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OFFICE OF CHEMICAL SAFETY AND
POLLUTION PREVENTION

MEMORANDUM

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1.0 Executive Summary

Background

This document presents the revised human health risk assessment for the organophosphate insecticide chlorpyrifos. Chlorpyrifos is currently being evaluated under the FIFRA section 3(g) registration review program. The preliminary human health risk assessment (HHRA) for chlorpyrifos registration review was completed on June 30, 2011 (USEPA, 2011). This revised assessment addresses issues raised in the comments received on the preliminary HHRA, as well as incorporates new information and new approaches that have also become available since the June 2011 risk assessment. The most significant changes include the following:

- Hazard
  - Findings from multiple lines of evidence from experimental toxicology and epidemiology with respect to acetylcholinesterase/cholinesterase (AChE/ChE) inhibition and neurodevelopmental outcomes are used in the weight-of-evidence determination supporting the retention of the 10X FQPA Safety Factor (SF).
  - Utilization of a physiologically-based, pharmacokinetic-pharmacodynamic (PBPK-PD) model for deriving toxicological points of departure (PoDs) and for determining the appropriate intra-species and inter-species uncertainty factors.
  - Findings from two vapor phase, inhalation toxicity studies indicating no adverse effects even at the saturation concentration for chlorpyrifos and chlorpyrifos oxon in vapor form.
- Dietary Exposure
  - Updated food monitoring data and percent crop treated information.
- Residential and Bystander Exposure
  - Use of the 2012 *Standard Operating Procedures for Residential Pesticide Exposure Assessment*[1] which prescribe updates to several elements of the assessment.
  - Use of the AgDISP model for estimation spray drift (deposition and airborne concentrations) following aerial mosquito adulticide application.
  - Updated methodology for determining the airborne concentration of chlorpyrifos following ground-based mosquito adulticide applications.
  - Completion of a dose reconstruction evaluation to further evaluate the epidemiological findings.
- Occupational Exposure
  - Inclusion of use patterns based on a Master Use Summary Document developed for chlorpyrifos.
  - Use of revised guidance for all of the currently recommended unit exposures (and body weights) for standard EPA occupational handler exposure scenarios[2], *Occupational Pesticide Handler Unit Exposure Surrogate Reference Table – Revised March 2013*.

[1] http://www.epa.gov/pesticides/science/USEPA-OPP-HED_Residential%20SOPs_Oct2012.pdf
[2] http://www.epa.gov/opp00001/science/handler-exposure-data.html

  - Use of the revised inputs for seed treatment use patterns.
- Aggregate Assessment
  - Use of a calculated drinking water level of comparison (DWLOC) approach for comparison to model estimated drinking water concentrations (EDWCs).

Use Profile

Chlorpyrifos (0,0-diethyl-0-3,5,6-trichloro -2-pyridyl phosphorothioate) is a broad-spectrum, chlorinated organophosphate (OP) insecticide. Registered use sites include a large variety of food crops (including fruit and nut trees, many types of fruits and vegetables, and grain crops), and non-food use settings (e.g., golf course turf, industrial sites, greenhouse and nursery production, sod farms, and wood products). Public health uses include aerial and ground-based fogger adulticide treatments to control mosquitoes. There are also residential uses of roach bait products and ant mound treatments. Permanent tolerances are established (40 CFR§180.342) for the residues of chlorpyrifos in/on a variety of agricultural commodities (including meat, milk, poultry and eggs). There are also tolerances for use in food handling establishments (FHE). Chlorpyrifos is manufactured as granular, microencapsulated liquid, soluble concentrate liquid, water dispersible granular in water soluble packets (WSP), wettable powders in WSPs, impregnated paints, cattle ear tags, insect bait stations and total release foggers. There is a wide range of application rates and methods. The residues of concern for risk assessment purposes are chlorpyrifos and chlorpyrifos oxon under some circumstances.

Hazard Characterization

The hazard characterization for chlorpyrifos and its oxon is based on adverse health effects in animals and humans related to two different endpoints - acetylcholinesterase (AChE) inhibition and potential for neurodevelopmental effects. A weight-of-the-evidence (WOE) analysis has been completed using the draft "Framework for Incorporating Human Epidemiologic & Incident Data in Health Risk Assessment." The WOE analysis integrated quantitative and qualitative findings from experimental toxicology studies, epidemiology studies and physiologically-based pharmacokinetic-pharmacodynamic (PBPK-PD) modeling does not support reduction of the 10X FQPA Safety Factor. In addition, the Agency is explicitly using the robust PBPK-PD model to estimate human equivalent doses/concentrations which are used as the toxicological points of departure (PoDs) and also to define inter- and intra-species factors for chlorpyrifos and/or its oxon from multiple exposure pathways (e.g. food, water, occupational, residential).

The key issues considered in the WOE are 1) whether chlorpyrifos causes long-term effects from prenatal and/or early lifestage exposure and 2) whether adverse effects can be attributed to doses lower than those which elicit 10% inhibition of RBC AChE. When taken together, the evidence from 1) the experimental toxicology studies evaluating outcomes such as behavior and cognitive function; 2) mechanistic data on possible /modes of action/ adverse outcome pathways (MOA/AOP); and 3) epidemiologic and biomonitoring studies, indicate that chlorpyrifos likely played a role in the neurodevelopmental outcomes reported by the epidemiologic study (Columbia University) investigators. However, uncertainties such as the lack of an established MOA/AOP for neurodevelopmental effects and the potential exposure to multiple AChE-inhibiting pesticides preclude definitive causal inference. However, there is sufficient

uncertainty in the human dose-response relationship for neurodevelopmental effects to prevent the Agency from reducing or removing the statutory 10X FQPA Safety Factor. The FQPA 10X Safety Factor will be retained for infants, children, youths, and women of childbearing age for all exposure scenarios.

EPA has applied the Data-Derived Extrapolation Factor (DDEF) guidance (USEPA, 2014), in its use of the PBPK-PD model; the model that was used to calculate a DDEF intra-species extrapolation for some populations. The PBPK-PD model estimates human RBC AChE inhibition from exposures via oral, dermal, and inhalation routes and thus obviates the need for the typical inter-species factor. Administered doses leading to the response level of interest (10% change in RBC AChE inhibition) are compared between a measure of average response and response at the tail of the distribution representing sensitive individuals in order to calculate the intra-species factor. Based on the 99th percentile of the distributions, the DDEF for intra-species extrapolation is 4X for chlorpyrifos and 5X for the oxon (Dow, 2014b). For this revised risk assessment, the 99th percentile is being used to account for variation of sensitivity (i.e., the intra-species factor used in the risk assessment is 4X for chlorpyrifos and 5X for the oxon for all groups except women who are pregnant or may become pregnant for whom the 10X intra-species factor was retained).

While the current PBPK-PD model accounts for age-related growth from infancy to adulthood by using polynomial equations to describe tissue volumes and blood flows as a function of age, the model does not include any descriptions on physiological, anatomical and biochemical changes associated with pregnancy. Due to the uncertainty in extrapolating the current model predictions among women of child bearing age, the Agency is applying the standard 10X intra-species extrapolation factor for women of childbearing age. For all other relevant populations, the Agency has reliable data to reduce the standard 10x intra-species factor.

The PBPK-PD model has been used to estimate exposure levels resulting in 10% RBC AChE inhibition following acute (single day, 24 hours) and steady state (21-day) exposures for a variety of exposure scenarios for chlorpyrifos and/or chlorpyrifos oxon. For OPs, repeated exposures generally result in more AChE inhibition at a given administered dose compared to acute studies. Moreover, AChE inhibition in repeated dosing guideline toxicology studies with OPs show a consistent pattern of inhibition reaching steady state at or around 2-3 weeks of exposure in adult laboratory animals (U.S. EPA, 2002). This pattern observed with repeated dosing is a result of the amount of inhibition comes at equilibrium (or steady state) with the production of new enzyme. As such, AChE studies of 2-3 weeks generally show the same degree of inhibition with those of longer duration (*i.e.*, up to 2 years of exposure).

Separate PoDs have been calculated for dietary (food, drinking water), residential, and occupational exposures by varying inputs on types of exposures and populations exposed. Examples of inputs include: duration; route (dermal, oral, inhalation); body weights which vary by lifestage; exposure duration (hours per day, days per week); and exposure frequency [events per day (eating, drinking)]. The predicted PoDs, used in this revised risk assessment can be seen in Table 4.8.4.

Exposure and Risk Assessment

The general approach for the chlorpyrifos exposure and risk assessment can be described as follows: The PBPK-PD model was used to calculate acute (24 hour) and steady state (21 day) points of departure dose levels which correspond to 10% RBC ChEI for the index lifestages relevant to chlorpyrifos risk assessment (children of various ages which differ due to exposure pattern, and adult females of childbearing age). The PBPK-PD PoD predictions for each exposure route and pathway were modeled separately (e.g., for residential exposure dermal, inhalation, and incidental oral calculations).

PoDs are divided by the total uncertainty factors to derive a population adjusted dose (PAD). There are potential risks of concern when estimated dietary risk exceeds 100% of the PAD. For the dietary (food only) assessment, the only residue of concern is chlorpyrifos (the oxon metabolite is not an expected residue on foods). The chlorpyrifos total uncertainty factors are 100X for adult females (10X FQPA SF and 10X intra-species extrapolation factor) and 40X for the other relevant populations (10X FQPA SF and 4X intra-species extrapolation factor). The chlorpyrifos exposure values resulting from dietary modeling are compared to the PAD.

For the dietary (water only) assessment, the only residue of concern assumed to occur in drinking water is chlorpyrifos oxon. It is assumed that all chlorpyrifos in water is converted to chlorpyrifos oxon upon treatment. The chlorpyrifos oxon total uncertainty factors are 100X for adult females (10X FQPA SF and 10X intra-species extrapolation factor) and 50X for the other relevant populations (10X FQPA SF and 5X intra-species extrapolation factor). A DWLOC approach is employed to determine the risk estimates for drinking water. Drinking water is addressed in the aggregate sections of the risk assessment.

For the residential and occupational assessment, margins of exposure (MOEs) were calculated by comparing the PoDs to the calculated exposures for each scenario and applicable level of personal protection (e.g., varied levels of protective clothing and equipment). The resulting MOEs were then compared to the appropriate level of concern (LOC) which are 100 for adult females and 40 for the other index lifestages evaluated in this risk assessment. If calculated MOEs were less than the applicable LOC then a risk of concern was identified for that exposure scenario.

Dietary Risk Assessment

Highly refined acute and steady state dietary (food only; parent chlorpyrifos only) exposure analyses were performed using the Dietary Exposure Evaluation Model (DEEM) and the Calendex models, respectively. Most food residues used were based upon U.S. Department of Agriculture's Pesticide Data Program (PDP) monitoring data. Percent crop treated and empirical food processing factors were used where available. Acute dietary (food only) risk estimates are all <100 % of the acute PAD for food ($aPAD_{food}$) at the 99.9th percentile of exposure. The subgroup with the highest exposure was females (13-49 years old) at 3.2 % $aPAD_{food}$. For the steady state dietary (food only) exposure analyses, children (1-2 years old) was the population subgroup with the highest exposure at 9.7% of the $ssPAD_{food}$ at the 99.9th percentile of exposure.

Residential (Non-occupational) Exposures

The residential exposure assessment for chlorpyrifos has been updated to reflect the use of the PBPK-PD model and updates to EPA's SOPs for Residential Exposure Assessment. Current chlorpyrifos residential uses include a granular ant mound use which is applied by commercial applicators only and as a roach bait which is available for homeowner users in child-resistant packaging. Additionally, chlorpyrifos is labeled for golf course turf applications and for public health aerial and ground-based fogger ultra-low volume (ULV) mosquito adulticide applications.

Residential post-application exposure is anticipated from playing golf on chlorpyrifos-treated courses and from exposures which can occur following aerial and ground-based ULV mosquito adulticide use. Chemical-specific turf transferable residue (TTR) data were used in the residential post-application assessment. The residue of concern for residential post-application exposure is the parent compound chlorpyrifos. A quantitative assessment was not performed for the roach bait product and ant mound treatments as residential exposures for these uses are negligible.

The assessment of steady state golfer post-application exposures (dermal only) to chlorpyrifos treated turf for the lifestages adults (females 13-49 years old), children (6 to < 11 years old), and youths (11 to < 16 years old), results in no risks of concern (i.e., for children and youths, MOEs are ≥ 40; for adults, MOEs are ≥ 100 on the day of application). For the assessment of post-application exposures from public health mosquitocide applications, no combined risks of concern were identified, even on the day of application, for adults (dermal and inhalation) and children (dermal, incidental oral, and inhalation).

In addition to the assessment of residential exposures for golf course and mosquitocide uses, the potential for post-application exposures to residential bystanders (who live on, work in, or frequent areas adjacent to chlorpyrifos-treated agricultural fields) from spray drift and volatilization were also considered. The potential risks from spray drift and the impact of potential risk reduction measures were assessed in a July 2012[3] memorandum. To increase protection for children and other bystanders, chlorpyrifos technical registrants voluntarily agreed to lower application rates and adopt other spray drift mitigation measures such as buffer zones.[4] The spray drift risk assessment results have been updated to incorporate the PBPK-PD modeling approach. The resulting buffer distances (feet) necessary to reach the level of concern for adults (females 13-49 years old) and children (1 to < 2 years old) (i.e., for adults, MOEs are ≥ 100; for children, MOEs are ≥ 40) with use of certain application rates, nozzle droplet types, and application methods range from 0 to 25 feet. The estimated buffer distances based on this revised assessment are less than previously predicted in July 2012.

[3] J. Dawson, W. Britton, R. Bohaty, N. Mallampalli, and A. Grube. Chlorpyrifos: Evaluation of the Potential Risks from Spray Drift and the Impact of Potential Risk Reduction Measures. 7/13/12. U.S. EPA Office of Chemical Safety and Pollution Prevention. D399483, D399485.
[4] R. Keigwin. Spray Drift Mitigation Decision for Chlorpyrifos (059101). 7/2012. U.S. EPA Office of Chemical Safety and Pollution Prevention. EPA-HQ-OPP-2008-0850-0103.

In January 2013, a preliminary assessment of the potential risks from chlorpyrifos volatilization was conducted.[5] However, this assessment was revised in June 2014[6] following submission of two vapor phase inhalation toxicity studies which indicate no adverse effects occurred even at the saturation concentration for chlorpyrifos and chlorpyrifos oxon.[7] Because these new studies demonstrated that no toxicity occurred even at the saturation concentration, which is the highest physically achievable concentration, there are no anticipated risks of concern from exposure to the volatilization of either chlorpyrifos or chlorpyrifos oxon.

Aggregate Assessment and Risk Estimates

The Agency has considered aggregate exposures and risks from combined food, drinking water, and residential exposures to chlorpyrifos and chlorpyrifos oxon. The acute aggregate assessment includes only food and drinking water. The steady state aggregate assessment includes exposures from food, drinking water and residential uses. Exposure to the parent compound chlorpyrifos is expected for food and residential uses. Exposure to either chlorpyrifos or chlorpyrifos oxon may be expected from drinking water sources. The drinking water assessment assumed 100% conversion of chlorpyrifos to the more toxic chlorpyrifos oxon (the predominant chlorpyrifos transformation product formed during drinking water treatment (*e.g.*, chlorination)).

For acute and steady state aggregate assessments, a DWLOC approach was used to calculate the amount of exposure which could occur without exceeding the risk level of concern (i.e., the available space in the total aggregate risk cup for exposures to chlorpyrifos oxon in drinking water after accounting for exposures to parent chlorpyrifos from food and residential uses). The calculated DWLOCs were then compared to the estimated drinking water concentrations (EDWCs) of oxon modeled under a variety of conditions. When the EDWC is less than the DWLOC, there are no risk concerns for exposures to the pesticide in drinking water which also indicates aggregate exposures are not of concern. Conversely, when the EDWC is greater than the DWLOC, then potential risks of concern have been identified. For chlorpyrifos, DWLOCs were calculated for both the acute and steady state aggregate assessments for infants, children, youths and adult females. However, only the steady state DWLOCs were compared to the EDWCs as the steady state aggregate risk estimates are protective of any acute exposures. The lowest DWLOC calculated was 3.9 ppb for infants (<1 year old) for the steady state exposure duration. This water concentration is the concentration used for comparison to the EDWCs. Drinking water concentrations of chlorpyrifos oxon above 3.9 ppb indicate a potential risk

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[5] R. Bohaty, C. Peck, A. Lowit, W. Britton, N. Mallampalli, A. Grube. Chlorpyrifos: Preliminary Evaluation of the Potential Risks from Volatilization. 1/31/13. U.S. EPA Office of Chemical Safety and Pollution Prevention. D399484, D400781.

[6] W. Britton. W. Irwin. J. Dawson. A. Lowit. E. Mendez. Chlorpyrifos: Reevaluation of the Potential Risks from Volatilization in Consideration of Chlorpyrifos Parent and Oxon Vapor Inhalation Toxicity Studies. 6/25/2014. U.S. EPA Office of Chemical Safety and Pollution Prevention. D417105.

[7] W. Irwin. Review of Nose-Only Inhalation of Chlorpyrifos Vapor: Limited Toxicokinetics and Determination of Time-Dependent Effects on Plasma, Red Blood Cell, Brain and Lung Cholinesterase Activity in Femal CD(SD): Crl Rats. U.S. EPA Office of Chemical Safety and Pollution Prevention. 6/25/14. D411959. TXR# 0056694. EPA MRID# 49119501.

W. Irwin. Review of Nose-Only Inhalation of Chlorpyrifos-Oxon Vapor: Limited Toxicokinetics and Determination of Time-Dependent Effects on Plasma, Red Blood Cell, Brain, and Lung Cholinesterase Activity in Female CD(SD):Crl Rats. U.S. EPA Office of Chemical Safety and Pollution Prevention. 6/25/14. D415447. TXR# 0056869. EPA MRID# 49210101.

concern.

EFED used modeling to perform a national screening bounding exercise to illustrate the range of estimated EDWC. Two maximum label rate application scenarios were selected to represent high and low end exposures, i.e., tart cherries at five applications totaling 14.5 pounds per acre per year, and bulb onions at a single application of one pound per acre per year, respectively. For surface water, the application of chlorpyrifos to tart cherries resulted in a 21-day average concentration that exceeded the drinking water level of comparison (DWLOC of 3.9 ppb). For surface water, applications to bulb onions resulted in a 21-day average concentration below the DWLOC. For groundwater, both tart cherry and onion scenarios resulted in EDWCs below the DWLOC.

To investigate whether other chlorpyrifos application scenarios may result in concentrations that exceed the DWLOC, a screen of all available surface water modeling scenarios was completed considering three different application dates and a single application at several different application rates that ranged from one to six pounds. This analysis showed that even with only one application, several chlorpyrifos uses may exceed the DWLOC at rates lower than maximum labeled rates (both single as well as yearly), including an application rate of one pound per acre per year. The analysis also showed that the DWLOC exceedances are not expected to be uniformly distributed across the country.

Further analysis was conducted to look at the spatial distribution of EDWCs at a regional level, as well as by using a drinking water intake watershed approach. This exercise demonstrated that chlorpyrifos applications will result in variable drinking water exposures that are highly localized and that the highest exposures generally occur in small hydrologic regions where there is a high percent cropped area on which chlorpyrifos use could occur.

Finally, EDWCs were compared to monitoring data. This analysis showed that when modeling scenarios are parameterized to reflect reported use and EDWCs are adjusted to reflect percent cropped area, the EDWCs are within an order of magnitude of the measured concentrations reported in the monitoring data. Therefore, although there are uncertainties associated with the model input parameters for which conservative assumptions were made (*e.g.*, one aerobic aquatic metabolism half-life value multiplied by the uncertainty factor of three, stable hydrolysis, 100% of the cropped waters is treated at the same time, and use of the Index Reservoir as the receiving waterbody), these assumptions do not appear to lead to an overly conservative estimate of exposure. In addition, evaluation of the monitoring data further illustrates that exposures are highly localized. Additional work can be done to examine EDWCs on a regional and/or watershed scale to pinpoint community drinking water systems where exposure to chlorpyrifos oxon as a result of chlorpyrifos applications may pose an exposure concern.

[For complete details of the DWA see Bohaty, R. 12/23/14, D424487, Chlorpyrifos: Updated Drinking Water Assessment for Registration Review.]

Occupational Exposures

Based on the anticipated chlorpyrifos use patterns and current labeling, a variety of equipment

and application techniques can potentially be used. These are expected to result in both occupational handler and post-application exposures. The 2011 occupational exposure assessment has been updated based on a number of changes that have occurred since that time, including use of the PBPK-PD modeling approach and incorporation of updated metrics (e.g., unit exposures for some scenarios). For the occupational risk assessment, the residue of concern is the parent chlopyrifos. Exposure to the oxon is only an issue under very limited circumstances in greenhouses for postapplication workers.

Current labels generally require that handlers use normal work clothing (i.e., long sleeved shirt and pants, shoes and socks) and coveralls, chemical resistant gloves, and dust/mist respirators. Also, some products are marketed in engineering controls such as water soluble packets. In order to determine what level of personal protection is required to alleviate risk concerns and to ascertain if label modifications are needed, steady state exposure and risk estimates were calculated for occupational handlers of chlorpyrifos for a variety of scenarios at differing levels of personal protection including engineering controls. In this assessment, a total of 285 total occupational handler exposure scenarios were assessed and 132 of them are not of concern (i.e., MOEs are ≥ 100) at current product label requirements. Risks of concern for 27 additional exposure scenarios could potentially be mitigated if additional personal protective equipment or engineering controls are used. Risks of concern were estimated for 126 exposure scenarios and remain a concern regardless of the levels of personal protection and engineering controls considered. These risks could be mitigated using the highest level of personal protection available and by modification to other label requirements such as application rate or, in some cases, deleting certain types of application equipment.

Additionally, occupational handler exposure for seed treatment activities were assessed (combined dermal and inhalation). Of the 68 exposure scenarios assessed, 36 resulted in risk estimates which were not of concern (i.e., MOEs are ≥ 100) at the level of personal protection currently required by product labels. Secondary handler (seed planter) scenarios were also evaluated and none were of concern at current label requirements. The remaining 32 seed treatment scenarios are of potential concern.

Steady state occupational post-application exposures and risks were assessed for any crops where hand labor is anticipated following applications of chlorpyrifos. The assessment was completed using seven chlorpyrifos dislodgeable foliar residue (DFR) studies. Chlorpyrifos parent compound is the residue of concern for occupational post-application exposures that occur outdoors; however, it may be possible that the formation of chlorpyrifos oxon is greater and its degradation slower in greenhouses when compared to the outdoor environment. Occupational post-application assessments were performed for: 1) exposures to the parent compound chlorpyrifos in outdoor environments (uses other than greenhouse), 2) exposures to the parent chlorpyrifos (only) in greenhouses and 3) exposures to both the parent and the oxon metabolite in greenhouses.

Current labels require a Restricted Entry Interval (REI) of 24 hours from most crops and activities, but in some cases such as tree fruit, REIs are up to 5 days after application. All post-application worker risks have been updated in the current assessment. Results indicate that no REI increase is required for the majority of outdoor environment crops and commodities with a

labeled REI (i.e., 43 of 55 total). However, for 12 crops, activities such as irrigation, hand harvesting, scouting, and thinning result in risks of concern up to as many as 10 days following application. For the assessment of post-application exposures to parent chlorpyrifos (only) from ornamental production in greenhouses, 4 formulations were assessed: emulsifiable concentrate, microencapsulated liquid, wettable powder in WSP, and total release fogger. An REI increase of up to 5 days may be needed to alleviate risks from with use of the emulsifiable concentrate, wettable powder in WSP and total release fogger formulations. For the microencapsulated liquid formulation, the estimated REIs range from 0 to > 35 days after treatment (the completion of the monitoring period) dependent on the exposure activity considered.

Due to uncertainty regarding the formation of chlorpyrifos oxon in greenhouses, HED also estimated risks for reentry into treated greenhouses (all 4 formulations) for the parent chlorpyrifos plus chlorpyrifos oxon using a total toxic residue approach. The total toxic residue approach was conducted with use of chlorpyrifos DFR studies and an assumed daily fraction, 5%, of chlorpyrifos oxon. When the total toxic residue approach is used to evaluate risks from exposure to the oxon with use of the emulsifiable concentrate, wettable powder in WSP, and total release fogger formulations, results indicate that an REI increase up to 6 days may be needed to alleviate risk concerns. For the microencapsulated liquid formulation, REIs range from 3 to > 35 days after treatment (the completion of the monitoring period) dependent on the exposure activity considered.

Tolerances and MRLs

The establishment of tolerances are being recommended for residues of chlorpyrifos on cotton gin byproducts, aspirated grain fractions, milled corn byproducts, and milled wheat byproducts. In addition, the current tolerance definition should be updated to be consistent with current policy.

U.S. tolerances and Codex maximum residue limits (MRLs) are based on the analysis of residues of chlorpyrifos only. There are differences for some commodities between the U.S. and Codex. Canadian MRLs are for chlorpyrifos only for some commodities and for both parent chlorpyrifos and its metabolite TCP (3,5,6-trichloro-2-pyridinol; not a U.S. residue of concern) for other commodities. The potential for tolerance harmonization will be revisited during Registration Review.

2.0 HED Recommendations

2.1 Data Deficiencies

Toxicology
None.

Residue Chemistry

860.1500:
Separate magnitude of the residue studies for lemons are needed after application of Lorsban 4E and 75% WDG formulations in order to reevaluate the existing tolerance for chlorpyrifos for the citrus fruit crop group.

Magnitude of the residue studies are needed to establish a tolerance for residues of chlorpyrifos on wheat hay.

