freedlander, P. Havens, S. Jackson, N. Peranginangin, A. Ritter, N. Synder, C. Smith, M. Winchell

The development of SAM provides a tool that will allow more realistic ecological assessments (for example, endangered species assessments) and human health assessments (for example drinking water) during the registration process for crop protection products. Since EPA has announced that the initial testing of SAM will be done for drinking water, CropLife America has examined the potential use of SAM in drinking water assessments. In the past much of the excessive conservatism in assessments of residues in drinking water from surface water have been associated with the estimate of product use in the watershed (these could include, for example, all the watershed area being planted to the crop, all

fields in which the crop is grown are treated with the maximum number of applications at the maximum rate, and all fields in the watershed are treated on the same days). The use of SAM allows for more realistic estimates of use in the watershed with the use of existing databases providing information on crops located in a watershed and regional product use on specific crops. The estimates can be further refined with information on the water body associated with the drinking water intakes when that information is available.

Higher tier modeling endpoints derivation from lab and field studies based on EFSA opinion on aged sorption

Prasesh Sharma, Corteva Agrisciences

Degradation behavior of pesticides in soil is investigated by means of aerobic soil degradation experiments, and degradation rates are commonly derived using FOCUS (or NAFTA) approaches, in order to derive exposure modeling endpoints (DT_{50}). However, pesticide degradation is not always of single first order and can be impacted by processes like non-equilibrium sorption (or aged sorption). As sorption increases over time, the substrate appears to become less bioavailable to microbes, leading to slower degradation. In such cases, modeling endpoints (with FOCUS or NAFTA) can only be derived using multi- or biphasic models, resulting in overestimated and, hence, too conservative DT_{50}. Based on the exposure modeling guidance (EU framework), modeling conducted with aged sorption parameters can be considered as higher tier.

EFSA (EFSA, 2018) recently published an opinion on the use of aged sorption endpoints in groundwater models. The opinion provides guidance on derivation of aged sorption parameters ($DegT50_{,eq}$,F_{ne} , K_{des}) from aged sorption experiments and conversion of regular aerobic soil degradation rates lab and field to equilibrium DegT50. Following the opinion, higher tier parameters were successfully derived from all three types of studies (aged sorption, lab aerobic deg and field dissipation studies). Results showed that the modeling endpoints derived by means of FOCUS (or NAFTA) procedures were significantly overestimated compared to the outcome of inverse modeling where aged sorption was included. Thus, the importance of aged sorption should be considered when performing soil metabolism experiments and when evaluating degradation kinetics for pesticides.

1)
2) References:
3) EFSA (2018) EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), Ockleford C, Hernandez-Jerez AF, Hougaard Bennekou S, Klein M, Adriaanse TP, Berny P, Brock T,Duquesne S, Grilli S, Kuhl T, Laskowski R, Machera K, Pelkonen O, Pieper S, Stemmer M, Sundh I, Teodorovic I, Topping CJ, Wolterink G, Smith RH, Gimsing AL, Kasteel R, Boivin A, van der Linden T, Oriol Magrans J, Egsmose M and Tiktak A, 2018. Scientific Opinion about the Guidance of the Chemical Regulation Directorate (UK) on how aged sorption studies for pesticides should be conducted, analysed and used in regulatory assessments. EFSA Journal 2018;16(8):5382, 86 pp. https://doi.org/10.2903/j.efsa.2018.5382

Message

From: Pease, Anita [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=DBBEF4B4951144499885D4CDF88D46D0-ANITA PEASE]
Sent: 1/24/2018 1:27:04 PM
To: Parsons, Laura [Parsons.Laura@epa.gov]; Erickson, William [Erickson.William@epa.gov]
Subject: more on ESA

Feel free to share w those who may be interested…

Daily News

EPA, FWS Delay Pesticide BiOps, Spurring Fears Of Trump Interference

January 23, 2018

EPA and the U.S. Fish and Wildlife Service (FWS) have delayed completing reviews of certain pesticides' risks to endangered species despite another agency finishing a similar review in advance of a court-ordered deadline, spurring environmentalist fears that the Trump administration is derailing a novel Obama-era review process.

EPA, FWS and the National Marine Fisheries Service (NMFS) had planned to complete by Dec. 31, 2017, inter-agency consultation on the first three pesticides -- chlorpyrifos, malathion and diazinon -- to undergo a new process crafted under the Obama administration for assessing pesticides risks under the Endangered Species Act (ESA).

But in November correspondence obtained by *Inside EPA* this month FWS requested and EPA agreed to extend consultation on the three pesticides, with FWS arguing that additional time was necessary. EPA backed FWS' request for further review, saying it would provide additional data within six months, though an EPA official defended that agency's portion of the review as complete.