860.1520:
Processing studies are needed for soybean meal, hulls and refined oil.

Occupational/Residential

No new data requirements have been identified for chlorpyrifos; however, in the 2011 preliminary HHRA, additional studies to address the uncertainties regarding the formation and degradation of chlorpyrifos oxon in greenhouses were recommended. To date, those data have not been submitted. In the absence of the recommended data, and to account for the potential for oxon to form in greenhouses, EPA has used a conservative total toxic residue approach for parent chlorpyrifos plus the chlorpyrifos oxon.

2.1 Tolerance Considerations

2.2.1 Enforcement Analytical Method

The methods in the PAM Volume II are adequate to analyze the residue of concern for tolerance enforcement purposes, chlorpyrifos only. The limit of detection of these methods is adequate to cover the lowest tolerance level included in the 40 CFR 180.342 for detection of chlorpyrifos only, 0.01 ppm. In addition, chlorpyrifos is completely recovered using FDA multiresidue protocols D and E (nonfatty matrices) and partially recovered using multiresidue method protocol E (fatty matrices).

2.2.2 International Harmonization

Current U.S. tolerances for chlorpyrifos are listed in 40 CFR§180.342 and are summarized in Appendix 8 of this document. The Codex Alimentarius Commission and Canada have established Maximum Residue Limits (MRLs) for chlorpyrifos. Mexico adopts U.S. tolerances and/or Codex MRLs for its export purposes. U.S. tolerances and Codex MRLs are based on the analysis of residues of chlorpyrifos. Canada MRLs are for chlorpyrifos for some commodities and for both parent chlorpyrifos and its metabolite TCP (3,5,6-trichloro-2-pyridinol) which is not a U.S. residue of concern, for other commodities.

With the exception of apple commodities, Canada MRLs are currently not harmonized with the U.S. tolerances. This is because the Canada MRL residue definition is for the combined residues

of chlorpyrifos and the TCP metabolite and in the U.S. the tolerance definition is just for chlorpyrifos (TCP is not a residue of concern for the U.S.). Harmonization between the U.S. tolerances and Codex MRLs is only possible for corn, field, grain; cranberry; egg; sorghum, grain, grain; sorghum, grain, stover; and wheat, grain. In addition, two commodities of the Leafy Vegetable (CG 5) can be harmonized with the Codex, head cabbage, and Chinese cabbage (type petsai). A summary of the U.S. and international tolerances and MRLs is included in Appendix 8 of this document.

2.2.3 Recommended/Reassessed Tolerances

The following tolerances for chlorpyrifos on cotton gin byproducts and aspirated grain fractions are necessary to address residues found in field trials:

Cotton, gin byproducts……..15 ppm
Grain, aspirated fractions…..22 ppm

The following tolerances should be reinstated to address residues of chlorpyrifos on the milled byproducts of corn and wheat:

Corn, milled byproducts…….0.1 ppm
Wheat, milled byproducts…..1.5 ppm

Table 2.2.3 Recommended Tolerances for Chlorpyrifos

Commodity	Established Tolerance (ppm)	Recommended Tolerance (ppm)	Comments *Correct Commodity Definition*
Aspirated grain fractions	none	22	
Cotton, gin byproducts	none	15	
Corn, milled byproducts	none	0.1	
Wheat, milled byproducts	none	1.5	

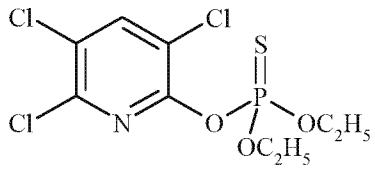
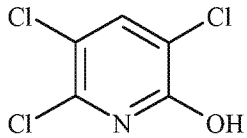
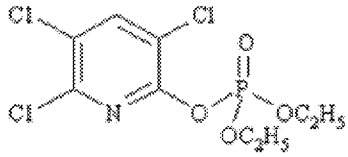
On 5/27/09 HED established interim guidance on writing tolerance expressions for enforcement purposes. According to the guidance, the tolerance expression in the 40 CFR § 180.342 should be revised to read as follows:

> "(a) General. (1) Tolerances are established for residues of chlorpyrifos, including its metabolites and degradates, in or on the commodities in the table below. Compliance with the tolerance levels specified below is to be determined by measuring only chlorpyrifos."

The current tolerance expression reads "chlorpyrifos *per se* (*O,O* -diethyl *O* -(3,5,6-trichloro-2-pyridyl) phosphorothioate".

3.0 Introduction

3.1 Chemical Identity

Table 3.1 Chlorpyrifos Degradate/ Residues of Concern Nomenclature.	
Chlorpyrifos	
IUPAC name	*O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate
CAS name	*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridinyl) phosphorothioate
CAS registry number	2921-88-2
End-use product (EP)	Lorsban 75% WDG and Lorsban 50% WP
TCP Metabolite/Degradate (Residue of Concern for Canada) IUPAC Name 3,5,6 Trichloro-2-pyridinol	
Oxon Metabolite/Degradate Common Name Chlorpyrifos Oxon IUPAC Name O,O-diethyl. O-3,5,6-trichloro-2-pyridyl phosphate	

3.2 Physical/Chemical Characteristics

Technical chlorpyrifos is a white crystalline solid. Chlorpyrifos is stable in neutral and acidic aqueous solutions; however, stability decreases with increasing pH. Chlorpyrifos is practically insoluble in water, but is soluble in most organic solvents (i.e., acetone, xylene and methylene chloride). Chlorpyrifos is moderately volatile based on its vapor pressure of 1.87×10^{-5} mmHg at 25°C. See Appendix 7.

Laboratory studies show chlorpyrifos is susceptible to hydrolysis under alkaline conditions and that volatilization and photo-degradation are not likely to play a significant role in the dissipation of chlorpyrifos in the environment. Nonetheless, chlorpyrifos has been detected in air samples, and so volatilization may play more of a role in dissipation than laboratory studies indicate. The major route of dissipation appears to be aerobic and anaerobic metabolism, as well as partitioning to the soil (partition coefficient of 6040). The aerobic aquatic metabolism half-life is 30.4 days (~6% remaining in 4 months). The water peak half-lives were ~1 day in a monitoring study (MRID 44711601). Based on available data, chlorpyrifos degrades slowly in soil under

both aerobic and anaerobic conditions. Degradation begins with cleavage of the phosphorus ester bond to yield 3,5,6-trichloro-2-pyridinol (TCP). Field dissipation studies show that chlorpyrifos is moderately persistent under field conditions—dissipation half-life less than 60 days. Chlorpyrifos is only slightly soluble in water (1400 ppb). However, if it reaches aquatic environments the Log K_{ow} (4.7) indicates that chlorpyrifos may bioaccumulate in fish and other aquatic organisms. A fish bioaccumulation study shows that chlorpyrifos is absorbed by fish; however, it rapidly depurates when exposure ceases.

Oxidation of chlorpyrifos to chlorpyrifos oxon could potentially occur through photolysis, aerobic metabolism, and chlorination as well as other oxidative processes. Chlorpyrifos oxon is expected to have similar fate characteristics as chlorpyrifos except chlorpyrifos oxon is more soluble in water and undergoes hydrolysis faster. The hydrolysis half-life of chlorpyrifos oxon is significantly shorter than that observed for chlorpyrifos (5 days vs 81 days). Chlorpyrifos oxon hydrolyses to form TCP. For chlorpyrifos, water purification (chlorination) has been shown to be a major route of chlorpyrifos oxon formation and degradation.

3.3 Anticipated Exposure Pathways

Chlorpyrifos applications may be made directly to growing crops (food and feedstuffs) which may result in human exposure to chlorpyrifos in food and to chlorpyrifos oxon in drinking water (from surface and ground water sources). Registered uses that may result in residential (non-occupational) exposures to chlorpyrifos include aerial and ground-based fogger adult mosquitocide applications and golf course turf applications. There are also potential exposures for residential bystanders who live on, work in, or frequent areas adjacent to chlorpyrifos-treated agricultural fields from spray drift and volatilization. In occupational settings, exposure may occur while handling the pesticide prior to application, as well as during application. There is also a potential for post-application exposure for workers re-entering treated fields.

3.4 Consideration of Environmental Justice

Potential areas of environmental justice concerns, to the extent possible, were considered in this human health risk assessment, in accordance with U.S. Executive Order 12898, "Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations," (http://epa.gov/compliance/ej/resources/policy/exec_order_12898.pdf). As a part of every pesticide risk assessment, OPP considers a large variety of consumer subgroups according to well-established procedures. In line with OPP policy, HED estimates risks to population subgroups from pesticide exposures that are based on patterns of that subgroup's food and water consumption, and activities in and around the home that involve pesticide use in a residential setting. Extensive data on food consumption patterns are compiled by the U.S. Department of Agriculture (USDA) under the NHANES/WWEIA (What We Eat in America) Survey; 2003-2008 and are used in pesticide risk assessments for all registered food uses of a pesticide. These data are analyzed and categorized by subgroups based on age, season of the year, ethnic group, and region of the country. Additionally, OPP is able to assess dietary exposure to smaller, specialized subgroups and exposure assessments are performed when conditions or

circumstances warrant. Whenever appropriate, non-dietary exposures based on home use of pesticide products and associated risks for adult applicators and for toddlers, youths, and adults entering or playing on treated areas post-application are evaluated. Potential exposures from spray drift and via volatilization have also been considered in this risk assessment. The spray drift assessment is revised and previously implemented measures such as buffer zones were refined.

4.0 Hazard Characterization and Dose-Response Assessment

This section provides summary information and weight of evidence findings integrating multiple lines of evidence from experimental toxicology and epidemiology with respect to AChE/ChE inhibition (acetylcholinesterase/cholinesterase) and neurodevelopmental outcomes. This section also describes the use of a robust physiologically-based pharmacokinetic-pharmacodynamic (PBPK-PD) model for deriving points of departure (PoDs) and refined intra-species factors. Details of the science and data analysis that support these conclusions can be found in Appendices 1-6, 10, and 11. Code for the PBPK-PD model can be found in the docket for this risk assessment.

Appendix 1: Evaluation of Experimental Toxicology Data
Appendix 2: Detailed Review and Synthesis of Three Children's Environmental Health Cohort Studies
Appendix 3: Epidemiology Study Specific Evaluations
Appendix 4: Detailed Summary Tables of Children's Environmental Health Epidemiology Studies
Appendix 5. Summary of OPP's ChE Policy & Use of BMD Modeling
Appendix 6. Columbia Center for Children's Environmental Health (CCCEH) Epidemiology Data Acquisition "Raw Data" Request
Appendix 10. Dose Reconstruction Analysis
Appendix 11. New Literature on Chlorpyrifos since the 2012 FIFRA SAP Meeting

4.1 Introduction & Background

Mode of action (MOA) and adverse outcome pathways (AOPs) provide important concepts and organizing tools for risk assessment (Boobis et al., 2008; Seed et al., 2005; Sonich-Mullin et al., 2001; Meek et al, 2014; Ankley et al, 2010). MOAs/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. An AOP further defines the initial step in the process as the molecular initiating event (MIE; Ankley, et al., 2010). Fundamentally, MOA and AOP are different terms for basically the same concept. Consistent with the World Health Organization/International Programme on Chemical Safety, for this risk assessment, the terms are considered equivalent and interchangeable (Meek et al, 2014). For chlorpyrifos two different adverse outcomes provide the focus of the revised chlorpyrifos risk assessment: AChE/ChE inhibition and neurodevelopmental outcomes.

It is well established that AChE inhibition is the MOA/AOP for the cholinergic toxicity of organophosphate pesticides (OPs), including chlorpyrifos. Because AChE inhibition is the initiating event for this MOA/AOP, using AChE inhibition as a regulatory endpoint is protective of downstream cholinergic effects. Moreover, given the sensitivity of AChE inhibition data for OPs, these data have historically been considered to be protective of other potential toxicities and/or MOAs/AOPs for OPs.

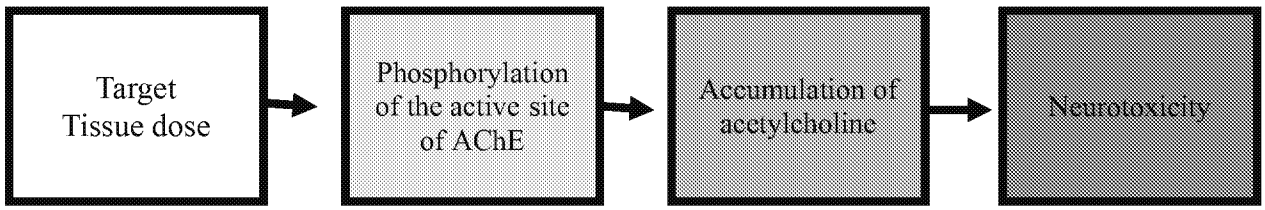


Figure 1. Adverse outcome pathway for OPs

Newer lines of research on chlorpyrifos, notably epidemiological studies in mothers and children, have raised some uncertainty about the Agency's risk assessment approach for chlorpyrifos with regard to the potential for neurodevelopmental effects. It is this uncertainty which provides the foundation for retaining the 10X FQPA Safety Factor as described in Section 4.6 and 4.7.

Over the last several years, the Agency has taken a stepwise, objective and transparent approach to evaluate, interpret, and characterize the strengths and uncertainties associated with all the lines of scientific information related to the potential for adverse neurodevelopmental effects in infants and children as a result of prenatal exposure to chlorpyrifos. The stepwise evaluation began with the September 2008 FIFRA SAP involving a preliminary review of the literature for chlorpyrifos, with a particular focus on women and children (USEPA, 2008). Subsequent Agency activities have involved developing approaches for performing risk assessment of semi-volatile pesticides (USEPA, 2009), and developing 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 early 2011, the FIFRA SAP reviewed the PBPK-PD model being used in this assessment to conduct quantitative risk assessment with estimates of human ChE inhibition following exposure to chlorpyrifos and/or the oxon from a variety of exposure pathways (FIFRA SAP 2011). In summer 2011, the Agency released its preliminary human health risk assessment that focused on the AChE inhibiting potential of chlorpyrifos (USEPA, 2011). This focus was consistent with the recommendation from the 2008 SAP that AChE data provide the most appropriate endpoint and dose-response data for deriving PoDs for purposes of risk assessment. Moreover, because of the Agency's long experience with assessing the potential risk to chlorpyrifos and other OPs, and because the dose response approaches based on AChE inhibition used in the 2011 preliminary assessment had been vetted by numerous SAPs, there was confidence in that approach.

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 epidemiologic studies from three major children's health cohort studies in the U.S., as well as plausible hypotheses on MOAs/AOPs leading to neurodevelopmental outcomes. Following the 2012 SAP meeting, the Agency solicited additional input from federal experts in the areas of MRI (Magnetic Resonance Imaging) and neurobehavioral testing in children to further clarify results obtained by examination of the epidemiological cohorts.[8] Also, the potential for exposure to lead and other environmental chemicals to affect the interpretation of the results from the Columbia University studies was investigated and EPA inquired about potential availability of additional information from investigators at Columbia University (Appendix 6). This effort has involved extensive collaboration across EPA and also within the Federal government.

Aspects of this risk assessment represent innovative approaches to pesticide human health risk assessment. For example, this risk assessment uses the draft "Framework for Incorporating Human Epidemiologic & Incident Data in Health Risk Assessment" (U.S. EPA, 2010) to develop a weight of evidence analysis integrating quantitative and qualitative findings across many lines of evidence, including experimental toxicology studies, epidemiology studies and PBPK-PD modeling, does not provide support for the reduction of the 10X FQPA Safety Factor. In addition, the Agency is explicitly using the robust PBPK-PD model to estimate human equivalent doses/concentrations and intra-species factors to chlorpyrifos and/or its oxon from multiple pathways (e.g. food, water, occupational, residential) of exposure.

4.2 Types of Studies Available for Analysis

Chlorpyrifos and its oxon are widely studied and thus have an extensive database of scientific studies. Included in the database are: guideline studies, special studies conducted by the registrant, and literature studies across many scientific areas reflective of different levels of biological organization (*e.g.* metabolism, MOA/AOP, *in vitro* and *in vivo* experimental toxicology, biomonitoring, epidemiology), various species (mouse, rabbit, dog, non-rodent, human) and across lifestages (fetal, postnatal, pregnant and non-pregnant adult). As reflected in the 2000 risk assessment, 2008 and 2012 draft issue papers for the SAP, the 2011 preliminary risk assessment, and this revised risk assessment, EPA has reviewed numerous studies for chlorpyrifos and the oxon and provided those citations in the various documents for public evaluation.

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 risk assessments to inform regulatory decision making[9]. EPA's Office of Chemical Safety and Pollution Prevention is currently developing systematic review policies and procedures. As part of the revised human health risk assessment, the Agency has reviewed and updated the experimental toxicology literature search since the 2012 SAP using the concepts consistent with systematic review such as detailed tracking of search terms and which literature have been included or excluded. The literature review was conducted in PubMed for the time

[8] http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2008-0850-0170
[9] 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"

period January 2012 up to August 2014 and was supplemented with ToxLine and Google Scholar (Appendix 11).

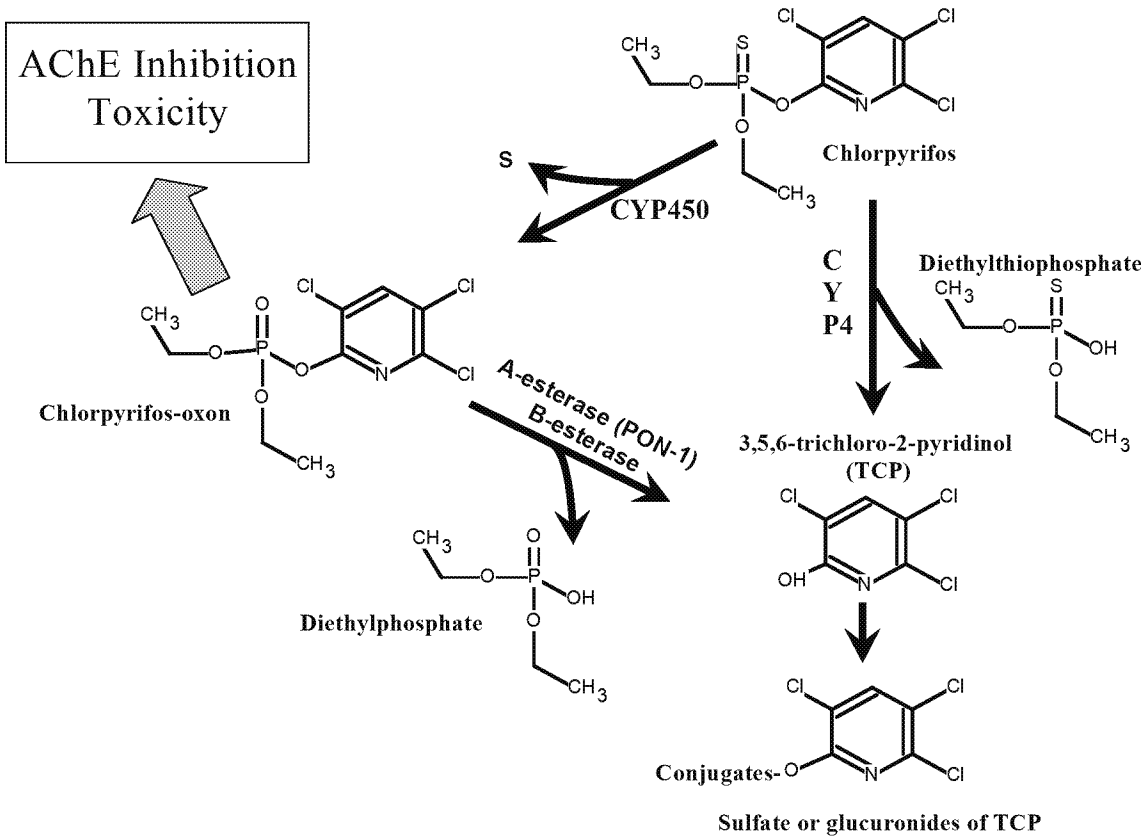
4.3 Absorption, Distribution, Metabolism, & Excretion (ADME)

The metabolism and pharmacokinetic (PK) profiles of chlorpyrifos and its oxon have been extensively studied in *in vitro* systems, *in vivo* laboratory animals, as well as humans. This large body of PK information is used in the PBPK-PD model which is described in more detail in Section 4.8. Only limited summary information is provided here.

Chlorpyrifos undergoes metabolic transformations mainly by the liver microsomal enzymes (*i.e.*, cytochrome P450s). Although, chlorpyrifos is lipophilic, its extensive metabolism into water soluble metabolites does not lead to any accumulation of the parent material or its metabolites in the body tissues. The initial metabolic action of chlorpyrifos is desulfuration, resulting in bioactivation of the parent compound to the more toxic and potent AChE inhibitor, the oxon form (Figure 2). However, the oxon is unstable and is rapidly deactivated through hydrolytic cleavage by a process called dearylation releasing 3,5,6-trichloro-2-pyridinol (TCPy). Simultaneous with the desulfuration process, dearylation acts on both the parent chlorpyrifos as well as on the oxon metabolite leading to the release of TCPy. TCPy is further conjugated to form glycine or glucuronide conjugates and eliminated into the urine. TCPy is the major excreted metabolite and used as the biomarker in PK, biomonitoring, and epidemiology studies. Diethylphosphate (DEP) is another metabolite often used in biomonitoring studies, but since it is produced by a number of OPs, DEP is not a specific marker for chlorpyrifos. An important aspect of the chlorpyrifos PBPK model is that chlorpyrifos and its oxon are approximately 98-99% protein bound in the blood.

There are several enzymes that have roles in the metabolism and toxicity of chlorpyrifos. In addition to inhibition of ChE, the oxon binds stoichometrically to B-esterases, the most important of which are butyrylcholinesterse (BuChE; abundant in blood, brain, and other tissues) and carboxylesterases (highest levels in liver). These B-esterases function as a scavenger, or "sink", of the oxon and may lessen its entry in the brain or peripheral targets to inhibit AChE. Another group of important enzymes in the detoxification of chlorpyrifos is the A-esterases; one such A-esterase is paraoxonase (i.e., PON1). These esterases are calcium-activated enzymes that are distributed in various tissues including the liver, brain and blood. These enzymes act on the oxon by hydrolyzing it before reaching its target AChE enzyme. The cytochrome P450 family of microsomal enzymes (CYPs) is also responsible for its metabolic activation and deactivation of chlorpyrifos. Glutathione-dependent enzymes play an important role in the secondary metabolism of chlorpyrifos producing water soluble metabolites that are readily excreted into the urine.

Figure 2. Major metabolic pathways of chlorpyrifos metabolism (Reproduced from Timchalk et al, 2002)



As summarized below and supported by the PBPK-PD model, there is lifestage sensitivity, particularly to the very young following acute exposures. This sensitivity is not derived from differential inhibition of the AChE enzyme itself as supported by *in vitro* studies (Benke and Murphy, 1975; Chanda *et al.*, 1995; Mortensen *et al.*, 1996; Atterberry *et al.*, 1997). Rat fetuses and juveniles and human fetuses, infants, and young children have lower capacity to detoxify chlorpyrifos than adults. Specifically, in rats, A-esterase activity is virtually nonexistent in the fetus (Lassiter et al., 1998) and increases from birth to reach adult levels around post-natal day 21 (PND21) (Mortensen et al., 1996; Li et al., 1997). Mortenson et al (1996) showed that in the plasma level of A-esterase in 4-day old rats was 1/11 that of adult animals. The animal data regarding the role of carboxylesterase in mediating OP toxicity are also quite extensive (e.g., Clement, 1984; Fonnum et al., 1985; Maxwell, 1992 a, b). Fetal rats and mice possess very little carboxylesterase activity with increasing activity as the postnatal rodent matures, reaching adult values around puberty (Lassiter et al., 1998; Morgan et al., 1994; Moser et al., 1998; Karanth and Pope, 2000; Zhu et al., 2009). The temporal pattern of A-esterase and carboxylesterase activity correlates well with studies on OP sensitivity. Several studies have shown an increased sensitivity of newborn rats to OP compounds which are detoxified via the A-esterase and/or carboxylesterase pathways (e.g., Gagne and Brodeur, 1972; Benke and Murphy, 1975; Pope et al., 1991; Chambers and Carr, 1993; Padilla et al., 2000; 2002; Karanth and Pope, 2000).

While there are fewer data in human tissues which could evaluate age-related maturation of carboxylesterase and A-esterase expression, there are numerous studies that present age differences in liver P-450 metabolism in humans that also play a role in OP metabolism (e.g., review by Hines, 2007). Studies evaluating maturational expression and activity of carboxylesterases in human liver tissues show lowest levels in fetal tissues, with increasing activity during childhood, although there are large individual differences and thus high variability (Pope et al., 2005; Yang et al., 2009; Zhu et al., 2009). Adult tissues were approximately 10-fold and 4-fold more active in hydrolyzing certain chemicals than fetal and child (0-10 years) tissues, respectively, and enzyme expression differences were much greater (Yang et al., 2009). In contrast, in Pope et al. (2005), the differences in activity were relatively small (and not statistically significant) between children ages 2–24 months and adults (20–36 years); however, that youngest age evaluated in the study was 2 months old and this individual had the lowest level of carboxylesterase.

Serum A-esterase levels also appear to be very low in human infants and children compared to adults (Augustinsson and Barr, 1962; Cole et al., 2003; Huen et al., 2009; Mueller et al., 1983; Ecobichon and Stephens, 1973; Gonzalez et al., 2012; Holland et al, 2006; Chen et al, 2003). While some studies have shown that PON1 activity reaches adult levels by about 2 years of age (e.g., Cole et al., 2003), recent papers have reported slightly lower levels as late as 9 years (Gonzalez et al., 2012). Reasons for these differences could include type of measurement (i.e., genotyping vs phenotyping), assay substrate, sample sizes, and population factors (e.g., ethnicity).

With respect to distribution to the fetus during gestation, there are multiple studies on chlorpyrifos (Mattsson et al., 1998, 2000;; Qiao et al, 2002) and other OPs (USEPA, 2006) which show that the pregnant dam exhibits similar or more AChE inhibition than the fetus at a given dose to the dam. As such, for AChE inhibition, protecting against AChE inhibition in the pregnant female is expected to be protective for AChE inhibition in the fetus. Biomonitoring data from rats and humans illustrate fetal exposure levels are similar to maternal levels which support these findings. Specifically, Whyatt et al (2003) have shown that levels of chlorpyrifos in maternal blood are similar to the levels measured in human umbilical cord blood. In Hunter et al. (1999), pregnant rats were orally dosed with chlorpyrifos for 5 days during late gestation at doses of 3 or 7 mg/kg/day, and the amounts of chlorpyrifos, chlorpyrifos-oxon, and TCPy were quantified in fetal and maternal brain and liver. No chlorpyrifos or its oxon were detected. The concentration of TCPy in the fetal brain was higher than the TCPy concentration in the maternal brain in time-course and dose-response studies but in liver the maternal levels were higher. In a study by Mattsson et al (1998, 2000), concentrations of chlorpyrifos, the oxon, and TCPy were measured in the blood of maternal and fetuses and TCPy levels in dam and fetal blood were similar and chlorpyrifos levels were approximately 2-fold higher in maternal blood than fetal blood. In another gavage gestational exposure study, Akhtar et al (2006) exposed rats to chlorpyrifos from GD 0-20 in fetal and maternal tissues on GD21 at high doses (9.6-15 mg/kg/day); while there is a high degree of variability in the Akhtar et al study, dams and fetuses showed similar levels of chlorpyrifos in liver and brain.

4.4 Summary of Experimental Toxicology Studies

Summarized below are key findings from experimental toxicology studies on AChE inhibition and neurodevelopmental outcomes. Details can be found in Appendix 1. Chlorpyrifos has also been evaluated for other adverse outcomes such as reproductive toxicity, developmental toxicity, cancer, genotoxicity, dermal toxicity, inhalation toxicity, and immunotoxicity. The summary of these findings can be found in the 2000 risk assessment which supported the Registration Eligibility Decision and the 2011 preliminary risk assessment. These adverse outcomes are less sensitive than AChE inhibition and neurodevelopmental effects and are thus not discussed in detail here.

4.4.1 AChE Inhibition in Experimental Laboratory Animal Studies

For the 2008 and 2012 SAP reviews and the 2011 preliminary risk assessment, the Agency performed comprehensive reviews of the literature for AChE inhibition. This risk assessment has been updated with newer studies. A summary of EPA's ChE policy with respect to the use of such data in risk assessment is provided in Appendix 5. AChE inhibition remains the most robust quantitative dose response data and thus continues to be the critical effect for the quantitative risk assessment. This approach is consistent with the advice of the SAP from 2008 and 2012. The Agency has conducted benchmark dose (BMD) analysis of numerous studies using empirical approaches previously accepted by the FIFRA SAP (USEPA, 2002) and consistent with the 2006 OP cumulative risk assessment (USEPA, 2006) and other single chemical OP risk assessments. Details on ChE studies and related analyses can be found in Appendix 1 and the preliminary HHRA (USEPA, 2011).

There are many chlorpyrifos studies evaluating AChE inhibition in red blood cell (RBC) or brain in multiple lifestages (gestational, fetal, post-natal, and non-pregnant adult), multiple species (rat, mouse, rabbit, dog, human), methods of oral administration (oral gavage with corn oil, dietary, gavage via milk) and routes of exposure (oral, dermal, inhalation via vapor and via aerosol). In addition, chlorpyrifos is unique in the availability of ChE data from peripheral tissues in some studies (e.g., heart, lung, liver). There are also literature studies comparing the *in vitro* ChE response to a variety of tissues (Chambers, 2013) which show similar sensitivity and intrinsic activity. Across the database, brain AChE tends to be less sensitive than RBC AChE or peripheral ChE. In oral studies, RBC AChE inhibition is generally similar in response to peripheral tissues. Thus, the *in vitro* data and oral studies combined supports the continued use of RBC AChE inhibition as the critical effect for quantitative dose-response assessment.

As with many OPs, female rats tend to be more sensitive than males to these AChE effects. For chlorpyrifos, there are data from multiple studies which provide robust RBC AChE data in pregnant, lactating, and non-pregnant female rats from oral exposure [e.g., DNT, reproductive, and subchronic rats], respectively. The $BMD_{10}/BMDL_{10}$[10] values from these studies range from 0.05/0.04 to 0.18/0.12 mg/kg/day (Table 4 in Appendix 1). Studies are available in juvenile pups which show age-dependent differences, particularly following acute exposures, in sensitivity to

[10] BMD_{10} is the estimated dose to yield 10% inhibition in RBC AChE inhibition compared to controls or background levels. The $BMDL_{10}$ is the lower 95% confidence limit on the BMD_{10}.

chlorpyrifos and its oxon (Tables 1, 2, 3, and 4 in Appendix 1). As discussed above, this sensitivity is not derived from differences in the AChE enzyme itself but instead are derived largely from the immature metabolic clearance capacity in the juveniles.

Multiple route-specific studies for the dermal and inhalation routes are available. Dermal AChE data are available from a 21-day study and 4-day probe study (MRID 40972801) in rats which together establish a NOAEL of 5 mg/kg/day and LOAEL of 10 mg/kg/day. Two subchronic inhalation toxicity studies (MRID Nos.40013901, 40166501, 40908401) in the rat are available using vapor phase chlorpyrifos which show no ChE effects up to a concentration of 20.6 ppb (287 $\mu g/m^3$ or 0.082 mg/kg/day (MRID 40013901, 40166501, 40908401). Multiple acute inhalation studies are also available. In a special acute inhalation study, female rats were exposed by nose only[11] to atmospheric concentrations of up to 53.9 mg/m^3 of particulate chlorpyrifos for six hours and allowed an additional 72 hours to recover (MRID No: 48139303 Hotchkiss et al. 2010, TXR # 0055409). Consistent and significant lung ChE inhibition were noted at the lowest concentration tested of 3.7 mg/m^3, which is a LOAEL. RBC and brain ChE inhibition were noted at $\geq$ 12.9 mg/m^3 and 53.9 mg/m^3, respectively, indicating they are less sensitive than lung and plasma ChE inhibition following acute inhalation exposures.

Since the 2011 preliminary risk assessment, two acute inhalation studies on the saturated vapor have been performed on the parent chlorpyrifos and chlorpyrifos oxon (MRID 49119501 and 49210101, respectively). In these studies, female rats were exposed by nose only to a saturated vapor of chlorpyrifos or its oxon for 6 hours to a time-weighted concentration of 17.7 ppb (0.254 mg/m^3) (MRID 49119501) or 2.58 ppb (35.3 $\mu g/m^3$) (MRID 49210101), respectively. There were no statistically-significant decreases in ChE activity in the RBC, lung, brain or plasma tissues. These acute studies along with the subchronic inhalation studies with vapor phase chlorpyrifos support a conclusion that acute exposure to the saturated vapor of chlorpyrifos or its oxon do not result in hazard.

4.4.2 Neurodevelopmental Outcomes in Laboratory Animals

There is a considerable and growing body of literature on the effects of chlorpyrifos on the developing brain of laboratory animals (rats and mice) indicating that gestational and/or postnatal exposure may cause persistent behavioral effects into adulthood. These data provide support for the susceptibility of the developing mammalian brain to chlorpyrifos exposure. In the 2008 and 2012 SAP reviews, the Agency evaluated the neurobehavioral studies available at that time; the literature review has been updated for the revised risk assessment (Appendix 1). Papers considered by EPA as addressing long-term outcomes from developmental exposure include only those where chlorpyrifos is administered during the pre-weaning period (gestational and/or postnatal) and the offspring are examined at some time after weaning. That is, papers reporting evaluations shortly after birth or during the pre-weaning period do not reflect long-term consequences and may also be confounded by AChE/ChE inhibition during concurrent or recent exposure. In addition, the Agency focused its efforts on studies using relatively low doses (e.g., 1 mg/kg/day), that is, doses that would not be expected to produce a considerable degree of brain AChE inhibition and resultant cholinergic toxicity. These constraints aid in the unencumbered

[11] Mass median aerodynamic diameter/geometric standard deviation was 1.9/1.51 , respectively

evaluation of longer-term effects compared to acute impacts of AChE inhibition. In total, the Agency has reviewed 31 papers generated from 14 different laboratories on areas such as cognitive function, anxiety/emotion, social behaviors/interaction, and motor activity. Twenty-five papers were reviewed for the 2012 SAP, and another six have been published and reviewed by ORD since then. The review of the new papers can be found in Appendix 1.

In spite of considerable differences in study design, upon review of the published literature a pattern of neurodevelopmental adverse outcomes emerges. Although the manifestations of these effects differ (nature and/or severity), these differences are likely due to the variability in study designs including developmental period of exposure, dosing scenarios, testing methods, ages of testing subjects, specific equipment used, choice of dependent variable, statistical analyses--all of which are critical features of all developmental neurotoxicity studies. While behavioral changes were consistently reported, they were somewhat inconsistent, possibly due to experimental design differences. Given the wide array of testing that has been conducted, some variability is not unexpected and in fact, the consistency of finding neurological effects is striking. At both the 2008 and 2012 SAP meetings, the Panel agreed that exposure to doses of 1 mg/kg/d and greater, during some developmental period, produced significant and long-term effects on animal behavior.

These studies report a range of neurobehavioral changes in rats and mice following developmental exposure to chlorpyrifos. Obvious species differences have not emerged, although effective doses are similar (1-6 mg/kg/d). Changes in various aspects of cognitive tests indicate perturbations of learning and/or memory, even though in some cases these may be manifested as improved function. Likewise, alterations in domains such as anxiety and social interactions may differ in direction of change, but are still suggestive of impacts on normal neuronal processing. There is replication of some effects across studies, and with some of the newer papers, across laboratories as well. Activity measures, on the other hand, still provide results as varied as the different measures of assessment. Taken together, these data do not provide evidence for a specific profile of effects but instead suggest more global alterations in neurobehavioral function.

All testing was conducted at various times after weaning (adolescents to adults), and there is a presumption that the effects are permanent; however, no study has directly addressed this issue, and there is a range in test ages. Dose-response is not always evident, since many studies only use one dose, and of those using two or more doses, there is not always a monotonic response. While there are demonstrated differences in uptake and persistence of chlorpyrifos given subcutaneously vs. oral, and with different oils or DMSO as vehicle, the developmental literature does not provide obvious differences in outcome based on these factors. Likewise, the experimental literature has not shown that any specific developmental period is critical overall to the long-term outcomes, since similar effects are shown with different exposure periods. For example, cognitive changes in the radial arm maze were observed following gestational and early postnatal (PND1-4), but not late (PND11-14), exposure (Aldridge, et al., 2005; Icenogle, et al., 2004; Levin, et al., 2002; Levin, et al., 2001). However, cognitive deficits were reported with the Morris water maze following both gestational and late postnatal exposures (Billauer-Haimovitch, et al., 2009; Jett, et al., 2001; Turgeman, et al., 2011). Similarly, 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, or definitive sensitive behavioral outcomes. Unfortunately, no laboratory has provided systematic comparisons across exposure period, dosing regimen, and age of testing; such studies would improve understanding of the impact of these critical factors.

These studies have almost exclusively focused on doses that could produce some degree, however minimal, of AChE inhibition. For example, a number of papers from Duke University researchers use a dose of 1 mg/kg/d administered 1-4 days after birth (e.g., Aldridge et al., 2005; Levin et al., 2001), but a recent publication (Slotkin et al., 2013) has reported that 5-10% inhibition of brain AChE activity is measured at 2 hours after the last dose. Given that this dose is widely used in many studies from several laboratories, this information clearly shows that while this dose does not induce toxic effects, it does however have a small but significant effect on pup brain AChE. Another study of relatively low doses administered chlorpyrifos in feed to pregnant rats, and even the lowest intake of 0.36 mg/kg/d produced about 20-25% RBC ChE inhibition (Ohishi et al., 2013). Thus it is not possible to know whether effects would be present at lower doses, since they have not been adequately studied.

Overall, across the literature on neurodevelopmental outcomes and including most recent publications, there continue to be inconsistencies in effects in relation to functional domains, dosing paradigms, and gender-specificity. The only studies reporting effects use doses that inhibit fetal/pup brain ChE activity to some degree, even though there are also negative effects at the same doses. The broad profile of neurological effects that have been reported do not aid in the development of a specific AOP (AChE inhibition or other mechanisms), and existing experimental studies have not been designed to examine and track possible mechanisms from early initiating events to the final neurological outcome.

4.4.3 Plausible hypotheses on MOA/AOP for neurodevelopmental outcomes

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.

- *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 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.

4.5 Epidemiology Studies in Mothers and Children

4.5.1 Review of Children's Environmental Health Epidemiology Studies

In this chlorpyrifos human health risk assessment (HHRA), EPA is including epidemiologic research results from three prospective birth cohort studies. These include: 1) The Mothers and Newborn Study of North Manhattan and South Bronx performed by the Columbia Children's Center for Environmental Health (CCCEH) at Columbia University; 2) the Mt. Sinai Inner-City Toxicants, Child Growth and Development Study or the "Mt. Sinai Child Growth and Development Study;" and 3) the Center for Health Assessment of Mothers and Children of Salinas Valley (CHAMACOS) conducted by researchers at University of California Berkeley. In these epidemiology 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.

These 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 chlorpyrifos 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 chlorpyrifos via the oral route through ingesting residues in the diet and from hand-to-mouth contact with in-home chlorpyrifos-contaminated surfaces, as well as possible dermal or inhalation exposure through contact with treated areas in the home environment (Berkowitz et al., 2003; Whyatt et al., 2003; Whyatt et al., 2009; Whyatt et al., 2007). 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) (Bradman et al., 2007). Reported use of chlorpyrifos in the CHAMACOS region was about 10% of total pesticide use (Eskenazi et al., 2004). In each of the children's health cohorts, the biological measurements in these cohorts were comparable to the general population (National Health and Nutrition Examination Survey; NHANES[12]).

The Columbia CCCEH study measured parent chlorpyrifos in cord blood, and other indicators (e.g., air sampling, behavioral information), as etiologic measures of exposure, while the other two birth cohorts measured non-specific urinary metabolites of chlorpyrifos and other OPs (TCPy, dialkyl phosphate metabolites) in the mothers to estimate pesticide exposure. Therefore, EPA considers the CCCEH Mothers and Newborn Study research results as most relevant to the chlorpyrifos HHRA; the other two cohorts provide important supporting information.

[12] http://www.cdc.gov/nchs/nhanes.htm

EPA performed its review and critical evaluation of these epidemiology studies in an open and transparent manner. The Agency presented an iterative evaluation of these data at two meetings of the FIFRA SAP, as well as discussed strengths and limitations of these studies in the preliminary human health risk assessment upon which EPA sought public comment in 2011 (EPA-HQ-OPP-2008-0850). In September 2008, EPA presented its initial review and assessment of the available epidemiological data (1999-2007) as well as supporting experimental studies as to the neurodevelopmental toxicity of chlorpyrifos to the Panel.[13] In April 2012, EPA presented an expanded and updated review of the available epidemiologic data (1999-2011) concerning the effect of chlorpyrifos exposure on children's environmental health in conjunction with a review of recent experimental studies as well as hypothesized adverse outcome pathways (AOP).[14] Published observational studies and supplemental analyses produced subsequent to the September 2008 FIFRA SAP evaluation expanded the knowledge base of potential long-term sequelae of prenatal chlorpyrifos exposure. Data included the results of supplemental analyses recommended by the 2008 SAP, additional data concerning intelligence measures at age 7 years from each study cohort, the effect of PON1 genotypic and phenotypic status on infant neurodevelopment, and several methodological studies relating to the accuracy and reliability of exposure and confounding variables. Both the 2008 and 2012 SAP panels agreed with EPA's assessment, and concluded that "chlorpyrifos likely played a role" in the observed neurodevelopmental outcomes.

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* chlorpyrifos 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 chlorpyrifos likely played a role in these outcomes. Although it cannot be stated the chlorpyrifos is the *sole* contributer to these outcomes; co-exposure to other OPs and mixtures of environmental exposures may also contribute to these outcomes.

4.5.2 Review of Study Design and Research Methods

The study design and research methods of these prospective cohort studies are summarized in this section. Appendices 2, 3, and 4 reflect a more detailed evaluation of these studies, study specific critical evaluations, and accompanying detailed evidence table for the included studies, respectively. These cohort studies each enrolled pregnant women from approximately 1997 through1999, measured both environmental exposure to the pesticide during pregnancy and also estimated 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.

[13] http://www.epa.gov/scipoly/sap/meetings/2008/091608_mtg.htm
[14] http://www.epa.gov/scipoly/sap/meetings/2012/041012meeting.html

EPA focuses its review on research results from these three epidemiological cohort studies into the chlorpyrifos HHRA due to the considerable strengths in study design, conduct, and analyses demonstrated in these investigations. 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 chlorpyrifos; 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. The lack of understanding of the critical window of exposure for these effects is a significant overarching uncertainty. Balancing the strengths as well as the limitations, EPA believes these data present an informative body of evidence with some notable consistencies across studies.

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 parent chlorpyrifos, TCPy and DEP to estimate chlorpyrifos and/or OP exposure in relation to neurodevelopment. There is uncertainty as to the extent measurement of non-specific metabolites of OP or chlorpyrifos accurately reflects chlorpyrifos exposure; for this reason, the Columbia CCCEH study provides the most valuable data for specifically assessing chlorpyrifos. The findings presented below in relation to DEP (chlorpyrifos or diazinon) and TCPy (chlorpyrifos or chlorpyrifos-methyl) provide supporting information. The Columbia CCCEH study does not estimate post-natal exposure to chlorpyrifos among child participants, therefore the influence of early life and childhood chlorpyrifos exposure is unaccounted for in these analyses. However, the pre- and post-natal chlorpyrifos levels would have to be strongly correlated to significantly affect the reported epidemiological risk estimates; the time period of the study reflects a rapid decline in use of the pesticide due to the voluntary cancellation of chlorpyrifos in the residential market. Further, the CHAMACOS cohort measured early life OP exposure in relation to neurodevelopment and did not observe significant associations (Eskenazi et al., 2007). The CHAMACOS cohort investigators also measured acetyl and butyl ChE as supplemental indicators of OP exposure. To overcome the limitations of a one-time measure of chlorpyrifos exposure, CCCEH authors performed additional analyses supporting the correlation of this measure with other measures of chlorpyrifos during the pregnancy period (TCPy[15] in meconium, air concentration of chlorpyrifos, and maternal urinary concentration of chlorpyrifos during the third trimester). Together, these multiple measures of exposure and internal dose improve our understanding of *in utero* chlorpyrifos exposure, although there are still uncertainties associated with the biomonitoring data collected at or near time of chlorpyrifos applications.

[15] Internal measures of TCPy (3,5,6-trichloro-2-pyridinol) need to evaluated with caution as they may also result from exposure to chlorpyrifos, chlorpyrifos-methyl, or TCPy in the environment.

Potential confounding bias is another major uncertainty within environmental epidemiology studies. Confounding variables, exposures that could be related to both chlorpyrifos 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).

EPA notes that researchers from the three birth cohort studies also investigated the possible role of prenatal chlorpyrifos exposure and fetal growth. These results were not consistent across these cohorts. Authors with CCCEH Mothers and Newborn Study 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 (Berkowitz et al., 2004; Eskenazi et al., 2004; R. M. Whyatt et al., 2004). Inconsistent results may be due to differences across study groups in exposure profiles as well as dissimilar methods of prenatal chlorpyrifos exposure assessment (Needham, 2005). The varied study results across cohorts and the limited biological rationale between reduced fetal growth and adverse neurodevelopment reduces the relevance of this evidence. The database of laboratory animal studies does not suggest that fetal growth is a key endpoint for concern with chlorpyrifos. Given the lack of consistency among cohorts for the fetal growth metrics and the lack of corroborating animal data, the proposed link between fetal growth and neurodevelopment is tenuous recalling that EPA's focus in the chlorpyrifos HHRA is on neurodevelopmental outcomes. These results are summarized below.

4.5.3 Neurological and Neurodevelopmental Health Effects in Children

4.5.3.1 The CCCEH Mothers and Newborn Study

Analysts with the CCCEH Mothers and Newborn Study have performed both etiologic studies into the relationship between prenatal chlorpyrifos exposure and adverse neurodevelopmental outcomes in children from infancy through early childhood, as well as important methodological research as to the validity and reliability of exposure and confounding variable measurement and statistical modeling. The result of this body of work extends the knowledge of the long-term sequelae of prenatal chlorpyrifos exposure and reduces uncertainty in important aspects of these analyses. Both are briefly described in this section.

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 (≤6.17pg/g) prenatal exposure, using categories informed by results of the previous study on birth characteristics (R. M. Whyatt et al., 2004). Authors reported that the difference in MDI scores was "marginally significant" (p = .06) between the "high" and "low" exposed groups; the high exposed group scoring an average of 3.3 points lower than the low exposed (V. A. Rauh et al., 2006). 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. 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 = .02) and PDI: 4.9 times greater (95% CI: 1.78-13.72; p = .002)) than those with lower prenatal exposure (V. A. Rauh et al., 2006).

Further, in supplemental analyses suggested by the 2008 FIFRA SAP, authors illustrated that when diazinon was added to the statistical model, the effect of chlorpyrifos on the MDI and PDI measure was greater (increasing the magnitude chlorpyrifos risk estimate for MDI and PDI 50-200% in the same direction (positively away from the null)) suggesting diazinon is a strong confounding variable in this association (correlation with chlorpyrifos 0.63), (R. Whyatt & Rauh, 2011). Lastly, Rauh et al. (2006) also investigated attention-problems in infants. When analyzing the 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).

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. (2011) 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 (Rauh et al., 2011). 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.

To ascertain whether observed differences in neurodevelopment by 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 (Rauh et al., 2012). Authors determined there were distinct morphological differences in brain areas associated with these neurodevelopmental outcomes. The pilot study

included only 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.[16] 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.

As mentioned above, CCCEH researchers have also performed epidemiologic methods research which in many ways reduces uncertainties related to key exposure and confounding measures within these studies. The extent to which the one (or the average of two) estimate of chlorpyrifos or DAP exposure during pregnancy reflects exposure(s) over critical windows of development during pregnancy is an uncertainty in these data. To address this uncertainty, investigators assessed the inter- and intra- individual variability in exposure measurements over time and determined that intra-individual variation is low such that the individual exposure profiles may be relatively stable over time (Whyatt et al., 2007). In a separate study, researchers measured the correlation between chlorpyrifos exposure measures taken at different time points in pregnancy as well as between internal dose (biomarker) and external exposure. They demonstrated that the one-time measure of cord blood chlorpyrifos levels at birth were moderately well correlated with measures of exposure earlier in pregnancy (meconium, 14-weeks gestation) and with environmental exposures (air concentration) during the last trimester of pregnancy (Whyatt et al. 2009). This additional information supports the validity of using the one-time measure of chlorpyrifos in cord blood as an approximation of *in utero* pesticide exposure during gestation as a method to correctly rank study participants by pesticide exposure category.

The appropriate control of potentially confounding variables is vital to an accurate assessment of the association under study. Both important characteristics of socio-economic status (SES) and estimates of other environmental exposures are important variables to consider in this analysis as inaccurate measurement of modeling of either type of variable could lead to a false positive result. With regard to both of these types of factors, the CCCEH researchers were able to perform additional analyses to clarify the estimated association, and, therefore, to reduce uncertainty in the interpretation of this study. SES is difficult to measure because the effects of variables such as race, class, education are related in complex ways. The CCCEH investigators augmented the understanding of SES using regression methods that model individual as well as group level variable contribution of overall model variability (Lovasi et al., 2011). This allows researchers to better isolate the effect of chlorpyrifos on neurodevelopment, holding constant the influence of socio-demographic influences. Using this approach, investigators demonstrated that the association between prenatal chlorpyrifos and neurodevelopmental outcomes remained significantly, positively related.

In addition, the authors were able to measure and model important environmental exposures including environmental tobacco smoke (ETS), polyaromatic hydrocarbons (PAHs), methylmercury and other ChE-inhibiting pesticides such as diazinon and propoxur (Whyatt & Rauh, 2011). Given the known relation between both lead and also methyl mercury and

[16] http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2008-0850-0170.

neurodevelopment, the authors performed supplemental analyses. The results of these analyses showed that neither pre-natal nor post-natal blood lead levels or methyl mercury levels measured in cord blood were significantly correlated with chlorpyrifos, and was therefore not considered a confounding variable in the association of interest within the Columbia cohort (Whyatt & Rauh, 2011; Rauh et al., 2006). This additional work significantly reduces uncertainty in research results.

Lastly, the authors demonstrated that chlorpyrifos exposure measures in the time periods immediately before and after the voluntary cancellation of chlorpyrifos for residential use reduced substantially (Whyatt & Rauh, 2011; Whyatt et al., 2004). CCCEH researchers illustrated that prior to the voluntary cancellation there were >80% detectable levels of chlorpyrifos in cord blood, in the time period after the cancellation, only 16% of the measured values were greater than the level of detection (LOD). Furthermore, although reported household use of any pesticide in general did not change over the same time period in this study, among newborns born before 1 January 2001, 34% had combined high level exposure levels whereas among newborns born after 1 January 2001, only one participant (1.5%) was in the highest chlorpyrifos exposure group—a difference that was highly significant (chi squared = 50, $p < 0.001$) (Whyatt et al., 2004).