"[A]fter further review and lessons learned in consideration of the [biological evaluations] the Service is requesting additional information necessary to complete formal consultation," Gary Frazer, FWS' assistant director of ecological services says in a Nov. 14 letter to Marietta Echeverria of EPA's pesticides office. "This letter also serves as a request to extend the consultation."

In a Nov. 17 response, Echeverria says, "We are pleased that the utility of the use and usage information is being reconsidered, and we anticipate being able to provide this information within approximately 6 months." She adds, "We agree that consultation should continue and be extended as necessary."

Despite the EPA and FWS delay, NMFS Jan. 9 issued its final biological opinion (BiOp) of the three substances conducted under the Obama-era process. NMFS faced a court-ordered year-end deadline for completing its review, while the EPA and FWS companion analysis was slated for completion under a legal settlement that set a non-mandatory schedule for completion.

Environmentalists and a fishing industry association are weighing lawsuits to press EPA to implement NMFS' recommendations in the BiOp for protecting endangered species of salmon and whales, according to press reports.

NMFS also sought to delay completion of its final BiOp of the pesticides' risks to species, in a Nov. 9 request to the U.S. District Court for the Western District of Washington for a two-year delay of the Dec. 31, 2017 deadline.

The court did not grant the request, and federal officials Dec. 29 notified the court that NMFS had completed the BiOps and forwarded recommendations for reducing risks to EPA.

ESA Consultation

Environmental groups have pursued multiple lawsuits seeking to compel EPA to correct its long-standing failure to comply with the ESA in its Federal Insecticide, Fungicide and Rodenticide Act registration process. While advocates won a court-ordered deadline for NMFS to finalize its BiOp, the FWS review was subject to an agreement that did not set a hard deadline for completion.

Prospects for timely completion of the reviews was cast in doubt after an April request from the pesticide industry to scrap the Obama-era review process, charging initial analyses produced overly-conservative and unrealistic results.

Nonetheless, Gina Shultz, FWS' deputy assistant director of ecological services, told *Inside EPA* in September that the service had completed drafts of the first three BiOps and was seeking Trump administration review before forwarding the documents to EPA.

"We are in the process of trying to brief our new policy leadership team," she said. "We want to make sure that everybody understands and is comfortable with what this is and the science behind" the new process and the reviews.

An official with the Center for Biological Diversity (CBD), which reached a July 2014 legal settlement that set a non-binding schedule for EPA and FWS to complete the reviews, argues that the agencies' delay of a final BiOp suggests that Trump administration officials are heeding pesticide producers' request to scrap the Obama-era process.

While the delay does not violate a mandatory court deadline, the source argues that the reviews of the first three chemicals are designed to serve as pilots for other substances for which EPA and FWS face strict deadlines and so delaying the reviews creates a backlog that the agencies will have to wrestle with in the future.

EPA and FWS staff have completed "one of the most rigorous BiOps in the history of the agency and accomplished something that was truly impressive, but the political leadership and donor class for the Trump administration don't want that to come out," the source says. "Elections have consequences -- a lot of people were trying to make this work better, and it's all been thrown into doubt now."

Effects Analysis

In the Nov. 14 letter to EPA, FWS requests revised effects analysis for the three pesticides and asks that EPA monitor pesticide use data to ensure that assumptions in the consultation process remain accurate. FWS also asks that EPA consult with industry and user groups in crafting any reasonable or prudent alternatives that may be necessary.

The FWS request for additional data says that the revised effects analysis should reflect "the best scientific and commercial data that is currently available or which can be obtained during the consultation."

The request also seeks a revised effects analysis "that eliminates from analysis geographic areas identified by EPA where these pesticides are not used and where such use is not likely during the time period of the label authorization."

EPA, in its response, defends the biological evaluations (BE) EPA staff conducted in support of FWS' BiOp, noting that the agency collaborated with FWS and provided all information that the service had requested.

"As you are aware, the BEs were developed with Services oversight and included all information and analyses as requested by [NMFS and FWS] during their development," EPA says. "We understand, however, that in the course of our consultation, FWS has indicated that additional information regarding use and usage information could be of value in the development of the FWS" BiOps.

NMFS final BiOp, released Jan. 9, found that proposed uses of the three pesticides are likely to harm numerous listed species and their habitats. The BiOp includes recommendations to EPA for reducing the substances' risks to listed species. -- *Dave Reynolds* (dreynolds@iwpnews.com)

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Appointment

From: Pease, Anita [Pease.Anita@epa.gov]
Sent: 1/12/2018 8:02:30 PM
To: Sims, Diann [Sims.Diann@epa.gov]
CC: Miller, Wynne [Miller.Wynne@epa.gov]

Subject: ESA use/useage data - reaction to FWS meeting agenda
Attachments: Draft agenda for pesticide use meeting_1_12_18.docx
Location: my office

Start: 1/16/2018 2:00:00 PM
End: 1/16/2018 2:30:00 PM
Show Time As: Tentative

Recurrence: (none)