In summary, CCCEH researchers, examined several aspects of infant and early childhood neurodevelopment in relation in chlorpyrifos exposure during the gestational period. The authors reported a 2-4 fold increase in reduced mental and also psychomotor development in infants exposed to chlorpyrifos *in utero* and these results are most strong at 36 months of age. At three years of age, the authors also demonstrated statistically significant evidence of differences in the proportion of pervasive developmental disorder diagnoses between children in the high and low exposure groups (+/- 6.17 pg/g). At seven years of age, the authors observed reduced measures of intelligence by increasing measures of prenatal chlorpyrifos exposure (1.4-2.4% decrease in intelligence score per 4.6 pg/g change, or 1 SD, of chlorpyrifos). Preliminary data from a small pilot study indicate, in principle, that differences in brain morphology between high and low chlorpyrifos exposed groups may exist, however, it is unclear whether these morphological differences are directly responsible for functional neurodevelopmental decrements observed (a conclusion supported by EPA's Federal Review Panel). In addition, these investigators performed several methodological investigations which reduce key uncertainties in these data, specifically with regard to exposure measurement error and potential confounding bias. This work reduces the probability that positive associations reported above are inaccurate, *i.e.*, they are false positive results.

4.5.3.2 Supporting Epidemiological Evidence: The Mt. Sinai and CHAMACOS Cohorts

As previously noted, for the purpose of informing the chlorpyrifos human health risk assessment, EPA has focused its review on the Columbia Mother's and Newborn Study among the three birth cohort studies reviewed due to researchers' measurement of parent chlorpyrifos *per se*. However, EPA also believes that it is valuable to comprehensively consider the results of all three of the children's environmental health cohorts together, given each study cohort contributes different

and unique information to the chlorpyrifos HHRA assessment. The Mt. Sinai and CHAMACOS cohorts examined other health outcomes (e.g., the Brazelton index of neonatal development and attentional difficulties in early childhood) and performed supplemental analyses (*e.g.,* effect modification by PON1 status) not reflected in the CCCEH Mothers and Newborn Study database.

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 (Engel et al., 2007; Young et al., 2005). 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.

Each of the three children's health cohorts utilized the Bayley scale (BSID-II) to generate an MDI and PDI score to assess neurodevelopment in early childhood (6-36 months). 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 Hispanics children; however, these associations either attenuated or were non-existent at the 24-month visit (Engel et al., 2011). In the CHAMCAOS cohort, Eskenazi et al. (2007) 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 (Eskenazi et al., 2010). 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 (Eskenazi et al., 2007).

Regarding intelligence measures, both research groups observed an inverse association between prenatal OP exposure and intelligence measures. In the Mt. Sinai study, prenatal maternal DEP urinary metabolite concentrations were associated with slight decrements in 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 (Engel et al., 2011). Similarly, in the CHAMACOS cohort, Bouchard et al. (2011) 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.

Results from the Mt. Sinai and CHAMACOS cohort studies support the results of the CCCEH study in several ways. Both of these studies also investigated infant mental and psychomotor development. With regard to mental development (as measured by the Bayley scale), both of these epidemiology studies reported increased risk of delays in appropriate mental development in relation to increasing DAPs, and the effects appeared to be greater at older ages tested; however, neither of these cohorts reported a link of psychomotor development. Similar to the CCCEH cohort study results, the CHAMACOS investigators also reported a significant increased odds of pervasive developmental disorder and attention difficulties in association with prenatal OP exposure; the Mt. Sinai study did not evaluate this outcome. Each study group reported an inverse relationship between increasing chlorpyrifos or OP exposure and intelligence measures using the same Wechslar scale of intelligence, when testing study participants at the same age and developmental time period. Researchers with each cohort reported evidence of an inverse exposure response relation, although the results were not consistently statistically significant. Taken together, these results bolster the findings reported by CCCEH's study authors.

4.5.4 Dose Reconstruction

In 2012, the SAP recommended that the Agency conduct a dose reconstruction analysis for the CCCEH study to help characterize the extent to which participants in the cohort may or may not have experienced RBC AChE inhibition. EPA has conducted such analysis and it is summarized here. In this analysis EPA has estimated potential exposures for pregnant women who may have used chlorpyrifos containing products and also to investigate possible post-natal exposures for children in the cohort. The primary goal was to estimate the degree to which adverse effects could be attributed to doses lower than those below the level eliciting elicit 10% inhibition of RBC AChE, which is the benchmark response level that has been used to establish the points of departure previously used for regulatory decision making.

Prior to 2000, chlorpyrifos was one of the most widely used insecticides for indoor pest control so there are many possible ways which exposures could have occurred as a result of these types of uses (e.g., use of a total release fogger, aerosol can sprays, or crack and crevice treatment by a pest control operator). Dose estimates for these uses were calculated based on the Agency's *SOPs for Residential Exposure Assessment*[17]. This SOP describes, on a scenario basis, specific algorithms and data used to predict the potential exposures which could have occurred in individuals in the cohort from these now canceled uses of chlorpyrifos. The potential for RBC AChE inhibition was then investigated using a chlorpyrifos specific PBPK-PD model that is described below. The scenarios which were considered represent the highest potential exposures for each population being evaluated. They include:

[17]See http://www.epa.gov/pesticides/science/residential-exposure-sop.html for further information. These SOPs provide the basis for health protective risk assessments for residential scenarios used routinely by EPA for regulatory purposes.

- Pregnant women who may have purchased a consumer aerosol can product and applied it in their homes;
- Exposures to pregnant women who may have had contact with residues in their homes after a previous treatment; and
- Exposures to young children (aged 1 to 2 years old) who may have contact with residues in their homes after a previous treatment.

The results of this analysis are presented below for pregnant women who potentially applied consumer products (Table 4.5.4.1). Details of the analysis can be found in Appendix 10. Results for pregnant women and children 1 to < 2 years old exposed from contact to residues following broadcast applications to hard surfaces in their homes are presented in Tables 4.5.4.2 and 4.5.4.3, respectively. Peak RBC AChE inhibition for pregnant women resulting from combined dermal and inhalation exposure occurring during the application, assuming continuous exposure for one hour, of a consumer product was 0.0012%. Similarly, peak RBC AChE inhibition for pregnant women (75 kg) resulting from dermal exposure occurring from contact with previously treated areas was 0.45% and from inhalation exposure immediately after application was 0.005%; combined peak % RBC AChE inhibition for pregnant women was 0.45%. For young children (11 kg), peak RBC AChE inhibition associated with the exposures from being in previously treated areas results in RBC AChE inhibition from dermal exposure of 0.14%, from mouthing behaviors of 2.2%, and from inhalation exposure of 0.014%; combined peak inhibition for children 1 to < 2 years old was 2.7%.

This dose reconstruction analysis only includes exposure to indoor uses of chlorpyrifos. At the time of recruitment into the CCCEH study, women could have been exposed to additional ChE-inhibiting pesticides indoors and likely were exposed to higher levels of ChE-inhibiting pesticides in food compared to current food residue levels of ChE-inhibiting pesticides (data from 1998 to 2012 indicate that total annual pounds of OPs applied to food crops has been steadily declining). For example, Whyatt et al (2003) report that 50% of cord blood samples in the CCCEH had detectable levels of diazinon, although all indoor uses of diazinon were removed from product labels in December 2002 due to concerns over human health. In addition other OPs were detected in some women at low frequency (0.5%-2%); included among these are some of the most potent OPs (methyl parathion, parathion, terbufos, phorate). The Agency's dose reconstruction analysis supports a qualitative conclusion that it is unlikely that > 10% RBC AChE inhibition would have occurred in the CCCEH participants; however, it is important to interpret the results in 4.5.4.1-4.5.4.3 with caution as it is possible that additional sources of exposure to OPs are not accounted for in this analysis.

Overall, the dose reconstruction results support a conclusion that indoor application of chlorpyrifos, when used as allowed prior to cancellation from the residential marketplace in 2000, likely would not have resulted in RBC AChE inhibition greater than 10% in pregnant women or young children. These findings are pertinent to the weight-of-evidence discussion for the FQPA 10X Safety Factor.

Table 4.5.4.1 Residential Handler (Pregnant Women in the Columbia Cohort) Estimated Exposures and Predicted % RBC AChE Inhibition (Route-specific and Combined)

Exposure Scenario	Formulation	Amount Handled	Dermal Dose (mg/kg/day)	Dermal: Peak % RBC AChE Inhibition	Inhalation Dose (mg/kg/day)	Inhalation: Peak % RBC AChE Inhibition	Combined Peak % RBC AChE Inhibition
Broadcast	Ready to Use: 1 % Aerosol Spray Can	1 can	0.049	0.0007%	0.030	0.0008%	0.0012%

Table 4.5.4.2 Residential Post-application (Pregnant Women in the Columbia Cohort) Estimated Dermal and Inhalation Exposures and Predicted % RBC AChE Inhibition (Route-specific and Combined)

Exposure Scenario	Formulation	Dermal Dose (mg/kg/day)	Dermal: Peak % RBC AChE Inhibition	Airborne Concentration of Chlorpyrifos (mg/m^3)	Inhalation: Peak % RBC AChE Inhibition	Combined: Peak % RBC AChE Inhibition
Broadcast (Hard Surfaces)	1% PCO[18] Application or RTU 1 16 oz can	0.71	0.45%	0.092	0.0049%	0.45%

Table 4.5.4.3 Residential Post-application (Children in the Columbia Cohort) Estimated Dermal and Inhalation Exposures and Predicted % RBC AChE Inhibition (Route-specific and Combined)

Exposure Scenario	Formulation	Dermal Dose (mg/kg/day)	Dermal: Peak % RBC AChE Inhibition	HTM Dose (mg/kg/day)	HTM Peak % RBC AChE Inhibition (mg/day)	Airborne Concn. of Chlorpyrifos (mg/m3)	Inhalation: Peak % RBC AChE Inhibition	Combined: Peak % RBC AChE Inhibition
Broadcast (Hard Surfaces)	1% PCO Application or RTU 1 16 oz can	1.3	0.14%	0.10	2.2%	0.092	0.014%	2.7%

4.5.4. Summary of EPA's Conclusions Regarding Children's Environmental Health Cohort Studies

Across these three children's environmental health studies, authors consistently identified associations with neurodevelopmental outcomes in relation to chlorpyrifos exposure. There is evidence of delays in mental development in infants (24-36 months), attention problems and pervasive developmental disorder in early childhood, and intelligence decrements in school age children who were exposed to chlorpyrifos or OP 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. Researchers with CCCEH study demonstrated that

[18] PCO: pest control operator; RTU: ready-to-use

environmental and biological measures of chlorpyrifos exposure decreased significantly in the time period subsequent to the voluntary cancellation of the pesticide.

These studies reflect several strengths and some notable limitations; however, upon analysis, EPA believes that it is more likely that research results were under-estimated, rather than over-estimated. Errors (or mistakes) in the measurement of exposure and confounding variables are major sources of biases that may result in an inaccurate measurement of risk. Within CCCEH study, researchers illustrated that due to low intra-individual variability in exposure over time, and the correlation between the (one-time) measure of cord blood chlorpyrifos and other measures of chlorpyrifos exposure throughout pregnancy, a one-time measure of exposure may accurately rank or order the study participants by chlorpyrifos exposure group in this cohort. EPA notes that given the nature of the study design, the most likely effect of any exposure measurement error would be to under-estimate rather than over-estimate risk (*i.e.* non-differential exposure misclassification). The lack of a post-natal estimate of chlorpyrifos exposure in the CCCEH study is a limitation; however, supporting data from the CHAMACOS cohort indicate post-natal OP exposure is not significantly related to neurodevelopmental outcomes. Further, both SES and other environmental exposures may be potential confounding variables in these studies, *i.e.*, they may be related to both chlorpyrifos exposure and neurodevelopment. However, EPA believes that authors were able to appropriately measure and model the effect of these variables on the study outcomes.

Therefore, as a result of the Agency's review and critical assessment of these data, including expert elicitation to characterize known uncertainties in these studies, EPA concludes that chlorpyrifos likely played a role in the neurodevelopmental outcomes observed in these epidemiology studies. It cannot be stated with certainty, however, that chlorpyrifos is the sole contributor to these effects.

4.6 Weight of Evidence Analysis

Sections 4.1-4.5 summarized key scientific information on the PK profile of chlorpyrifos and its oxon along with adverse health effects in animals and humans related to the effects of chlorpyrifos and its oxon on two different adverse health outcomes, AChE inhibition and potential for neurodevelopmental effects. The Agency has conducted a WOE analysis implementing the draft "Framework for Incorporating Human Epidemiologic & Incident Data in Health Risk Assessment" focused on two key scientific questions: 1) the degree to which scientific data suggest that chlorpyrifos causes long-term neurodevelopmental effects from fetal or early life exposure and 2) the degree to which adverse effects can be attributed to doses lower than those which elicit 10% inhibition of AChE, *i.e.*, the dose levels previously used for regulatory decision making.

4.6.1 Summary of the draft "Framework for Incorporating Human Epidemiologic & Incident Data in Health Risk Assessment"

In 2010, OPP developed a draft "Framework for Incorporating Human Epidemiologic & Incident Data in Health Risk Assessment" which provides the foundation for evaluating multiple lines of

scientific evidence in the context of the understanding of the adverse outcome pathway (or mode of action (U.S. EPA, 2010). The draft framework, which includes two key components: problem formulation and use of the MOA/AOP frameworks, was reviewed favorably by the SAP in 2010 (FIFRA SAP, 2010).

OPP's draft framework is consistent with updates to the World Health Organization/International Programme on Chemical Safety mode of action/human relevance framework, which highlight the importance of problem formulation and the need to integrate information at different levels of biological organization (Meek et al, 2014). Consistent with recommendations by the NRC in its 2009 report on *Science and Decisions*[19], OPP's draft framework describes the importance of using problem formulation at the beginning of a complex scientific analysis. The problem formulation stage starts with planning dialogue with risk managers to identify goals for the analysis and possible risk management strategies. This initial dialogue provides the regulatory context for the scientific analysis and helps define the scope of such an analysis. The problem formulation stage also involves consideration of the available information regarding the pesticide use/usage, toxicological effects of concern and exposure pathways and duration along with key gaps in data or scientific information. Specific to chlorpyrifos, the 2008 and 2012 SAP reviews represent the problem formulation analyses for the weight-of-evidence (WOE) evaluation. Appendix 1 specifically describes a conceptual framework which integrates pesticide use/usage, toxicological effects of concern and exposure pathways and duration in on effects of chlorpyrifos to key lifestages.

MOA (Boobis et al., 2006; Boobis et al., 2008; USEPA, 2005; Simon et al, 2014; Meek et al, 2014) and AOP (Ankley et al., 2010) provide important concepts in this integrative analysis. Both a MOA and an AOP are based on the premise that an adverse effect caused by exposure to a compound can be described by a series of causally linked biological key events that result in an adverse human health or ecological outcome. One of the key components of the Agency's draft framework is the use the MOA framework /AOP concept as a tool for organizing and integrating information from different sources to inform the causal nature of links observed in both experimental and observational studies. Specifically, the modified Bradford Hill Criteria (Hill, 1965) are used to evaluate the experimental support that establishes key events within a mode of action or an adverse outcome pathway, and explicitly considers such concepts as strength, consistency, dose response, temporal concordance and biological plausibility in a weight of evidence analysis. Section 4.4.3 and Appendix 1 provide the state of science on MOAs/AOPs for both AChE mediated cholinergic toxicity and neurodevelopmental effects.

4.6.2 Integration Across Multiple Lines of Evidence

Dose-response relationships & temporal concordance.
Since the MOA(s)/AOP(s) is/are not established for neurodevelopmental outcomes as described in section 4.4.3, it is not possible to describe the concordance in key events or biological steps leading to neurodevelopmental outcomes. As such, the quantitative linkages between MIEs,

[19] NRC (National Research Council). (2009). Science and decisions: Advancing risk assessment. Washington, DC: The National Academies Press. http://www.nap.edu/openbook.php?record_id=12209

intermediate steps, and ultimately the adverse outcome (i.e., neurodevelopmental effects) cannot be determined. Experimental toxicology studies in rodents suggest that long-term effects from chlorpyrifos exposure may occur. Due to the dose selections in most of these *in vivo* studies evaluating effects such as behavior and cognition, it is not known whether such adverse effects would be shown at doses lower than those which elicit 10% RBC AChE inhibition. It is notable, however, that comparing the lowest NOAEL observed in the *in vivo* animal studies (0.2 mg/kg/day; Billauer-Haimovitch et al., 2009) for the neurodevelopmental outcomes to the repeated dosing reliable $BMDL_{10}$ ranging from 0.05-0.17 mg/kg/day for RBC AChE inhibition suggests that AChE inhibition is a sensitive endpoint.

Within the epidemiology studies, the relationship in time between prenatal chlorpyrifos exposure and adverse neurodevelopmental outcomes is concordant. Specifically, with regard to the children's environmental health epidemiology studies, each of the three study cohorts utilized a prospective birth cohort study design in which mothers were recruited into study prior to the birth of the infants and development and identification of adverse effects; therefore, it is known with certainty that exposure preceded effect. In addition, because the time period under study within these cohorts, and specifically the Columbia University (CCCEH) study, spanned the point in time in which pesticide manufacturers voluntarily cancelled the use of chlorpyrifos in the home environment, 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 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 limit of detection as well as the observation of an absence of an association between prenatal chlorpyrifos exposure among infants born after the voluntary cancellation of chlorpyrifos 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.

With respect to the timing of exposure, the cord blood and other (meconium) measures from the Columbia University study 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 three children's health cohorts, particularly during the time period prior to the voluntary cancellation of indoor residential uses of chlorpyrifos containing pesticide products, exposures measured in the range reported in the epidemiology studies (pg/g plasma) are likely low enough that is unlikely to result in AChE inhibition. The FIFRA SAP (2012) concurred with the conclusion that measured levels of chlorpyrifos among epidemiology study participants were unlikely to have resulted in AChE inhibition. The urinary TCPy concentrations among mothers were comparable to the general population levels measured in NHANES. Comparing cord blood concentrations with the concentrations in which AChE inhibition was observed in adult volunteers indicates AChE inhibition would likely not have occurred at levels

observed in the epidemiology studies (6.17 pg/g). Therefore, while uncertainty exists as to actual chlorpyrifos exposure at (unknown) critical windows of exposure, EPA believes it is unlikely mothers enrolled in the birth cohort studies experienced RBC AChE inhibition.

This biomarker data from the Columbia University studies are supported by the Agency's dose reconstruction analysis using the PBPK-PD model. In accordance with 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. Based on the output from the PBPK-PD model, for the highest exposure considered (i.e., indoor broadcast use of a 1% chlorpyrifos formulation), <10% RBC AChE inhibition in pregnant women and young children would be expected from residential uses. It is noteworthy that all estimates of exposure based on conservative assumptions lead to predicted AChE inhibition levels < 10%.

Strength, consistency, and specificity.

As stated in the EPA neurotoxicity guidelines[20], 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 these two databases, 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. For example, chlorpyrifos studies in rats and/or mice have reported impaired cognition (spatial learning and working memory; e.g., Icenogle et al., 2004, Billauer-Haimovitch et al., 2009); changes in locomotor activity levels (exploration, rearing; e.g., Levin et al., 2002; Ricceri et al., 2003); and altered social interaction (aggression, maternal behavior; Venerosi et al., 2006, 2010); and effects on brain morphometrics (MRID no. 44787301; Chen et al, 2012). Similarly, epidemiologic investigations have reported effects on cognition (Bayley scale indices; Rauh et al. 2006; Eskenazi et al. 2007), abnormal motor development in neonates (reflexes, Brazelton score; Young et al., 2005; Engel et al. 2007), altered social development (*e.g.,* ADHD; Rauh et al. 2006; Bouchard et al. 2010), and MRI brain scans (Rauh et al., 2012). 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 the behavior studies (1 and 5 mg/kg/day) are sufficient to elicit approximately ≥10% brain inhibition and ≥30% in RBC inhibition ,

[20] http://www.epa.gov/raf/publications/pdfs/NEUROTOX.PDF

depending on the study design, age of the animal, and sampling time. In the epidemiology studies, based on the comparisons with biomonitoring data and the results of the 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 (although the Agency has not investigated the degree to which exposure to multiple AChE-inhibiting pesticides indoors simultaneously could impact this conclusion). 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 chlorpyrifos application and blood collections are not coupled---thus higher levels of blood chlorpyrifos were likely missed (albeit the results of the dose reconstruction analysis reduce the likelihood of this hypothesis).

In making a weight-of-evidence analysis, it is important to consider the strength of the statistical measures of association between prenatal chlorpyrifos 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. While it cannot be stated that chlorpyrifos alone is the sole contributor to the observed outcomes (specificity), since other environmental, demographic or psychosocial exposures may also play a part in these outcomes, this does not obviate the contribution of prenatal chlorpyrifos exposure in the development of adverse neurodevelopmental outcomes as echoed by the FIFRA SAP (2012).

The CCCEH study, which measures chlorpyrifos specifically, provides a number of notable associations. Regarding infant and toddler neurodevelopment, the CCCEH authors also reported statistically significant deficits of 6.5 points on the Bayley Psychomotor Development Index (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.

Importantly, across the three children's environmental health birth cohorts, decrements in intelligence measures were identified in relation to increasing levels of prenatal chlorpyrifos exposure. Authors from the Columbia cohort (CCCEH) reported statistically significant decreases of 1.4% in full scale IQ and 2.8% in working memory among seven-year olds for each standard deviation increase in chlorpyrifos exposure (Rauh *et al.*, 2011). These results persist

even when performing sensitivity analyses including only those with detectable chlorpyrifos levels. In addition, no evidence was provided of mediation by child behavior on the measure of working memory instrument.

Biological plausibility and coherence. EPA's Cancer Guidelines (2005) includes guidance which are also applicable to this current evaluation of chlorpyrifos. 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 chlorpyrifos and/or its oxon are biologically active on a number of processes that affect the developing brain. Moreover, there is a large body of *in vivo* laboratory studies which show long-term behavioral effects from early life exposure. 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. The CCCEH data are not considered in isolation, but rather are strengthened when considered in concert with the results from the other two cohort studies, as noted by the FIFRA SAP (July 2012). As noted above, the CHAMACOS and Mt. Sinai cohorts that measured neurological effects at birth (the Brazelton index), observed an association with chlorpyrifos (Engel et al., 2007; Young et al., 2005). Similarly, while not consistent by age at time of testing (ranging from 6 month to 36 months across the three cohorts), each cohort reported evidence of impaired mental and psychomotor development. Attentional problems and ADHD were reported by both Mt. Sinai and CHAMACOS investigators. Finally, each of the three cohort study authors observed an inverse relation between the respective prenatal measures of chlorpyrifos and intelligence measures at age 7 years.

Although uncertainties remain as articulated above, these uncertainties are diminished in the context of the qualitative similarity between the databases, and the concern for long-term neurodevelopmental effects as a result of prenatal, perinatal and possibly early life exposure.

4.7 Safety Factor for Infants and Children (FQPA Safety Factor)

The key issues being considered by the Agency in the WOE are 1) whether chlorpyrifos causes long-term effects from fetal or early life exposure and 2) whether adverse effects can be attributed to doses lower than those which elicit 10% inhibition of AChE). When taken together the evidence from 1) the experimental toxicology studies evaluating outcomes such as behavior

and cognitive function; 2) mechanistic data on possible adverse outcome pathways/modes of action; and 3) epidemiologic and biomonitoring studies lead to the following:

- Qualitatively, the Agency concludes that these lines of evidence together support a conclusion that exposure to chlorpyrifos results in adverse neurodevelopmental outcomes in humans, at least under some conditions.

- Quantitatively, the dose –response relationship of AChE inhibition across different life stages is established, but MOAs/AOPs for neurodevelopmental outcomes are not established.

  The database of *in vivo* animal toxicology neurodevelopmental studies on adverse outcomes includes only a small number of studies at doses lower than 1 mg/kg/day. Despite this uncertainty, the Agency noted that the BMD values in adult (pregnant and nonpregnant) female rats (0.05-0.15 mg/kg/day) are generally 10-fold or more lower than the doses where effects on neurodevelopmental outcomes in laboratory rats are observed.

  With respect to the mechanistic data, there are some effects which are similarly sensitive 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.

  As noted above, 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. Despite this uncertainty, the cord blood and other measures (meconium) provide evidence of exposure to the fetus during gestation. Moreover, exposure levels in the range measured in the epidemiology studies (pg/g) are likely low enough that is unlikely to result in AChE inhibition, as supported by the dose reconstruction analysis of residential use prior to 2000 (although the agency has not investigated the degree to which exposure to multiple AChE-inhibiting pesticides indoors simultaneously could impact this conclusion).

Given the totality of the evidence, the agency concludes that chlorpyrifos likely played a role in the neurodevelopmental outcomes reported by the Columbia University investigators but uncertainties such as the lack of an established MOA/AOP for neurodevelopmental effects and the exposure to multiple AChE-inhibiting pesticides precludes definitive causal inference. However, 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. **The FQPA 10X Safety Factor will be retained for infants, children, youths, and women of childbearing age for all exposure scenarios.**

4.8 Dose-Response Assessment

4.8.1 Durations of Exposure, Critical Windows of Exposure, & Temporality of Effects

In risk assessment, exposure is evaluated in conjunction with the toxicology profile. More specifically, a variety of toxicokinetic and toxicodynamic factors are considered. In the case of chlorpyrifos, exposure can occur from a single exposure (*e.g.*, eating a meal) or from repeated days of exposure (*e.g.*, worker, residential).

With respect to AChE inhibition, these effects can occur from a single exposure or from repeated exposures. For OPs, repeated exposures generally result in more AChE inhibition at a given administered dose compared to acute studies. Moreover, AChE inhibition in repeated dosing guideline toxicology studies with OPs show a consistent pattern of inhibition reaching steady state at or around 2-3 weeks of exposure in adult laboratory animals (U.S. EPA, 2002). This pattern observed with repeated dosing is a result of the amount of inhibition comes at equilibrium with production of new enzyme. As such, AChE studies of 2-3 weeks generally show the same degree of inhibition with those of longer duration (*i.e.*, up to 2 years of exposure). Thus, for most of the human health risk assessments for the OPs, the agency is focusing on the critical durations range from a single day up to 21 days (*i.e.*, the approximate time to reach steady state for most OPs). As described below, points of departure for various lifestages, routes, and scenarios have been derived at the acute and steady state durations.

With respect to effects on the developing brain, very little is known about the duration of chlorpyrifos exposure needed to precipitate adverse effects in the developing brain. There are critical windows of vulnerability (Rice & Barone, 2000; Rodier, 2004) with regard to toxicant effects on brain development. This vulnerable period in humans spans early pregnancy to adolescence (Rice & Barone, 2000). In fact, evidence shows that synapse formation peaks quite late in human brain development at 4-8 years of age (Glantz et al, 2007). Within these vulnerable periods there are key neurodevelopmental processes (*e.g.* cell division, migration, differentiation, synaptogenesis, and myelination) and each of these is region and stage specific. Consequently, the time of toxicant exposure will be a major determinate in the spectrum of neurotoxic effects. Because of the dynamic processes in the developing brain (*i.e.*, vulnerable windows) it is difficult to determine if the effect or differences in effects is due to duration of exposure or if different vulnerable windows were affected. As such, it is impossible at this time to rule out even a single day of high exposure to chlorpyrifos having a potential adverse neurodevelopmental effect in humans.

For the chlorpyrifos risk assessment, PoDs for various lifestages, routes, and scenarios have been derived at the acute and steady state durations.

4.8.2. Introduction to the PBPK-PD Model

As described in detail in EPA's 2006 document entitled, "*Approaches for the Application of Physiologically Based Pharmacokinetic (PBPK) Models and Supporting Data in Risk Assessment,*" physiologically based pharmacokinetic (PBPK) modelling is a scientifically sound and robust approach to estimating the internal dose of a chemical at a target site and as a means to evaluate and describe the uncertainty in risk assessments. PBPK models consist of a series of mathematical representations of biological tissues and physiological processes in the body that

simulate the absorption, distribution, metabolism, and excretion (ADME) of chemicals that enter the body. Examples of PBPK model applications in risk assessments include interspecies extrapolation, intra-species extrapolation, route-to-route extrapolation, estimation of response from varying exposure conditions, and high-to-low dose extrapolation. PBPK models can be used in conjunction with an exposure assessment to improve the quantitative characterization of the dose-response relationship and the overall risk assessment. These models can also be used to evaluate the relationship between an applied dose and biomonitoring data.

The chlorpyrifos PBPK-PD model includes the description of a molecular initiating event in the cholinergic toxicity MOA/AOP: AChE inhibition. Thus, the PBPK-PD model can be used to predict the critical dose metrics associated with cholinergic toxicity following chlorpyrifos exposure: RBC and brain AChE inhibition. Also predicted by the model are chlorpyrifos, its oxon, and TCPy levels in various tissues, such as plasma and urine. Age-specific parameters are incorporated allowing for lifestage-specific evaluations from infant through adulthood. The model can be run in two modes: deterministic and variation. In the deterministic mode, the output accounts for human specific metabolism and physiology thus obviating the need for the inter-species extrapolation factor for all for all age groups. In variation mode, distributions for16 parameters, which are critical for determining human variations in RBC AChE inhibition, are incorporated and thus the output accounts for intra-species extrapolation for infants, toddler, youths, and non-pregnant adults. As described below, the agency is not using the PBPK-PD model for pregnant females due to uncertainty associated with lack of appropriate mathematical equations to account for physiological changes during pregnancy that could impact population variability.

4.8.3 Description & Structure of the PBPK-PD Model

The PBPK-PD model for chlorpyrifos that was originally developed by Timchalk and coworkers in 2002 (Timchalk et al., 2002a, b) has been refined over the years as more data has become available (Busby-Hjerpe et al., 2010; Cole et al., 2005; Garabrant et al., 2009; Lee et al., 2009; Lowe et al., 2009; Lu et al., 2010; Marty et al., 2007; Timchalk and Poet, 2008; Timchalk et al., 2005; Timchalk et al., 2006). The model will not be described in detail here as it is described in numerous publications, including a report reviewed by the FIFRA SAP in 2011; summary information is provided here. All model code for the PBPK-PD model are provided in the public docket for the chlorpyrifos risk assessment. Developers of the chlorpyrifos PBPK-PD model sponsored a third-party quality assurance assessment to verify model parameter values and their respective sources. The Agency has conducted a review of this third-party assessment by randomly checking a subset of the values and sources in the model parameters. The agency also conducted a mass balance analysis. Minor inconsistencies were identified, and developers of the chlorpyrifos PBPK-PD model have since made the corrections, or provided additional references to justify their parameterization choices.

In recent collaborative research effort between Battelle Pacific Northwest National Laboratory and Dow (DAS et al., 2011; Poet et al, 2014; MRIDs 49074901, 48913401), the chlorpyrifos PBPK-PD model has been expanded from the 'typical adult' model to include other lifestages, specific examples were infants (6 months), children (3 year-olds), and adults (30 year olds).

Body weight can be calculated at any given age using a modified Gompertz growth function for both male and female (Smith et al., 2014). Subsequently, tissue volumes, blood flows to tissue, metabolism rates, ChE enzyme activities, and exposure doses are calculated as a function of body weight for infants through adulthood, which in turn is also a function of age. Furthermore, the PBPK-PD model was refined to include probabilistic capabilities to allow for refining the intra-species extrapolation factor. The FIFRA SAP reviewed the initial multiple lifestage, probabilistic model at its February 2011 meeting and recommended some additional improvements (FIFRA SAP, 2011; Hinderliter et al, 2011; Price et al, 2011). In response to the SAP, DAS made multiple changes and performed additional analyses, including a global sensitivity analysis, improvements to the quantitative approach to evaluating population variability across individuals at a given age, and an uncertainty analysis on metabolism data from human hepatic microsomes and plasma to address variation in response that occurs from metabolism (Dow, 2014a, b). Recently, code has been developed to simulate tissue dosimetry from dermal and inhalation exposure routes (Poet et al, 2014). The deterministic multi-route, lifestage PBPK-PD model code used in the chlorpyrifos risk assessment was submitted to the agency in 2013. The model code to evaluate population variability and to derive intra-species data-derived extrapolation factors was most recently updated and submitted in 2014 (Dow, 2014a, b)

The chlorpyrifos PBPK-PD model includes descriptions of the ADME of chlorpyrifos, and its metabolites, oxon and TCPy (Figure 3). The metabolic profile for chlorpyrifos is provided above in Figure 2. The PBPK-PD model contains descriptions of metabolism to account for chlorpyrifos, its oxon, and TCPy in liver, blood, brain, small intestine, lungs, diaphragm, and skin. Model parameterization was achieved most often by extrapolation from *in vitro* studies (animal and human tissues), in some incidences by extrapolation from rat *in vivo* studies, and, for TCPy pharmacokinetics, by fitting to human data[21]. The PD portion of the model is an extension of the PBPK model and relates the prediction of the formation of oxon to activity changes in AChE, BuChE, and carboxylesterase in brain, diaphragm, liver, lungs, plasma, and RBC.

To simulate exposures to infants and children, the model incorporates age-specific body weight prediction (Leucke *et al.* (2007) and Young *et al.*, (2009)), which is then used to scale the tissue volumes, blood flows to tissues, and ChE activities (Figure 4). In addition to age-related physiology, the model also incorporates age-specific metabolism based on *in vitro* data, allowing the model to assess changes in metabolism from infancy to adulthood. Total V_{max} of human enzymatic metabolism of CPF to TCPy, CPF to CPF-oxon, and CPF-oxon to TCPy in the liver over various ages were scaled to age-dependent volume of the liver (Table 4.8.3.1) and concentration of microsomes in the liver, which is approximately 37 mg/g tissue (Barter *et al.*, 2008). Thus, even in cases where the enzymatic measures show no *in vitro* age- or physiological-dependences (on a per mg protein basis), the total enzymatic capacity of the individual varies with age. In plasma, however, *in vitro* PON1 metabolism of CPF-oxon to TCPy was age-specific on a per ml plasma volume basis, as described previously. Therefore, the age-

[21] Two human deliberate dosing studies (Nolan *et al.* 1982; Kisicki et al, 1999) are available which have been reviewed by EPA's Human Studies Review Board
http://www.epa.gov/osa/hsrb/files/june2009finalreport92609.pdf
http://www.epa.gov/osa/hsrb/files/meeting-materials/apr-13-14-2011/appendix1.pdf
_These studies have been used in the development and evaluation of the PBPK-PD model.

specific V_{max} was scaled to age-dependent volume of the blood, resulting in plasma metabolism varying by almost 2 orders of magnitude. Values and sources of model parameters can be found in Tables 4.8.3.1-4.8.3.2 and were extracted from Dow 2011 and MRID 49248201.

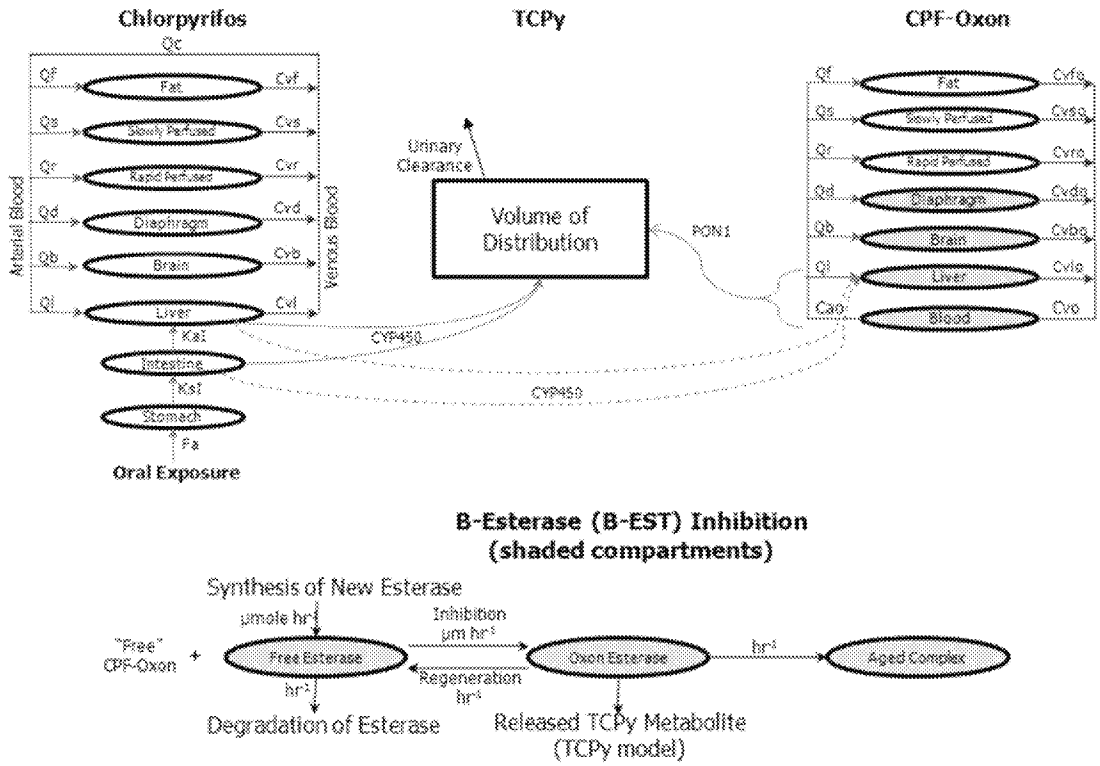


Figure 3. PBPK/PD model (Typical Adult model) structure. The shaded compartments denote tissues which contain B-esterases (bottom panel). Tissue volumes and enzyme activities (V_{max}) change with age based on liver and/or blood compartmental growth (Extracted from Dow, 2011).

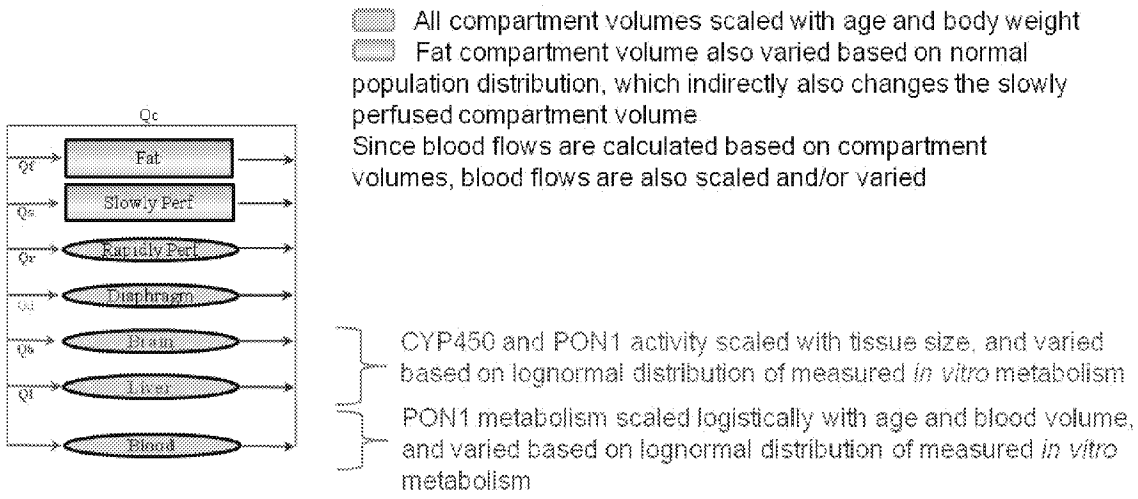


Figure 4. Schematic of age and body weight dependences in the PBPK-PD model. All compartment volumes and blood flows vary with age and body weight. *In vivo* metabolic rates are scaled based on tissue size (measured *in*

vitro values scaled to describe tissue-specific (brain, blood, and liver) metabolism); in blood, PON1 metabolism of oxon is not only blood volume but also age-dependent (Extracted from Dow, 2011).

Table 4.8.3.1. Pharmacokinetic model parameters.

Parameter	Value	Source
Tissue Ontogeny		
All	Scaled by age and body weight	See Table 4.8.3.2
Flows (L/hr/kg tissue volume)		
Cardiac Output	Summed from total tissue flow	See "*Blood flows*" in this section
Brain	30.6	Price *et al.*, 2003
Diaphragm	85.2	Luecke *et al.*, 2007
Fat	1.45	Luecke *et al.*, 2007, Cowles *et al.*, 1971, Price *et al.*, 2003
Liver	50.4	Price *et al.*, 2003
Rapidly Perfused	61.8	Adrenal/Spleen average: Price *et al.*, 2003
Slowly Perfused	1.80	Bone: Price *et al.*, 2003
Partition Coefficients for CPF (tissue:blood)		
Brain	16.5	Lowe *et al.*, 2009
Diaphragm	3.85	Lowe *et al.*, 2009
Fat	250	Lowe *et al.*, 2009
Liver	12.8	Lowe *et al.*, 2009
Rapidly Perfused	16.5	Lowe *et al.*, 2009
Slowly Perfused	3.85	Lowe *et al.*, 2009
Partition Coefficients for CPF-oxon (tissue:blood)		
Brain	5.6	Lowe *et al.*, 2009
Diaphragm	1.8	Lowe *et al.*, 2009
Fat	75	Lowe *et al.*, 2009
Liver	4.5	Lowe *et al.*, 2009
Rapidly Perfused	5.6	Lowe *et al.*, 2009
Slowly Perfused	1.8	Lowe *et al.*, 2009
Hepatic CYP Metabolic Constants (per tissue wt)		
CPF → TCPy Vmax (nmol/hr/kg)	1580	Measured *in vitro*, Median: See Table 4.8.3.2
CPF → TCPy Km (μM)	53.8	Measured *in vitro*, Median: See Table 4.8.3.2
CPF → Oxon Vmax (μmol/hr/kg)	689	Measured *in vitro*, Median: See Table 4.8.3.2
CPF → Oxon Km (μM)	85.8	Measured *in vitro*, Median: See Table 4.8.3.2
Intestinal CYP Metabolic Constants (per tissue wt)		
CPF → TCPy Vmax (μmol/hr/kg)	36.8	Poet *et al.*, 2003

Parameter	Value	Source
CPF → TCPy Km (μM)	55	Poet *et al.*, 2003
CPF → Oxon Vmax (μmol/hr/kg)	10.0	Poet *et al.*, 2003
CPF → Oxon Km (μM)	8.1	Poet *et al.*, 2003
Brain CYP Metabolic Constants (per tissue wt)		
CPF → TCPy Vmax (μmol/hr/kg)	3.85	Extrapolated: CPF metabolism/mechanism of action
CPF → TCPy Km (μM)	5.38	See Section 2: CPF metabolism/mechanism of action
CPF → Oxon Vmax (μmol/hr/kg)	0.91	Extrapolated: CPF metabolism/mechanism of action
CPF → Oxon Km (μM)	8.60	See CPF metabolism/mechanism of action
PON1 Metabolic Constants		
Plasma Vmax (μmol/hr/kg)	Logistic Fit	Measured *in vitro*, Median: See Table 4.8.3.2
Plasma Km (μM)	192	Measured *in vitro*, Median: See Table 4.8.3.2
Liver Vmax (μmol/hr/kg)	3902	Measured *in vitro*, Median: See Table 4.8.3.2
Liver Km (μM)	498	Measured *in vitro*, Median: See Table 4.8.3.2
Intestine Vmax (μmol/hr/mg)	246	Poet *et al.*, 2003
Intestine Km (μM)	328	Poet *et al.*, 2003
Oral Absorption		
Stomach →Intestine	0.5	Fitted: Nolan *et al.*, Timchalk *et al.*, 2002
Intestinal Absorption	0.2	Fitted: Nolan *et al.*, Timchalk *et al.*, 2002
Plasma Protein Binding (%)		
CPF	99	Lowe *et al.*, 2009
Oxon	99	Lowe *et al.*, 2009
TCPy Compartmental Model		
Vd (L)	$0.2 \times BW^{1.117}$	Fitted: Nolan *et al.*, 1984, Timchalk *et al.*, 2002
Ke (/hr)	0.013	Fitted: Nolan *et al.*, 1984, Timchalk *et al.*, 2002
Cholinesterase Degradation Rates (hr-1)		
Butyryl	0.004	Fitted (repeat dose data in rats: DOW unpublished)
Acetyl	0.01	Standardized, Timchalk *et al.*, 2002b
Bimolecular Inhibition Rate (μM hr^{-1})		
Butyryl	2000	Timchalk *et al.*, 2002

Parameter	Value	Source
Acetyl	220	Kousba *et al.* (2007)
Enzyme Turnover Rate (hr^{-1})		
Butyryl	$1.17x10^7$	Maxwell *et al.*, 1987, Timchalk *et al.*, 2002b
Acetyl	$3.66x10^6$	Maxwell *et al.*, 1987, Timchalk *et al.*, 2002b
Carboxyl	$1.086x10^5$	Maxwell *et al.*, 1987, Timchalk *et al.*, 2002b
Enzyme Turnover Rate (hr-1) Enzyme Turnover Rate (hr-1)		
Butyryl	11700000	Maxwell *et al.*, 1987, Timchalk *et al.*, 2002b
Acetyl	3660000	Maxwell *et al.*, 1987, Timchalk *et al.*, 2002b
Carboxyl	108600	Albers *et al.*, 2010
Enzyme Activity (µmol/kg/hr)		
Brain ACHE	440000	Hojring *et al.*, 1976
Diaphragm ACHE	77400	Maxwell *et al.*, 1987, Timchalk *et al.*, 2002b
Liver Carboxyl	1920000	Maxwell *et al.*, 1987, Timchalk *et al.*, 2002b; Pope *et al.*, 2005
Plasma Carboxyl	NA	Li *et al.*, 2005
Brain Butyryl	46800	Maxwell *et al.*, 1987, Timchalk *et al.*, 2002b
Diaphragm Butyryl	26400	Maxwell *et al.*, 1987, Timchalk *et al.*, 2002b
Liver Butyryl	30000	Maxwell *et al.*, 1987, Timchalk *et al.*, 2002b
Plasma Butyryl	263000	Sidell *et al.*, 1975
Enzyme Reactivation Rate (hr^{-1})		
Butyryl	0.0014	Carr and Chambers, 1996, Timchalk *et al.*, 2002b
Acetyl	0.014	Carr and Chambers, 1996, Timchalk *et al.*, 2002b
Carboxyl	0.014	Carr and Chambers, 1996, Timchalk *et al.*, 2002b
Enzyme Aging Rate (hr^{-1})		
Butyryl	0.0113	Carr and Chambers, 1996, Timchalk *et al.*, 2002b
Acetyl	0.0113	Carr and Chambers, 1996, Timchalk *et al.*, 2002b
Carboxyl	0.0113	Carr and Chambers, 1996, Timchalk *et al.*, 2002b

Table 4.8.3.2. Compartmental Growth.

Compartment		Fraction of Body Weight Equation[1] Eqn. Format $V0+V1\times BW+V2\times BW^2+V3\times BW^3+V4\times BW^4+V5\times BW^5+V6\times BW^6$							Source for LifeStage Parameters (BW: Body Weight in g)
		V0	V1	V2	V3	V4	V5	V6	
Blood	Leucke	$9.15e^{-2}$	$-8.59e^{-7}$	$1.25e^{-11}$	$-6.46e^{-17}$	-	-	-	Young *et al.* 2009
	Young	$8.97e^{-2}$	$-3.50e^{-7}$	$6.54e^{-13}$					
	LifeStage	$8.970e^{-2}$	$-3.500e^{-7}$	$6.540e^{-13}$	-	-	-	-	
Brain	Leucke	$1.19e^{-1}$	$-3.51e^{-6}$	$4.28\ e^{-11}$	$-1.82\ e^{-16}$	-	-		Fit to Valentin *et al.* 2002 (bwt≤70) & Young *et al.* 2009 data (body weight>70 kg) and extrapolated values based on Table 4A (Young *et al.* 2009)
	Young	$1.41e^{-1}$	$-5.54e^{-6}$	$9.30\ e^{-11}$	$-6.83e\ e^{-16}$	$1.80e^{-21}$			
	LifeStage	$1.216e^{-1}$	$-3.465e^{-6}$	$4.354e^{-11}$	$2.463e^{-16}$	$5.132e^{-22}$			
Diaphragm	Leucke	$3.000e^{-4}$	-	-	-	-	-	-	Luecke *et al.* 2007
	Young	NA	-	-	-	-	-	-	
	LifeStage	$3.000e^{-4}$	-	-	-	-	-	-	
Fat*									
Female	Leucke	$5.91e^{-2}$	$1.20e^{-5}$	$-5.80e^{-10}$	$1.12e^{-14}$	$-6.36e^{-20}$	-	-	Fit to Valentin *et al.* 2002 & Lafortuna *et al.* 2005 data and extrapolated data based
	Young	$1.84e^{-2}$	$-6.86e^{-6}$	$2.46e^{-10}$	$-2.11e^{-15}$	$7.58e^{-21}$	$-9.94e^{-27}$	-	

Compartment	Fraction of Body Weight Equation[1] Eqn. Format $V0+V1\times BW+V2\times BW^2+V3\times BW^3+V4\times BW^4+V5\times BW^5+V6\times BW^6$								Source for LifeStage Parameters (BW: Body Weight in g)
		V0	V1	V2	V3	V4	V5	V6	
	LifeStage	$9.217e^{-02}$	$1.401e^{-05}$	$-6.787e^{-10}$	$1.540e^{-14}$	$-1.558e^{-19}$	$7.249e^{-25}$	$1.273e^{-30}$	on equation in Fig 1B (Lafortuna *et al.* 2005)
Male	Leucke	$3.95e^{-2}$	$1.59e^{-5}$	$-6.99e^{-10}$	$1.09e^{-14}$	$-5.26e^{-20}$	-	-	Fit to Valentin *et al.* 2002 & Lafortuna *et al.* 2005 data and extrapolated data based on equation in Fig 1B (Lafortuna *et al.* 2005)
	Young	$1.61e^{-2}$	$-3.59e^{-6}$	$-8.28e^{-11}$	$-3.57e^{-16}$	$4.73e^{-22}$	-	-	
	LifeStage	$3.484e^{-2}$	$2.803e^{-5}$	$-1.422e^{-9}$	$2.892e^{-14}$	$-2.718e^{-19}$	$1.203e^{-24}$	$-2.036e^{-30}$	
Liver	Leucke	$3.49e^{-2}$	$-3.23e^{-7}$	$2.13e^{-12}$	-	-	-	-	Fit to Valentin *et al.* 2002 (body weight≤ 70) & Young *et al.* 2009 extrapolated values based on Table 4A
	Young	$4.25e^{-2}$	$-1.01e^{-6}$	$1.99e^{-11}$	$-1.66e^{-16}$	$4.83e^{-22}$	-	-	
	LifeStage	$3.917e^{-2}$	$-6.789e^{-7}$	$1.082e^{-11}$	$-7.393e^{-17}$	$1.701e^{-22}$	-	-	
Rapid									
Lung	Leucke	$1.67e^{-2}$	$-9.96e^{-8}$	$-1.09e^{-13}$	$1.13e^{-17}$	-	-	-	Young *et al.* 2009
	Young	$1.860e^{-2}$	$-4.550e^{-8}$	-	-	-	-	-	
	LifeStage	$1.860e^{-2}$	$-4.550e^{-8}$	-	-	-	-	-	
Kidney	Leucke	$7.31e^{-3}$	$-8.29e^{-8}$	$2.13e^{-12}$	-	-	-	-	Young *et al.* 2009
	Young	$7.26e^{-3}$	$-6.69e^{-8}$	$3.33e^{-13}$	-	-	-	-	
	LifeStage	$7.260e^{-3}$	$-6.690e^{-8}$	$3.330e^{-13}$	-	-	-	-	
Pancrease	Leucke	$1.17e^{-3}$	$-1.18e^{-8}$	$1.81e^{-13}$	-	-	-	-	Brown *et al.* 1997 and Young *et al.* 2009
	Young	$1.48e^{-3}$	-	-	-	-	-	-	
	LifeStage	$1.480e^{-3}$	-	-	-	-	-	-	
Spleen	Leucke	$3.05e^{-3}$	$-2.09e^{-8}$	$1.24e^{-13}$	-	-	-	-	Young *et al.* 2009
	Young	$3.12e^{-3}$	$5.57e^{-9}$	-	-	-	-	-	
	LifeStage	$3.120e^{-3}$	$5.570e^{-9}$	-	-	-	-	-	

Compartment		Fraction of Body Weight Equation[1] Eqn. Format $V0+V1\times BW+V2\times BW^2+V3\times BW^3+V4\times BW^4+V5\times BW^5+V6\times BW^6$							Source for LifeStage Parameters (BW: Body Weight in g)
		V0	V1	V2	V3	V4	V5	V6	
GI	Leucke	$1.93e^{-2}$	$-4.42e^{-7}$	$9.28e^{-12}$	$-4.88e^{-17}$	-	-	-	Brown *et al.* 1997
	Young	NA	-	-	-	-	-	-	
	LifeStage	$1.650e^{-2}$	-	-	-	-	-	-	
Slow									
Non-Fat adipose*	Leucke	NA	NA	NA	NA	NA	NA	NA	Fit to Valentin *et al.* 2002 & Lafortuna *et al.* 2005 fat data/0.8 (Valentin 2002) data and extrapolated data based on equation in Fig 1A (Lafortuna *et al.* 2005)
	Young	NA	NA	NA	NA	NA	NA	NA	
	LifeStage	$2.044e^{-1}$	$2.617e^{-5}$	$-1.542e^{-9}$	$3.268e^{-14}$	$-3.116e^{-19}$	$1.387e^{-24}$	$-2.35e^{-30}$	
Muscle	Leucke	$9.61e^{-2}$	$-4.88e^{-6}$	$3.05e^{-10}$	$-3.62e^{-15}$	$1.22e^{-20}$	-	-	Fit to Valentin *et al.* 2002 & Janssen *et al.* 2000 data and extrapolated values based on Fig 2A (Janssen *et al.* 2000)
	Young	$9.68e^{-1}$	$-3.32e^{-6}$	$1.83e^{-10}$	$-1.24e^{-15}$	-	-	-	
	LifeStage	$1.251e^{-1}$	$1.458e^{-5}$	$-2.927e^{-10}$	$2.114e^{-15}$	$-5.250e^{-21}$	-	-	
Skin	Leucke	$1.07e^{-1}$	$-3.26e^{-6}$	$6.11e^{-11}$	$-5.43e^{-16}$	$1.83e^{-21}$	-	-	Young *et al.* 2009
	Young	$1.03e^{-1}$	$-2.56e^{-6}$	$3.68e^{-11}$	$-2.580e^{-16}$	$8.620e^{-22}$	$-1.100e^{-27}$	-	
	LifeStage	$1.030e^{-1}$	$-2.560e^{-6}$	$3.680e^{-11}$	$-2.580e^{-16}$	$8.620e^{-22}$	$-1.100e^{-27}$	-	
Bone Marrow	Leucke	$5.19e^{-2}$	$8.06e^{-7}$	$-1.96e^{-10}$	$7.63e^{-15}$	$-1.08e^{-19}$	$5.14e^{-25}$	NA	Brown *et al.* 1997 (red only, yellow is in adipose)
	Young	NA	NA	NA	NA	NA	NA	NA	
	LifeStage	$2.100e^{-2}$	-	-	-	-	-	-	

4.8.4 Use of the PBPK-PD Model

Evaluation of PBPK-PD models intended for risk assessments includes a review of the model purpose, model structure, mathematical representation, parameter estimation (calibration), and computer implementation (USEPA, 2006b). The chlorpyrifos PBPK-PD model has been through several quality assurance reviews by various individuals or groups, including the agency, and found that the mathematical equations in this model were adequate to reasonably predict both blood/urine dosimetry and ChE inhibition in two controlled, deliberate oral human dosing studies (Nolan et al., 1984; Kisicki et al., 1999) and a dermal human study (Nolan et al., 1984). The PBPK-PD model predictions for rats inhaled chlorpyrifos compared well with animal data (Hotchkiss et al., 2013) with respect to chlorpyrifos, oxon, and TCPy concentrations in plasma, and ChE in plasma, RBC and brain (Poet et al., 2014). In addition, the agency has continued to critically evaluate the model code and related inputs during the risk assessment process. Significant improvements have been made to the PBPK-PD model in response to the 2008, 2011, and 2012 SAPs, the agency, and peer reviewers from academic journals in addition to the input of new data. The agency believes that the model is sufficiently robust for use in human health risk assessment. Because age-specific parameters are incorporated allowing for lifestage-specific evaluations from infant through adulthood, in the deterministic mode, the output accounts for human specific metabolism and physiology thus obviating the need for the inter-species extrapolation factor. The deterministic model can be used to simulate an "average individual" for all age groups. As such, as described below, the agency is using the PBPK-PD model to determine the points of departure (PoDs) for all age groups (See Table 4.8.4 below).

At the 2011 SAP meeting, the Panel specifically noted the lack of maternal and fetal PK and PD compartments in the current PBPK-PD model to inform about tissue dosimetry and AChE inhibition during lactation (FIFRA SAP 2011). As described in detail below, the agency has assessed exposure to bottle-feeding infants exposed to the oxon through water used with infant formula. With respect to chlorpyrifos or oxon exposure to infants through breast milk, any exposure to chlorpyrifos would be far lower than drinking water levels predicted by EFED. Thus, the agency is already accounting for oral exposure to chlorpyrifos to infants via bottle-feeding and a lactation component in the PBPK-PD model is not necessary.

The SAP also noted the lack of maternal and fetal PK and PD compartments in the current PBPK-PD model to inform about tissue dosimetry and AChE inhibition to pregnant women and their fetuses (FIFRA SAP 2011). With respect to exposure to the fetus during gestation, there are multiple studies on chlorpyrifos (Mattsson et al., 1998, 2000) and other OPs (U.S. EPA, 2006a) which show that the pregnant dam exhibits similar or more AChE inhibition than the fetus at a given dose to the dam. As such, for AChE inhibition, by protecting against AChE inhibition in the pregnant female is expected to be protective for AChE inhibition in the fetus. Biomonitoring data from rats and humans support the findings of the AChE studies. Specifically, Whyatt et al (2003) have shown that levels of chlorpyrifos in maternal blood are similar to the levels measured in human umbilical cord blood (Whyatt et al, 2003). With respect to the pregnant dam during gestation, metabolic activities and physiological parameters can be altered during pregnancy (for citations, see Appendix 1). While the current PBPK-PD model

accounts for age-related growth from infancy to adulthood by using polynomial equations to describe tissue volumes and blood flows as a function of age, the model does not include any descriptions on physiological, anatomical and biochemical changes associated with pregnancy. Due to the uncertainty in extrapolating the current model predictions among women who may be pregnant, **the agency is applying the standard 10X intra-species extrapolation factor for women of child bearing age.**

4.8.4.1 Derivation of Human Equivalent Doses/Concentrations

In typical risk assessments, PoDs are derived directly from laboratory animal studies and inter- and intra-species extrapolation is accomplished by use of 10X factors. In the case of chlorpyrifos and its oxon, PBPK-PD modeling is being used as a data-derived approach to estimate PoDs for all age groups and Data-Derived Extrapolation Factors (DDEF) for intra-species extrapolation for some groups (USEPA, 2014). The agency typically uses a 10% response level for AChE inhibition in human health risk assessment. This response level is consistent with the 2006 OP cumulative risk assessment (USEPA, 2006a) and other single chemical OP risk assessments. As such, the model has been used to estimate exposure levels resulting in 10% RBC AChE inhibition following single day (acute; 24 hours) and 21-day exposures for a variety of exposure scenarios (For discussion of durations see Section 4.8.1).

The PBPK-PD model accounts for PK and PD characteristics to derive age, duration, and route specific PoDs (Table 4.8.4 below). Separate PoDs have been calculated for dietary (food, drinking water), residential, and occupational exposures by varying inputs on types of exposures and populations exposed. Specifically, the following characteristics have been evaluated: duration [acute, 21 day (steady state)]; route (dermal, oral, inhalation); body weights which vary by lifestage; exposure duration (hours per day, days per week); and exposure frequency [events per day (eating, drinking)].

For each exposure scenario, the appropriate body weight for each age group or sex was modeled as identified from the Exposure Factors Handbook (USEPA, 2011) for occupational and residential exposures and from the NHANES/What We Eat in America (WWEIA) Survey[22] for dietary exposures. All body weights used are consistent with those assumed for dietary, occupational and residential exposure assessments. For infants from birth to < 1 year old, the agency has selected the body weight for the youngest age group, birth to < 1 month old, 4.8 kg (Exposure Factors Handbook, Table 8-3, mean body weight for the birth to < 1 month age group). For children between 1-2 years old, the body weight was set to 12.6 for dietary exposures (NHANES/WWEIA) and was set to 11 kg for residential exposures (Exposure Factors Handbook, Table 8-3, mean body weight for the 1 to < 2 year old age group). For female adults, the body weight was set to 72.9 kg for dietary exposures (NHANES/WWEIA) and was set to 69 kg for residential exposures (Exposure Factors Handbook, Table 8-5, mean body weight for females 13 to < 49 years old).

[22] http://www.ars.usda.gov/Services/docs.htm?docid=13793

The agency assesses dietary exposures for children 6-12 years old, and children between 6-11 years old for residential exposures. For purpose of aggregate assessment, these age groups are combined. The body weight for children 6-12 years old was set to 37.1 for dietary exposures (NHANES/WWEIA); the body weight for children 6-11 years old was set to 32 kg for residential exposures (Exposure Factors Handbook, Table 8-3, mean body weight for the 6 to < 11 year old age group). The agency assesses dietary exposures for youths 13-19 years old, and youths between 11-16 years old for residential exposures. For purpose of aggregate assessment, these age groups are combined. For youths 13-19 years old, the body weight was set to 67.3 kg for dietary exposures (NHANES/WWEIA); the body weight for youths 11-16 years old was set to 57 kg for residential exposures (Exposure Factors Handbook, Table 8-3, mean body weight for the 11 to < 16 year old age group).

The following scenarios were evaluated: dietary exposure to the oxon exposures via drinking water (24-hour and 21-day exposures for infants, children, youths, and female adults); exposure to chlorpyrifos exposures via food (24-hour and 21-day exposures for infants, children, youths, and female adults); 21-day residential exposures to chlorpyrifos via skin for children, youths, and female adults; 21-day residential exposures to chlorpyrifos via hand-to-mouth ingestion for children 1- 2 years old; 21-day residential exposures to chlorpyrifos via inhalation for children 1 -2 years old and female adults.

Dietary exposure was estimated for 7 days/week. For the dietary cases in which people are exposed to oxon via drinking water, the daily water consumption volume was set to 0.688557 L for infants, children between 1-2 year old, and children 6-12 years old, and 1.71062 L for youths 13-19 years old and female adults. Infants and children were assumed to consume water six times a day; youths and female adults were assumed to consume water four times a day. For the dietary cases in which people are exposed to chlorpyrifos via food, the eating event was set to one meal per day. The daily volumes consumed and number of daily consumption events for all populations are mean values by age group based on USDA What We Eat in America, NHANES survey for dietary exposures. The mean daily water consumption for children 1- 2 years old, 0.35 L, and children 6-12 years old, 0.58 L, was less than that for the infants, 0.688557 L; however, the infant daily water consumption volume was selected to be protective for PBPK-PD PoD derivation for these age groups. For youths 13-19 years old, the mean daily water consumption, 0.93 L, was less than that for the female adults; however, the adult daily water consumption was also selected to be protective.

For all residential dermal exposures to chlorpyrifos, the fraction of skin in contact with chlorpyrifos was set to 50%. A daily shower (i.e., washing off the chlorpyrifos) was assumed following chlorpyrifos exposure. All residential exposures were set to be continuous for 21 days. For residential exposures via golfing on treated turf, the daily exposure time is assumed to be 4 hours/day; for residential exposures via contact with turf following public health mosquitocide application, the daily exposure duration is assumed to be 1.5 hours. For residential inhalation exposures following public health mosquitocide application, the exposure duration was set to 1 hour per day for 21 days. The exposure times selected are based on those recommended in the 2012 Residential SOPs.

In addition to dietary and residential exposures, the PBPK-PD model was also used to estimate exposure levels resulting in 10% RBC AChE inhibition following occupational exposures (Table 4.8.4). Dermal exposures for workers assumed even distribution across the entire body surface area. A daily shower (i.e., washing off the chlorpyrifos) was assumed following chlorpyrifos exposure. The worker was assumed to be a female adult between the ages of 13 to 49, and had a body weight of 69 kg. This worker is exposed to chlorpyrifos either via inhalation or skin for 8 hours/day, 5 days/week, for a total of 21 days.

Table 4.8.4. Chlorpyrifos PBPK Modeled Doses (PoDs) Corresponding to 10% RBC AChE Inhibition

RA Type	Exposure Pathway (all chlorpyrifos unless noted)	Infants (< 1 yr old)		Young Children (1 - 2 years old)		Children (Residential:6-11 years old; Dietary:6-12 years old)		Youths (Residential:11-16 years old; Dietary:13-19 years old)		Females (13 – 49 years old)	
		Acute	Steady State (21 day)	Acute	Steady State (21 day)	Acute	Steady State (21 day)	Acute	Steady State (21 day)	Acute	Steady State (21 day)
Dietary	Drinking Water (oxon conc, ppb)	1,183	217	3,004	548	7,700	1,358	4,988	878	5,285	932
	Food (ug/kg/day)	600	103	581	99	530	90	475	80	467	78
Residential (Golfers)	Dermal (ug/kg/day)						25,750		13,950		11,890
Residential (Mosquitocide Application)	Dermal (ug/kg/day)				134,250						23,600
	Oral (ug/kg/day)				101						
	Inhalation (concn. in air mg/m3)				2.37						6.15
Occupational	Dermal (ug/kg/day)										3,630
	Inhalation (ug/kg/day)										138

*PoDs and exposure and risk estimates for females 13-49 yrs covers all youths >13 yrs

4.8.4.2 Intra-species extrapolation

With respect to intra-species extrapolation, the PBPK-PD model can be run in 'variation' mode which allows for age-specific parameters to vary across a distribution of values. The model will not be described in detail here as it is described in multiple recent publications, including a detailed report reviewed by the FIFRA SAP in 2011; summary information is provided here. All model code for the PBPK-PD variation model are available to the public.

Significant improvements have been made to the PBPK-PD model in response to the 2008, 2011, and 2012 SAPs, the agency, and peer reviewers from academic journals in addition to the input of new data. At the 2011 SAP, the panel was critical of some aspects of how Dow proposed to assess intra-species extrapolation. DAS made multiple changes, including the addition of a global sensitivity analysis, improvements to the quantitative approach to evaluating population variability across individuals at a given age, and an uncertainty analysis on metabolism data from human hepatic microsomes to address variation in response that occurs from metabolism. In addition, Dow performed additional analysis leading to further refinement of the derivation of intra-species extrapolation across multiple dose levels by incorporating human variation in 12 additional parameters in the predictions of RBC AChE responses to chlorpyrifos exposures.

Of the more than 120 parameters in the PBPK-PD model, 16 parameters were selected for varying in the DDEF intra-species analysis. They were selected using local and global sensitivity analyses (MRID 49248201, Dow, 2014a,b). The distributions for these 16 are provided in Table 4.8.4.2 (below). Interindividual variations for the 16 sensitive parameters (listed above) were assumed to follow a lognormal distribution. The distributions are truncated at far extreme values only to permit the model to compute but functionally not truncated with respect to assessing human variability. References cited in the table are listed in the report "Development of Chemical Specific Adjustment Factors for Chlorpyrifos and Chlorpyrifos Oxon" (MRID number 49248201) and also provided in Dow, 2014a,b,c.

hepatic CYP450 activation of chlorpyrifos to chlorpyrifos oxon	total blood volume	RBC ChE degradation rate	transfer rate of chlorpyrifos or oxon from the stomach to the intestine
hepatic PON1 detoxification of chlorpyrifos oxon to TCPy	hepatic blood flow	RBC ChE reactivation rate	volume of the liver
PON1 detoxification of chlorpyrifos oxon to TCPy in plasma	RBC AChE inhibition rate	intestinal CYP bioactivation to chlorpyrifos oxon	hepatic carboxyl basal activity rate
hepatic PON1 detoxification of chlorpyrifos oxon to TCPy	hematocrit	intestinal CYP detoxification to TCPy	hepatic carboxyl reactivation rate

Of these 16, four metabolism-related parameters (hepatic CYP450 activation of chlorpyrifos to chlorpyrifos oxon, hepatic CYP450 detoxification of chlorpyrifos oxon to TCPy, hepatic PON1 detoxification of chlorpyrifos oxon to TCPy, PON1 detoxification of chlorpyrifos oxon to TCPy in plasma) were found to drive more than 80% of the total variation in RBC AChE inhibition.

The human variability for these four parameters were assessed using *in vitro* data from 30 human hepatic microsome samples and 20 human plasma samples (Smith et al., 2011). Twenty of the hepatic microsome samples came from individuals < 12 years of age; and 10 of the samples came from adults > 17 years old. Ten of the plasma sample came from individuals < 2 years of age; and 10 of the samples came from adults. Because the findings from Smith et al (2011) account for more than 80% of the total variation in RBC AChE inhibition, it was determined that evaluating the uncertainty associated with the data (i.e., small number of samples compared to the large U.S. population) from this study was important to having confidence in the DDEFs derived from the variation model. Although some other *in vitro* studies shown in Table 4.8.4.2 also have small numbers of samples, these parameters make relatively small contributions to the overall variability. As such, additional quantitative uncertainty analysis on these *in vitro* studies is not needed.

The uncertainty associated with these four critical parameters were incorporated in the subsequent Monte Carlo analysis by generating 50 sets of unbounded parametric distributions using the following approach. First, the parametric bootstrap approach was used to sample 1000 values, with replacement, from the *in vitro* data. Then, this process was repeated for 50 iterations, and the resulting 50 sets of distribution all have equally probable sets of means and coefficient of variation as the observed data, except for the coefficient of variation of the plasma PON1 metabolism rate. Since the liver is the origin of PON1 in plasma, the variation of the plasma PON1 metabolism rate was set to be the same as the hepatic PON1 metabolism rate. Even though the distributions have similar means and coefficient of variation as the observed data, they included values outside of the range of the observed data because the distributions were assumed to be unbounded. These 50 sets of distributions, for each of the four parameters, were found to cover the entire range of the observed data; and the ratios of maximum value to minimum value in the simulated distributions were at least three times the ratios of maximum value to minimum value in the observed data.

Table 4.8.4.2 Sixteen parameters in variation model. Extracted from Dow, 2014c

Parameter	Mean value	Standard Deviation	CV	Variability Reference
Total Blood Volume (L/kg body weight)	0.08	0.0022	0.027	P^3M; Price *et al.*, 2003
Plasma PON1 (µmol/hr×L)	162000	92000	0.57	Smith et al., 2011
Hepatic Blood Flow (L/hr×kg tissue)	50	14	0.27	Materne et al., 2000
RBC ChE Inhibition Rate (l/µmol×hr)	100	17	0.17	Dimitriadis and Syrmos, 2011
Hepatic PON1 (µmol/hr×kg tissue)	154000	88000	0.57	Smith et al, 2011
Hematocrit (%)	0.45	0.031	0.068	P^3M; Price *et al.*, 2003
RBC ChE Degradation Rate (l/hr)	0.01	0.0014	0.14	Chapman *et al.*, 1968
Hepatic P450 Bioactivation to Oxon (µmol/hr×kg tissue)	690	410	0.59	Smith et al., 2011
Hepatic P450 Detoxification to TCPy (µmol/hr×kg tissue)	1500	800	0.53	Smith et al., 2011
RBC ChE Reactivation Rate (l/hr)	0.014	0.0050	0.36	Mason et al., 2000
Intestinal CYP Bioactivation to Oxon (µmol/hr×kg tissue)	82	43	0.52	Obach *et al.*, 2001
Intestinal CYP Detoxification to TCPy (µmol/hr×kg tissue)	53	28	0.52	Obach *et al.*, 2001
Transfer Rate to Intestine (hr-1)	0.31	0.081	0.26	Singh et al., 2006
Volume of the Liver (L/kg body weight)	0.032	0.0010	0.032	P^3M; Price *et al.*, 2003
Hepatic Carboxyl Basal Activity Rate (l/hr/kg tissue)	1270000	460000	0.36	Pope *et al.*, 2005
Hepatic Carboxyl Reactivation Rate (l/hr)	0.014	0.0050	0.36	Mason et al., 2000

According to EPA's Data-Derived Extrapolation Factor guidance, when calculating a DDEF intra-species extrapolation (USEPA, 2014), administered doses leading to the response level of interest (10% change in RBC AChE inhibition) are compared between a measure of average response and response at the tail of the distribution representing sensitive individuals. DAS has conducted an analysis to derive the oral doses that cause 10% RBC AChE inhibition in both adults and 6-month old infants (example provided in Figure 5a, b). The ratio of the adult ED_{10} to the infant ED_{10} was then used to derive intraspecies extrapolation factors. In the subsequent Monte Carlo simulations, the target age group is six month old individuals. Some model parameters are specific to this age group (e.g., PON1 metabolism in plasma), and some parameters are scaled by body weight that reflect this age group (e.g., tissue volume). Based on the 5th percentile of the distributions, the DDEF for intraspecies extrapolation is 2.8X for chlorpyrifos and 3.1X for the oxon (Dow, 2014b). Based on the 99th percentile of the distributions, the DDEF for intraspecies extrapolation is 4X for chlorpyrifos and 5X for the oxon (Dow, 2014b). For this revised HHRA, the 99th percentile is being used to account for sensitivities (i.e., the intra-species factor is 4X for chlorpyrifos and 5X for the oxon for all groups except women who are pregnant or may become pregnant). As shown in Figure 5b, at the 99th-ile, only 1% of infants will experience 10% or greater RBC AChE inhibition at the POD.

Figure 5a. Simulated population of 6 month olds for intra-species extrapolation DDEF derivation. Percent RBC AChE inhibition from exposure to single oral doses of chlorpyrifos ranging from 0.05 to 5.0 mg/kg/day (X and Y axes provided on the log scale).

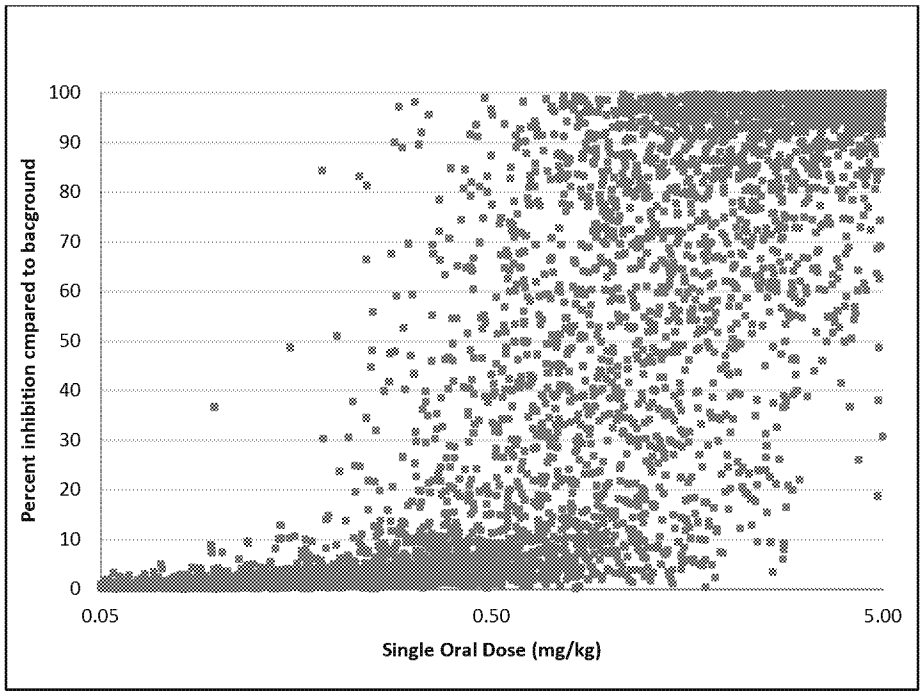
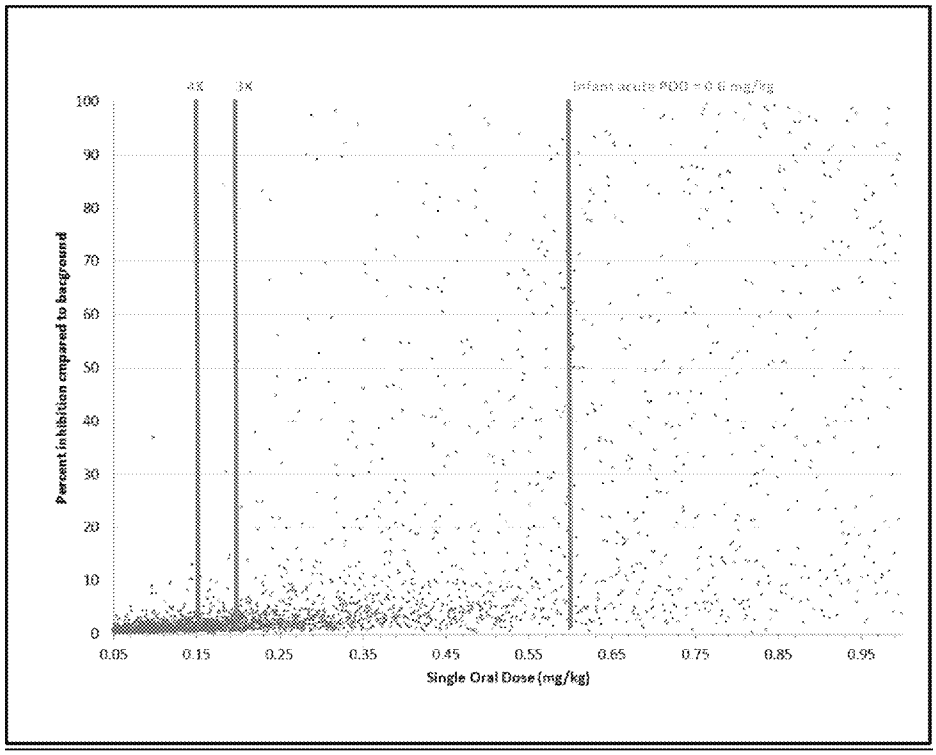


Figure 5b. Simulated population of 6 month olds for intra-species extrapolation DDEF derivation. Percent RBC AChE inhibition from exposure to single oral doses of chlorpyrifos ranging from 0.05 to 1.0 mg/kg/day. Green lines represent the infant acute PoD for chlorpyrifos, the PoD adjusted for the 3X and 4X intraspecies factors for the 95 and 99th-%ile, respectively.



In summary, for the chlorpyrifos HHRA, the PBPK-PD model has been used to derive PODs for RBC AChE inhibition for various populations, durations, and routes (Table 4.8.4). As such, the interspecies factor is not needed. To account for variations in sensitivities, an intra-species factor of 4X for chlorpyrifos and 5X for the oxon is applied for all groups except women of childbearing age. For women of childbearing age, the typical 10X intra-species factor is being applied due the lack of appropriate information and algorithms to characterize physiological changes during pregnancy. The 10X FQPA SF is being applied to women of childbearing age, infants, youths,

5.0 Dietary Exposure and Risk Assessment

5.1 Residues of Concern Summary and Rationale

The qualitative nature of the residue in plants and livestock is adequately understood based on acceptable metabolism studies with cereal grain (corn), root and tuber vegetable (sugar beets), and poultry and ruminants. The residue of concern, for tolerance expression and risk assessment, in plants (food and feed) and livestock commodities is the parent compound chlorpyrifos.

Based on evidence (various crop field trials and metabolism studies) indicating that the metabolite chlorpyrifos oxon would be not be present in edible portions of the crops (particularly at periods longer than the currently registered PHIs), it is not a residue of concern in food or feed at this time. Also, the chlorpyrifos oxon is not found on samples in the USDA PDP monitoring program. In fact, from 2007 to 2012, out of several thousand samples of various commodities, only one sample of potato showed presence of the oxon at trace levels, 0.003 ppm where the LOD was 0.002 ppm, even though there are no registered uses of chlorpyrifos on potato in the U.S.

The oxon metabolite was not found in milk or livestock tissues in cattle and dairy cow feeding studies, at all feeding levels tested, and is not a residue of concern in livestock commodities.

Oxidation of chlorpyrifos to chlorpyrifos oxon could potentially occur through photolysis, aerobic metabolism, and chlorination as well as other oxidative processes. Because of the toxicity of the oxon and data indicating that chlorpyrifos rapidly converts to the oxon during typical drinking water treatment (chlorination), the drinking water risk assessment considers the oxon as the residue of concern in treated drinking water and assumes 100% conversion of chlorpyrifos to chlorpyrifos oxon (see DWA, D424487).

The chlorpyrifos degradate TCP is not considered a residue of concern for this assessment as it does not inhibit cholinesterase (a separate human health risk assessment has been performed for TCP, which has its own toxicity database. TCP (derived from triclopyr, chlorpyrifos, and chlorpyrifos-methyl) was previously assessed on 6/6/2002 (W. Donovan, D283101)).

Table 5.1 Summary of Metabolites and Degradates to be included in the Risk Assessment and Tolerance Expression

Matrix		Residues included in Risk Assessment	Residues included in Tolerance Expression
Plants	Primary Crop	Chlorpyrifos	Chlorpyrifos
	Rotational Crop	Chlorpyrifos	Chlorpyrifos
Livestock	Ruminant	Chlorpyrifos	Chlorpyrifos
	Poultry	Chlorpyrifos	Chlorpyrifos
Drinking Water		Chlorpyrifos Oxon	Not Applicable

5.2 Food (Residue Chemistry) Profile

As mentioned in the 2011 assessment, newer crop field trial studies had been submitted for cotton gin byproducts (MRID 46651202), tart cherries (MRID 46651201), aspirated grain fractions for soybean, sorghum and wheat (MRID 46640901), and grass forage and hay as part of a data call in related to the 2002 chlorpyrifos IRED. These studies were reviewed previously and regulatory conclusions are included here for all the commodities with the exception of grass forage and hay (original petition withdrawn). The data are considered adequate to conclude that tolerances of 15 ppm and 22 ppm would cover any residues of chlorpyrifos on cotton gin by-products and aspirated grain fractions, respectively, and would support the current tolerance level for tart cherries under the condition that only dormant/delayed dormant and trunk spray applications are allowed on the label of the 75% WDG end use product.

The dietary burden to livestock was recalculated to consider residues at tolerance level in the feedstock commodities (aspirated grain fractions and cotton gin byproducts) and to use the most current version of Table 1 of the OPPTS Test Guidelines 860.1000, released on June 2008. Based on the residues observed in the feeding study of cattle beef, dairy cow, swine and poultry at the 1x level or higher, HED concludes that the possible residues observed on livestock commodities (from animals fed with feedstock that may contain residues resulting from legal applications) are covered by the current tolerances established in the 40 CFR §180.342.

There are some outstanding residue chemistry data requirements for chlorpyrifos. A magnitude of the residue study to establish a tolerance for wheat hay was required in the previous RED and has not been received. For soybean, processing studies are required to determine if tolerances are needed for chlorpyrifos in soybean meal, hulls and refined oil.

Studies submitted to support the registration of a microencapsulated formulation of chlorpyrifos showed over-tolerance residues after a foliar application of Lorsban 4E end use product to lemon with a rate of 6 lb ai/A. The maximum residue observed was 1.41 ppm while the tolerance for citrus fruit (CG 10) is 1.0 ppm. The label of the existing Lorsban 4E end use product (44.9% chlorpyrifos) allows a maximum application rate of 6.4 lb ai/A and addition of oil to the spray mixture. Under these use conditions residues over tolerance may occur. Magnitude of the residue studies are needed with lemon after application of Lorsban 4E and 75% WDG formulations separately in order to reevaluate the tolerance for the citrus fruit crop group.

Since the 2011 preliminary risk assessment, cotton processing studies have been submitted (MRID 00037455). The cotton processing study is considered scientifically acceptable. Following 16 broadcast foliar applications of the 4 lb ai/gal EC formulation of chlorpyrifos made at a total rate of 13.0 lb ai/A using aerial equipment, quantifiable (trail average) residues of chlorpyrifos were 0.103 ppm in/on cottonseed and 0.074 ppm in hulls harvested at a 18-day PHI. Residues of chlorpyrifos were nonquantifiable (<0.01 ppm) in solvent extracted meal and refined-bleached oil. A comparison of the residues in cottonseed with those in processed cottonseed fractions indicated that residues of chlorpyrifos do not concentrate in hulls (processing factor of 0.7x), solvent extracted meal (<0.1x), or refined-bleached oil (<0.1x). Separate tolerances are not needed for the cotton processed commodities. This data requirement is considered fulfilled.

A tolerance was previously established for chlorpyrifos in wheat milling fractions at 1.5 ppm. Also, for corn milled byproducts a tolerance of 0.1 ppm was previously recommended based on concentration factors from 1.25x in grits to 2x in flour (D188151, S. Knizner, 8/20/1993). Tolerances for residues of chlorpyrifos on wheat milled byproducts (1.5 ppm) and corn milled byproducts (0.1 ppm) should be included in the 40 CFR §180.342 as the current tolerance levels on the associated raw agricultural commodities, wheat grain and corn grain, are insufficient to cover any residues that may occur on these livestock feedstuffs.

[See Negrón-Encarnación, 5/24/11, D388164, *Chlorpyrifos. Registration Review Action for Chlorpyrifos. Summary of Analytical Chemistry and Residue Data* for complete details regarding label revision recommendations, chemistry data requirements and tolerance recommendations].

5.3 Water Residue Profile

EFED has provided a revised drinking water assessment (DWA) for chlorpyrifos (Bohaty, R., 12/23/14, D424487, *Chlorpyrifos: Updated Drinking Water Assessment for Registration Review*) which includes the EDWCs that are used in the aggregate assessment. The DWA also serves as the human health aggregate risk assessment since the EDWCs are compared to the DWLOC within that document.

See the Aggregate section 7.0 below for a summary of the DWA including the aggregate assessment results (comparison of DWLOCs to EDWCs). For complete details on the assumptions, results, and characterization of the drinking water and aggregate assessment refer to EFED's DWA (2014, D424487).

5.4 Dietary (Food Only) Risk Assessment

The general approach for the chlorpyrifos exposure and risk assessment can be described as follows: The PBPK-PD model was used to predict acute (24 hour) and steady state (21 day) points of departure dose levels (PoDs) which correspond to 10% RBC ChEI for the index lifestages relevant to chlorpyrifos risk assessment (children of various ages which differ due to exposure pattern, and adult females of childbearing age). The PoDs are then divided by the total uncertainty factor to determine the population adjusted dose (PAD). For food, the residue of concern is chlorpyrifos (the oxon metabolite is not an expected residue on foods). The chlorpyrifos total uncertainty factors are 100X for adult females (10X FQPA SF and 10X intra-species extrapolation factor) and 40X for the other populations (10X FQPA SF and 4X intra-species extrapolation factor). The chlorpyrifos exposure values resulting from dietary modeling are compared to the PAD. There are potential risks of concern when estimated dietary risk exceeds 100% of the PAD.

For the dietary risk assessment for food only, the exposure values resulting from Dietary Exposure Evaluation Model (DEEM) and the Calendex model are compared to the PBPK-PD-based acute PAD and steady state PAD, respectively. When estimated dietary risk estimates exceeds 100% of the PAD there may be a risk concern.

5.4.1 Description of Residue Data Used in Dietary (Food Only) Assessment

Acute and steady state dietary (food only) exposure analyses for chlorpyrifos were conducted using the Dietary Exposure Evaluation Model (DEEM) and Calendex software with the Food Commodity Intake Database (FCID) (D. Drew, 11/18/14, D424486, *Chlorpyrifos Acute and Steady State Dietary (Food Only) Exposure Analysis to Support Registration Review*). This software uses 2003-2008 food consumption data from the U.S. Department of Agriculture's (USDA's) National Health and Nutrition Examination Survey, What We Eat in America, (NHANES/WWEIA). The most recent previous dietary assessment was performed in 2011 to support chlorpyrifos registration review (D. Soderberg, 6/30/11, D388166, *Chlorpyrifos: Revised Acute (Probabilistic) and Chronic Dietary Exposure and Risk Assessments for Food Only (with and without Food Handling Use included) and for Water Only for the Registration Review Action – Typical Use Rates/Water Included)*. This current analyses reflect the latest consumption data as well as more recent food monitoring and percent crop treated data. These analyses was performed for the purpose of obtaining food exposure values for comparison to the chlorpyrifos doses predicted by the PBPK-PD model to cause RBC ChEI. The acute and steady state exposure analyses do not include drinking water which is assessed separately as discussed in Section 7.

All residues in food are assumed to be parent chlorpyrifos since the chlorpyrifos oxon is not typically found in foods in monitoring data or crop field trials. Food exposures are based only upon field and livestock use of chlorpyrifos and do not incorporate potential exposure from food handling establishment (FHE) uses since no active registered FHE uses have been identified by BEAD. The previous (2011) dietary risk assessment did include a chronic analysis for FHE use based on <2% establishments treated (BEAD could not confirm that there was any actual usage although there was a registered use at the time) and half the analytical limit of detection (½ LOD; 0.01 ppm) based on all nondetectable residues in a chlorpyrifos FHE study. The exposure from any potential FHE uses may be considered negligible compared to exposures from field uses.

Both the acute and steady state dietary exposure analyses are highly refined. The large majority of food residues used were based upon U. S. Department of Agriculture's Pesticide Data Program (PDP) monitoring data except in a few instances where no appropriate PDP data were available. In those cases, field trial data or tolerance level residues were assumed. The same data were used for both the acute and steady state analyses. BEAD of OPP provided percent crop treated information. Food processing factors from submitted studies were used as appropriate.

5.4.2 Percent Crop Treated Used in Dietary Assessment

The acute and steady state dietary exposure assessment used percent crop treated information from BEAD's Screening Level Usage Analysis (SLUA; May 1, 2014).

5.4.3 Acute Dietary (Food Only) Risk Assessment

Chlorpyrifos acute (food only) dietary exposure assessments were conducted using the Dietary Exposure Evaluation Model software with the Food Commodity Intake Database DEEM-FCID™, Version 3.16, which incorporates consumption data from USDA's National Health and

Nutrition Examination Survey, What We Eat in America, (NHANES/WWEIA). This dietary survey was conducted from 2003 to 2008. Acute dietary risk estimates are presented below for the sentinel population subgroups for acute risk assessment: infants (< 1 year old), children (1-2 years old), youths (6-12 years old) and adults (females 13-49 years old). The assessment of these index lifestages will be protective for the other population subgroups.

Acute dietary (food only) risk estimates are all <100 % of the acute PAD for food ($aPAD_{food}$) at the 99.9th percentile of exposure. The subgroup with the highest risk estimate was females (13-49 years old) at 3.2 % $aPAD_{food}$.

Table 5.4.3 Acute Dietary (Food Only) Exposure and Risk Estimates for Chlorpyrifos

Population Subgroup	$aPoD_{food}$[1] (ug/kg/day)	$aPAD_{food}$[2] (ug/kg/day)	Food Exposure[3] (ug/kg/day)	% of $aPAD_{food}$
Infants (< 1 yr)	600	15	0.273	1.8
Children (1-2 yrs)	581	14	0.423	3.0
Youths (6-12 yrs)	530	13	0.189	1.4
Adults (Females 13-49 yrs)	469	4.7	0.150	3.2

[1] acute point of departure; daily dose predicted by PBPK-PD model to cause RBC ChEI of 10% for acute dietary (food) exposures.
[2]aPAD= acute population adjusted dose = PoD (Dose predicted by PBPK-PD model to cause 10% RBC ChEI) ÷ total UF; Total uncertainty factor =100X for females 13-49 yrs (10X intraspecies factor and 10X FQPA uncertainty factor) and 40X for other populations (4X intraspecies factor and 10X FQPA uncertainty factor).
[3] Acute food only exposure estimates from DEEM (at 99.9th percentile). Refined with monitoring data and %CT.

5.4.4 Steady State Dietary (Food Only) Risk Assessment

A chlorpyrifos steady state dietary (food only) exposure analysis was conducted using Calendex-FCID™. HED's steady state assessment considers the potential risk from a 21-day exposure duration using a 3-week rolling average (sliding by day) across the year. For this assessment, the same food residue values used in the acute assessment were used for the 21-day duration. In the Calendex software, one diary for each individual in the WWEIA is selected to be paired with a randomly selected set of residue values for each food consumed. The steady state analysis calculated exposures for the sentinel populations for infant, child, youths, and adult (infants <1 yr, children 1-2 yrs, youths 6-12 yrs, females 13-49 yrs).

Calendex reported dietary exposures for each population subgroup at several percentiles of exposure ranging from 10th percentile to 99.9th percentile. The dietary (food only) exposures for

chlorpyrifos were all <100% $ssPAD_{food}$ (all populations, at all percentiles of exposure). Only the 99.9th percentile of exposure is presented in Table 5.4.4 below. Calendex results for other percentiles of exposure can be found in D424486.

For the steady state dietary (food only) exposure analyses, children (1-2 years old) was the population subgroup with the highest risk estimate at 9.7% of the $ssPAD_{food}$ at the 99.9th percentile of exposure.

Table 5.4.4 Steady State Dietary (Food Only) Exposure and Risk Estimates for Chlorpyrifos

Population Subgroup	$_{SS}PoD_{food}$[1] (ug/kg/day)	$ssPAD_{food}$[2] (ug/kg/day)	Food Exposure[3] (ug/kg/day)	% of $ssPAD_{food}$
Infants (< 1 yr)	103	2.6	0.186	7.2
Children (1-2 yrs)	99	2.5	0.242	9.7
Youths (6-12 yrs)	90	2.2	0.128	5.8
Adults (Females 13-49 yrs)	78	0.78	0.075	9.6

[1] Steady state point of departure; daily dose predicted by PBPK-PD model to cause RBC ChEI of 10% for steady state (21 day) dietary (food) exposures.
[2]ssPAD= Steady state population adjusted dose = PoD (Dose predicted by PBPK-PD model to cause 10% RBC ChEI) ÷ total UF; Total uncertainty factor =100X for females 13-49 yrs (10X intraspecies factor and 10X FQPA uncertainty factor) and 40X for other populations (4X intraspecies factor and 10X FQPA uncertainty factor).
[3]Steady state (21 day) food only exposure estimates from Calendex (at 99.9th percentile). Refined with monitoring data and %CT.

6.0 Residential (Non-Occupational) Exposure/Risk Characterization

The 2011 residential assessment has been updated[23] to reflect the use of the PBPK-PD modeling approach and EPA's SOPs for Residential Pesticide Exposure Assessment (USEPA, 2012). In 1997, the registrant, Dow AgroSciences, voluntarily agreed to cancel chlorpyrifos registrations for indoor broadcast use and direct pet treatments, except pet collars. In December 2001, the majority of the remaining chlorpyrifos residential products were subject to voluntary phase out/cancellation. Current chlorpyrifos residential uses include a granular ant mound use (commercial applicator only) and roach bait in child-resistant packaging (homeowner applicator). Additionally, chlorpyrifos is labeled for public health aerial and ground-based fogger ULV mosquito adulticide applications and for golf course turf applications. For the purpose of residential exposure assessment, the parent compound chlorpyrifos is the residue of concern.

This assessment also includes a retrospective dose reconstruction of the exposures which could

[23] W. Britton. Chlorpyrifos: Updated Occupational and Residential Exposure Assessment for Registration Review. 12/29/2014. U.S. EPA Office of Chemical Safety and Pollution Prevention. D424484.

have potentially occurred as a result of previous (now cancelled) chlorpyrifos uses in indoor residential environments (see Section 4.5.4).

6.1 Residential Handler Exposure

HED uses the term "handlers" to describe those individuals who are involved in the pesticide application process. HED believes that there are distinct tasks related to applications and that exposures can vary depending on the specifics of each task. Residential (non-occupational) handlers are addressed somewhat differently by HED as homeowners are assumed to complete all elements of an application without use of any protective equipment.

Based upon review of all chlorpyrifos registered uses, only the roach bait products can be applied by a homeowner in a residential setting but the application of roach bait products has not been quantitatively assessed because these exposures are negligible and do not pose a risk concern. The roach bait product is designed such that the active ingredient is contained within a bait station which eliminates the potential for contact with the chlorpyrifos-containing bait material.

6.2 Residential Post-Application Exposure

There is the potential for post-application exposures as a result of being in an environment that has been previously treated with chlorpyrifos. Chlorpyrifos can be used in areas frequented by the general population including golf courses. It is also applied as an aerial and ground-based ULV mosquito adulticide applications directly in residential areas. These uses were previously assessed in the 2011 preliminary risk assessment. Post-application exposure from residential ant mound treatment is not quantitatively assessed as exposures are considered to be negligible and do not pose a risk concern; these products can only be applied professionally and direct exposure with treated ant mounds is not anticipated.

The residential post-application exposure scenarios and application rates assessed in 2011 remain unchanged. However, the post-application exposure assessment has been updated to reflect the following changes: 1) use of the PBPK-PD model for determining toxicological PoDs, 2) use of the 2012 *Standard Operating Procedures for Residential Pesticide Exposure Assessment*[24], 3) use of the AgDISP model for estimation of airborne concentrations and residue dissipation following chlorpyrifos mosquito adulticide applications, 4) updated methodology for determining the airborne concentration of active ingredient following ground-based mosquito adulticide applications, and 5) use of updated body weights for all residential populations assessed.

In addition, steady state durations of exposure are assumed in the updated residential assessment. The steady state endpoint selection for chlorpyrifos overlaps HED's traditional short-term exposure duration endpoint selection and is considered health protective for both short- and intermediate- term exposures (additional explanation is available in section 4.8.1 above).

The quantitative exposure/risk assessment for residential post-application exposures is based on

[24] http://www.epa.gov/pesticides/science/USEPA-OPP-HED_Residential%20SOPs_Oct2012.pdf

the following scenarios:

Golf Course Use (Emulsifiable concentrate (EC) and Granular (G) formulations)

- Children 6 to < 11 years old, youths 11 to < 16 years old, and adult post-application dermal exposure from contact with treated turf while golfing.

Public Health Mosquito Adulticide Use (aerial and ground applications)

- Children 1 to < 2 years old and adult post-application dermal exposure from contact with turf following the deposition of chlorpyrifos residues from public health mosquito adulticide application.
- Children 1 to < 2 years old and adult post-application inhalation exposure from airborne chlorpyrifos following public health mosquito adulticide application.
- Children 1 to < 2 years old post-application incidental oral (hand-to-mouth) exposure from contact with turf following the deposition of chlorpyrifos residues from public health mosquito adulticide application.
- Children 1 to < 2 years old post-application incidental oral (object-to-mouth) exposure from contact with toys containing residues from turf following the deposition of chlorpyrifos residues from public health mosquito adulticide application.

A series of assumptions and exposure factors served as the basis for completing the residential post-application risk assessment and have been described in brief. The assumptions and factors are described in detail in the updated occupational and residential exposure and risk assessment (D424484).

Exposure Duration: Residential post-application exposures to chlorpyrifos are assumed to be steady state (i.e., 21 days or longer).

The application of mosquitocide in residential areas may result in the potential for post-application inhalation exposures. The aerosolized particulate remaining following application is assumed to persist for no longer than one hour in proximity to the application source and, accordingly, would be most appropriately defined as acute in duration. However, this assessment assumes that post-application inhalation exposures are steady state, which is a highly conservative approach given how infrequently mosquitocides are repeatedly applied to the same locations and how rapidly aerosols dissipate after these types of applications. The parameters used to define this exposure scenario in the PBPK-PD model conservatively reflect daily, one hour exposures for 21 days.

Application Rates: In order to seek clarification of chlorpyrifos usage, the Agency compiled a master use summary document reflective of the use profile of all active product labels. The document, among other information, presents all registered uses of chlorpyrifos and corresponding maximum single application rates, equipment types, re-entry intervals (REIs), etc. This assessment assumes that the detailed information on application rates and use patterns presented in Appendix 9 (Master Use Summary Document) will be implemented on all chlorpyrifos labels and is the basis of the occupational and residential risk assessment. If, for any reason, the final chlorpyrifos labels contain higher application rates, the actual risks posed by

those products may exceed the risks estimated in this assessment.

Body Weights: The body weights assumed for this assessment differ from those used in 2011 residential exposure assessment and are based on the recommendations of the 2012 Residential SOPs. These body weights are the same as selected for derivation of PBPK-PD PoDs for use in assessment of residential exposures.

The standard body weights are as follows: youths 11 to < 16 years old, 57 kg; children 6 to < 11 years old, 32 kg; and children 1 to < 2 years old, 11 kg. For adults when an endpoint is not sex-specific (i.e., the endpoints are not based on developmental or fetal effects) a body weight of 80 kg is typically used in risk assessment. However, in this case, a female-specific body weight of 69 kg was used. While the endpoint of concern, RBC AChE inhibition, is not sex-specific, the female body weight was used due to concerns for neurodevelopmental effects related to early life exposure to chlorpyrifos.

Post-application exposures from golfing have been assessed using the 2012 Residential SOPs and with use of exposure data from a chemical-specific turf transferable residue (TTR) study. The study was conducted with an emulsifiable concentrate, a granular, and a wettable powder formulation. Only the emulsifiable concentrate and granular data were used because there are no currently registered wettable powder formulations. The study was conducted in 3 states, California, Indiana and Mississippi, with use of the emulsifiable concentrate and wettable powder formulations. Exposure was estimated by normalizing Day 0 TTR measures from study application rates to the current maximum application rate allowable by the label. Chlorpyrifos oxon residues were not analyzed.

The post-application exposure potential from public health mosquito adulticide applications has been considered for both ground based truck foggers and aerial applications. For assessment of the mosquito adulticide use, the algorithms and inputs presented in the 2012 Residential SOP Lawns/Turf section were used coupled with the available TTR data described above. The deposition of chlorpyrifos from these applications are not based on the application rate alone, but also using the AgDISP (v8.2.6) model (aerial applications, the currently recommended model for assessment of mosquito adulticide applications) or empirical data (ground applications) to determine how much pesticide is deposited on residential lawns as a result of mosquito adulticide treatments at the maximum application rates for each. The TTR data are then used to determine the fraction of the total residue deposited following the mosquitocide application which can result in exposures to impacted individuals. Inhalation exposures are also estimated using AgDrift for aerial application and a recently developed well-mixed box (WMB) model approach for outdoor foggers.

As described above, the AgDISP (v8.2.6) model was used to estimate the deposition of chlorpyrifos from aerial applications and the airborne concentration of chlorpyrifos following public health mosquitocide application. AgDISP predicts the motion of spray material released from aircraft, and determines the amount of application volume that remained aloft and the amount of the resulting droplets deposited on the surfaces in the treatment area, as well as downwind from the treatment area. The model also allows for the estimation of air concentrations in the breathing zones of adults and children for use in calculating the post-application inhalation risks to individuals residing in areas being treated by aerial application of

chlorpyrifos. The aerial fraction of the mosquito adulticide application rate applied (0.010 lb ai/A) is 0.35 (i.e., 35 percent of application rate is deposited on turf); and the airborne concentration at the breathing height of adults and children of chlorpyrifos 1 hour following aerial mosquito adulticide application is 0.00060 mg/m^3.

Empirical data were used to derive the ground-based deposition of chlorpyrifos following public health mosquitocide application. These data, conducted by Moore *et al.* (1993)[25] and Tietze *et al.* (1994)[26], measured the deposition of malathion via ultra-low volume (ULV) ground equipment as applied for mosquito control. Based on these data, an off-target deposition rate of 5 percent of the application rate was used by HED to evaluate ground-based ULV applications (i.e., 5 percent of the target application rate deposits on turf). A value slightly higher than the mean values for both studies was selected because of the variability in the data and the limited number of data points. The adjusted application rate was then used to define TTR levels by scaling the available TTR data as appropriate.

In order to calculate airborne concentrations from ULV truck fogger applications, HED used the 2012 Residential SOPs for Outdoor Fogging/Misting Systems, with minimal modification to the well-mixed box (WMB) model. The WMB model allows for the estimation of air concentrations in the breathing zones of adults and children for use in calculating the post-application inhalation exposure to individuals residing in areas being treated by ground application of chlorpyrifos. This methodology is a modification of the previous method used in the 2011 occupational and residential exposure assessment to evaluate post-application inhalation exposure resulting from truck mounted mosquito fogger. The revised methodology more accurately accounts for dilution.

Combining Exposure and Risk Estimates

Since dermal, incidental oral, and inhalation exposure routes share a common toxicological endpoint, RBC AChE inhibition, risk estimates have been combined for those routes. The incidental oral scenarios (i.e., hand-to-mouth and object-to-mouth) should be considered inter-related and it is likely that they occur interspersed amongst each other across time. Combining these scenarios with the dermal and inhalation exposure scenarios would be unrealistic because of the conservative nature of each individual assessment. Therefore, the post-application exposure scenarios that were combined for children 1 < 2 years old are the dermal, inhalation, and hand-to-mouth scenarios (the highest incidental oral exposure expected). This combination should be considered a protective estimate of children's exposure to pesticides.

Summary of Residential Post-application Non-Cancer Exposure and Risk Estimates

The assessment of steady state golfer post-application exposures (dermal only) to chlorpyrifos treated turf for the lifestages adults, children 6 to < 11 years old, and youths 11 to < 16 years old,

[25] J.C. Moore, J.C. Dukes, J.R. Clark, J. Malone, C.F. Hallmon, and P.G. Hester. Downwind Drift and Deposition of Malathion on Human Targets From Ground Ultra-Low Volume Mosquito Sprays; Journal of the American Mosquito Control Association; Vol. 9, No. 2 (June, 1993)
[26] N.S. Tietze, P.G. Hester, and K.R. Shaffer. Mass Recovery of Malathion in Simulated Open Field Mosquito Adulticide Tests: Archives of Environmental Contamination and Toxicology; 26: 473-477 (1994)

results in no risks of concern (i.e., children 6 to < 11 and youths 11 to < 16 years old, MOEs are ≥ 40; adults, MOEs are ≥ 100). For the assessment of post-application exposures from public health mosquitocide applications, no combined risks of concern were identified for adults (dermal and inhalation) and children 1 to < 2 years old (dermal, incidental oral, and inhalation). A summary of risk estimates is presented in Table 6.2 below

Table 6.2. Residential Post-application Non-cancer Exposure and Risk Estimates for Chlorpyrifos.

Lifestage	Post-application Exposure Scenario		Application Rate[1]	State (TTR Data)	Dose (mg/kg/day)[3]	MOEs[4]	Combined Routes[5]	Combined MOEs[6]
	Use Site	Route of Exposure						
Adult (Females)	Golf Course Turf	Dermal	1.0 (Emulsifiable Concentrate)	CA	0.010	1,200	NA	NA
				IN	0.0069	1,700		
				MS	0.012	1,000		
				Mean	0.0095	1,200		
Youths 11 to < 16 years old				CA	0.010	1,400		
				IN	0.0069	2,000		
				MS	0.012	1,200		
				Mean	0.0096	1,500		
Children 6 to < 11 years old				CA	0.012	1,900		
				IN	0.0082	2,800		
				MS	0.014	1,600		
				Mean	0.011	2,000		
Adult (Females)			1.0 (Granular)	CA	0.0088	1,400		
Youths 11 to < 16 years old					0.0088	1,600		
Children 6 to < 11 years old					0.010	2,200		
Adult (Females)	Aerial and Ground Based ULV Mosquitocide Applications	Dermal	0.010 (Aerial)	MS	0.00052	46,000	X	8,400
		Inhalation		NA	0.00060 (mg/m^3)	10,300	X	
Children 1 to < 2 years old		Dermal		MS	0.00088	150,000	X	2,300
		Inhalation		NA[2]	0.00060 (mg/m^3)	4,000	X	
		Hand-to-Mouth		MS	0.000018	5,600	X	
		Object-to-Mouth		MS	6.1×10^{-6}	180,000	NA	NA
		Soil Ingestion		NA[2]	1.3×10^{-6}	4,900,000	NA	NA
Adult (Females)		Dermal	0.010 (Ground)	MS	0.000074	320,000	X	1,200
		Inhalation		NA	0.0051 (mg/m^3)	1,200	X	
Children 1 to < 2 years old		Dermal		MS	0.00013	1,100,000	X	460
		Inhalation		NA[2]	0.0051 (mg/m^3)	460	X	
		Hand-to-Mouth		MS	2.8×10^{-6}	39,000	X	
		Object-to-Mouth		MS	8.6×10^{-7}	1,300,000	NA	NA
		Soil Ingestion		NA[2]	1.7×10^{-8}	34,000,000	NA	NA

1 Based on the maximum application rates registered for golf course turf and ULV mosquito adulticide uses.
2 The airborne concentrations of chlorpyrifos following ULV mosquito adulticide applications was determined with use of the AgDISP (v8.2.6) model.
3 Dose (mg/kg/day) equations for golfing and mosquitocide applications are provided in Appendices B and C of the updated occupational and residential exposures assessment. For calculation of doses (i.e., dermal, hand-to-mouth, and object-to-mouth) from exposure to ULV mosquito adulticide, TTR data was used. The MS TTR data was selected for use because it is the worst

case and, as a result, most protective of human health. Additionally, the fraction of chlorpyrifos residue deposited following mosquitocide application, 35% (0.35), was determined with use of the AgDISP (v8.2.6) model and used for dose calculation. The fraction of chlorpyrifos deposited following ground ULV application, 5% (0.050), is based on surrogate exposure data (malathion). For dose estimation from exposures to golfing on treated turf, on the TTR data was used. Doses have been presented for all State sites, including the mean of all State sites.

4 MOE = PoD (mg/kg/day) ÷ Dose (mg/kg/day).

5 X indicates the exposure scenario is included in the combined MOE

6 Combined MOE = 1 ÷ (1/dermal MOE) + (1/inhalation MOE) + (1/incidental oral MOE), where applicable.

NA = Not applicable.

6.3 Residential Bystander Post-application Inhalation Exposure

6.3.1 Spray Drift

In the 2011 occupational and residential exposure assessment the potential risks to bystanders from spray drift were identified as possible concerns. Spray drift is the movement of aerosols and volatile components away from the treated area during the application process. The chemical may then be deposited on areas such as home lawns. Post-application exposures may occur to adults (dermal) or children (dermal and incidental oral) during activities on treated turf. The potential risks from spray drift and the impact of potential risk reduction measures were assessed in July 2012.[27] This evaluation supplemented the 2011 assessment where limited monitoring data indicate risks to bystanders. To increase protection for children and other bystanders, chlorpyrifos technical registrants voluntarily agreed to lower application rates and to other spray drift mitigation measures.[28] As of December 2012, spray drift mitigation measures and use restrictions appear on all chlorpyrifos agricultural product labels. Because the use of the PBPK-PD model impacts spray drift risks, an updated assessment was conducted.

Table 6.3.1 presents the buffer distances (feet) necessary to reach the level of concern for adults and children 1 to < 2 years old (i.e., adults, MOEs are ≥ 100; children 1 to < 2 years old, MOEs are ≥ 40) with use of certain application rates, nozzle droplet types, and application methods. The estimated buffer distances are less than those agreed to by the technical registrants in July 2012. All drift risk estimates are presented in the occupational and residential exposure assessment (Appendix D of D424484).

Table 6.3.1. Summary of Spray Drift Buffers for Chlorpyrifos[1]

Application Rate (lb ai/A)	Nozzle Droplet Type	Adult Buffer Summary			Children 1 to < 2 Years Old Buffer Summary (Dermal + Incidental Oral)		
		Buffers Necessary to reach MOE of 100 (Feet)			Buffers Necessary to reach MOE of 40 (Feet)		
		Aerial	Groundboom	Airblast	Aerial	Groundboom	Airblast
6.0	Medium or Coarse	NA[2]	NA	0	NA	NA	25
4.3		NA	0	0	NA	0	10
4.0		NA	0	0	NA	0	10
3.76		NA	0	0	NA	0	10
3.0	Coarse or	NA	0	0	NA	0	0

[27] J. Dawson, W. Britton, R. Bohaty, N. Mallampalli, and A. Grube. Chlorpyrifos: Evaluation of the Potential Risks from Spray Drift and the Impact of Potential Risk Reduction Measures. 7/13/12. U.S. EPA Office of Chemical Safety and Pollution Prevention. D399483, D399485.

[28] R. Keigwin. Spray Drift Mitigation Decision for Chlorpyrifos (059101). 7/2012. U.S. EPA Office of Chemical Safety and Pollution Prevention. EPA-HQ-OPP-2008-0850-0103.

Table 6.3.1. Summary of Spray Drift Buffers for Chlorpyrifos[1]							
Application Rate (lb ai/A)	Nozzle Droplet Type	Adult Buffer Summary			Children 1 to < 2 Years Old Buffer Summary (Dermal + Incidental Oral)		
		Buffers Necessary to reach MOE of 100 (Feet)			Buffers Necessary to reach MOE of 40 (Feet)		
		Aerial	Groundboom	Airblast	Aerial	Groundboom	Airblast
	Very Coarse						
3.0	Medium	NA	0	0	NA	0	0
2.3	Coarse or Very Coarse	50	0	0	0	0	0
2.3	Medium	100	0	0	10	0	0
2.0	Coarse or Very Coarse	25	0	0	0	0	0
2.0	Medium	75	0	0	0	0	0
1.5	Coarse or Very Coarse	25	0	0	0	0	0
1.5	Medium	50	0	0	0	0	0
1.0	Coarse or Very Coarse	10	0	0	0	0	0
1.0	Medium	25	0	0	0	0	0

1 Per December 2012 spray drift mitigation memorandum, aerial application of greater than 2 lb ai/A is only permitted for Asian Citrus Psylla control, up to 2.3 lb ai/A.
2 NA is not allowable.

6.3.2 Volatilization

In January 2013, a preliminary assessment of the potential risks from volatilization was conducted.[29] The assessment evaluated the potential risks to bystanders, or those who live and/or work in proximity to treated fields, from inhalation exposure to vapor phase chlorpyrifos and chlorpyrifos-oxon emitted from fields following application of chlorpyrifos. The results of the January 2013 assessment indicated that offsite concentrations of chlorpyrifos and chlorpyrifos-oxon may exceed the target concentration based on the toxicological endpoints used at that time.[30]

One significant area of uncertainty described in the preliminary assessment was the use of the aerosolized chlorpyrifos inhalation toxicity study -- as opposed to chlorpyrifos vapor -- for evaluation of lung AChE resulting from field volatilization. Because field volatilization is the production and release of vapor into the atmosphere after sprays have settled on treated soils and plant canopies, the vapor, rather than the aerosol, is the relevant form for evaluation of bystander volatilization exposures. However, EPA lacked chlorpyrifos vapor toxicity data at the time it conducted the preliminary volatilization assessment in 2013. Following the release of the preliminary volatilization assessment, Dow AgroSciences LLC conducted, high quality nose-

[29] R. Bohaty, C. Peck, A. Lowit, W. Britton, N. Mallampalli, A. Grube. Chlorpyrifos: Preliminary Evaluation of the Potential Risks from Volatilization. 1/31/13. U.S. EPA Office of Chemical Safety and Pollution Prevention. D399484, D400781.
[30] EPA MRID# 48139303:Acute Inhalation Exposure of Adult Crl:CD(SD) Rates to Particulate Chlorpyrifos Aerosols: Kinetics of Concentration-Dependent Cholinesterase (ACHE) Inhibition in Red Blood Cells, Plasma, Brain and Lung; Authors: J. A. Hotchkiss, S. M. Krieger, K. A. Brzak, and D. L. Rick; Sponsor: Dow AgroSciences LLC.

only vapor phase inhalation toxicity studies for both chlorpyrifos and chlorpyrifos-oxon[31] to address this uncertainty.

In June 2014, a reevaluation of the 2013 preliminary volatilization assessment was conducted to present the results of the vapor studies and their impact. In the vapor studies, female rats were administered a saturated vapor, meaning that the test subjects received the highest possible concentration of chlorpyrifos or chlorpyrifos-oxon which can saturate the air in a closed system. At these saturated concentrations, no statistically significant inhibition of AChE activity was measured in RBC, plasma, lung, or brain at any time after the six-hour exposure period in either study. Under actual field conditions, indications are that exposures to vapor phase chlorpyrifos and its oxon would be much lower as discussed in the January 2013 preliminary volatilization assessment.

Because these new studies demonstrated that no toxicity occurred even at the saturation concentration, which is the highest physically achievable concentration, then there is no anticipated risks of concern from exposure to the volatilization of either chlorpyrifos or chlorpyrifos oxon. In June 2014, the January 2013 volatilization assessment was revised to reflect these findings.[32]

7.0 Aggregate Exposure/Risk Characterization

In accordance with the FQPA, HED must consider and aggregate (add) pesticide exposures and risks from three major sources: food, drinking water, and residential exposures. In an aggregate assessment, exposures from relevant sources are added together and compared to quantitative estimates of hazard, or the risks themselves can be aggregated. The durations of exposure identified for chlorpyrifos uses are acute and steady state. The acute aggregate assessment includes food and drinking water only. The steady state aggregate assessment includes food, drinking water, and residential exposures.

A drinking water level of comparison (DWLOC) approach to aggregate risk was used to calculate the amount of exposure available in the total 'risk cup' for chlorpyrifos oxon in drinking water after accounting for any chloropyrifos exposures from food and/or residential use.

The DWLOC approach used a reciprocal MOE calculation method for adults (females of childbearing age) since the target MOEs are the same for all relevant sources of exposure, i.e., 100X for residential dermal and for dietary food and water. This entailed calculating the MOE

[31] W. Irwin. Review of Nose-Only Inhalation of Chlorpyrifos Vapor: Limited Toxicokinetics and Determination of Time-Dependent Effects on Plasma, Red Blood Cell, Brain and Lung Cholinesterase Activity in Femal CD(SD): Crl Rats. U.S. EPA Office of Chemical Safety and Pollution Prevention. 6/25/14. D411959. TXR# 0056694. EPA MRID# 49119501.
W. Irwin. Review of Nose-Only Inhalation of Chlorpyrifos-Oxon Vapor: Limited Toxicokinetics and Determination of Time-Dependent Effects on Plasma, Red Blood Cell, Brain, and Lung Cholinesterase Activity in Female CD(SD):Crl Rats. U.S. EPA Office of Chemical Safety and Pollution Prevention. 6/25/14. D415447. TXR# 0056869. EPA MRID# 49210101.
[32] W. Britton. W. Irwin. J. Dawson. A. Lowit. E. Mendez. Chlorpyrifos:Reevaluation of the Potential Risks from Volatilization in Consideration of Chlorpyrifos Parent and Oxon Vapor Inhalation Toxicity Studies. 6/25/2014. U.S. EPA Office of Chemical Safety and Pollution Prevention. D417105.

for water (MOE_{water}) by deducting the contributions from food (MOE_{food}) and dermal (MOE_{dermal}) from the aggregate MOE (MOE_{agg}) of 100. The aggregate MOE value is the same as target MOE (level of concern). The DWLOC is then calculated by dividing the PoD_{water} by the MOE_{water}. The general reciprocal MOE formula is as follows:

$MOE_{agg} = 1/ [(1/MOE_{water}) + (1/MOE_{food}) + (1/MOE_{dermal})]$

$MOE_{water} = 1/ [(1/MOE_{agg}) - ((1/MOE_{food}) + (1/MOE_{dermal}))]$

$DWLOC= PoD_{water}/ MOE_{water}$

When target MOEs (levels of concern) are not the same across the relevant sources of exposure, the reciprocal MOE approach for calculating DWLOCs is not appropriate; instead an aggregate risk index (ARI) method is used. This ARI method was employed for infants, children and youths aggregate assessments for chlorpyrifos uses since the target MOEs for the relevant sources of exposure are not the same i.e., the target MOE for dietary food and for residential dermal exposures is 40X while the target MOE for drinking water exposure is 50X. In this approach, the aggregate, or 'total', ARI value is assigned as 1 (HED is generally concerned when any calculated ARIs are less than 1). Similar to the reciprocal MOE approach, the ARIs for food and dermal are deducted from the aggregate ARI to determine the ARI for water. The water ARI is multiplied by the target MOE for water to determine the calculated water MOE (MOE_{water}). The DWLOC is then calculated by dividing the PoD_{water} by the MOE_{water}. The general ARI method formula is as follows:

ARIs for food or dermal are calculated as $ARI_{food\ or\ dermal} = [(MOE_{food\ or\ dermal})/(MOE_{target\ for\ food\ or\ dermal})]$.

$ARI_{agg} = 1/ [(1/ARI_{water}) + (1/ARI_{food}) + (1/ARI_{dermal})]$

$ARI_{water}= 1/[(1/ARI_{agg}) - ((1/ARI_{food}) + (1/ARI_{dermal}))]$; Where $ARI_{agg}=1$

$MOE_{water} = ARI_{water} \times MOE_{target}$.

$DWLOC= PoD_{water}/ MOE_{water}$

ARIs for food and dermal are calculated as $ARI_{food\ or\ dermal} = [(MOE_{food\ or\ dermal})/(MOE_{target\ for\ food\ or\ dermal})]$.

Aggregate risk estimate calculations and related DWLOCs are based on guidance in the HED SOP 99.5 (*Updated "Interim Guidance for Incorporating Drinking Water Exposure into Aggregate Risk Assessments,"* 8/1/99).

7.1 Acute Aggregate Risk

The acute aggregate assessment includes only food and drinking water. DWLOCs were calculated for infants, children, youths, and adults. The lowest acute DWLOC calculated was for

infants (<1 year old) at 24 ppb.

Table 7.1 Acute Aggregate (Food and Drinking Water) Calculation of DWLOCs[1,2]

Population	Food Exposure (chlorpyrifos)[3]		Drinking Water Exposure (chlorpyrifos)[4]		Acute DWLOC [5] (ppb chlorpyrifos oxon)
	MOE	ARI	MOE	ARI	
Infants[1] (<1 yr)	2200	55	50	1.0	24
Children[1] (1-2 yrs)	1400	35	50	1.0	60
Youths[1] (6-12 yrs)	2800	70	50	1.0	150
Adults[2] (Females 13-49 yrs)	3100	NA	100	NA	53

[1] DWLOCs for infants, children and youths are calculated using the ARI (Aggregate Risk Index) approach since target MOEs are different for drinking water (chlorpyrifos oxon target MOE=50) and for food and residential (chlorpyrifos target MOE= 40) exposure.
[2] DWLOCs for adults (females 13-49 yrs) are calculated using the reciprocal MOE approach since the target MOEs are the same for drinking water (chlorpyrifos oxon target MOE=100) and for food and residential (chlorpyrifos target MOE= 100) exposure.
[3] **FOOD:** MOE_{food} = PoD_{food} (ug/kg/day) (from Table 4.8.4)/ Food Exposure (ug/kg/day) (from Table 5.4.3).
ARI_{food} = [(MOE_{food})/(MOE_{target})].
[4] **WATER (ARI approach):** ARI_{water}= 1/[(1/ARI_{agg}) – ((1/ARI_{food}) + (1/ARI_{dermal}))]; Where ARI_{agg}=1 (Note:HED is generally concerned when calculated ARIs are less than 1).
MOE_{water} = ARI_{water} x MOE_{target}.
WATER (Reciprocal MOE approach): MOE_{water} = 1/ [(1/MOE_{agg}) – ((1/MOE_{food}) + (1/MOE_{dermal}))]; Where MOE_{agg} =Target MOE.
[5] **DWLOC:** DWLOC ppb= PoD_{water} (ppb; from Table 4.8.4) /MOE_{water}

7.2 Steady State Aggregate Risk

The steady state aggregate assessment includes dietary exposures from food and drinking water and dermal exposures from residential uses (dermal exposures represent the highest residential exposures). DWLOCs were calculated for infants, children, youths, and adults. The lowest steady state DWLOC calculated was for infants (<1 year old) at 3.9 ppb.

Table 7.2 Steady State Aggregate (Food, Drinking Water, Residential) Calculation of DWLOCs[1,2]

Population	Food Exposure (chlorpyrifos)[3]		Dermal Exposure (chlorpyrifos)[4]		Drinking Water Exposure (chlorpyrifos oxon)[5]		Steady State DWLOC [6] (ppb chlorpyrifos oxon)
	MOE	ARI	MOE	ARI	MOE	ARI	
Infants[1] (<1 yr)	550	14	NA	NA	55	1.1	3.9
Children[1] (1-2 yrs)	410	10	NA	NA	55	1.1	10
Youths[1] (6-12 yrs)	700	18	1600	40	54	1.1	16
Adults[2] (Females 13-49 yrs)	1000	NA	1000	NA	125	NA	7.5

[1] DWLOCs for infants, children and youths are calculated using the ARI (Aggregate Risk Index) approach since target MOEs are different for drinking water (chlorpyrifos oxon target MOE=50) and for food and residential (chlorpyrifos target MOE= 40) exposure.
[2] DWLOCs for adults (females 13-49 yrs) are calculated using the reciprocal MOE approach since the target MOEs are the same for drinking water (chlorpyrifos oxon target MOE=100) and for food and residential (chlorpyrifos target MOE= 100) exposure.
[3] **FOOD:** MOE_{food} = PoD_{food} (ug/kg/day) (from Table 4.8.4)/ Food Exposure (ug/kg/day) (from Table 5.4.4).
ARI_{food} = [(MOE_{food})/(MOE_{target})].
[4] **DERMAL:** MOE_{dermal} = PoD_{dermal} (ug/kg/day) (from Table 4.8.4)/ Dermal Exposure (ug/kg/day) (from Table 6.2).
ARI_{dermal} = [(MOE_{dermal})/(MOE_{target})].
[5] **WATER (ARI approach):** ARI_{water}= 1/[(1/ARI_{agg}) – ((1/ARI_{food}) + (1/ARI_{dermal}))]; Where ARI_{agg}=1 (Note:HED is generally concerned when calculated ARIs are less than 1).
MOE_{water} = ARI_{water} x MOE_{target}.
WATER (Reciprocal MOE approach): MOE_{water} = 1/ [(1/MOE_{agg}) – ((1/MOE_{food}) + (1/MOE_{dermal}))]; Where MOE_{agg} =Target MOE.
[6] **DWLOC:** DWLOC ppb= PoD_{water} (ppb; from Table 4.8.4) /MOE_{water}

7.3 Aggregate Risk Estimates

The acute aggregate assessment includes food and drinking water only. The steady state aggregate assessment includes food, residential and drinking water exposures. Acute and steady state risk estimates for dietary (food only) exposures or for residential only (dermal, inhaltion and incidental oral) exposures to chlorpyrifos are not of concern. Steady state risk estimates from food and residential exposures combined are also not of concern. For the chlorpyrifos aggregate assessments that include *drinking water* along with the food and residential components, a DWLOC approach is used to calculate the amount of exposure available in the total 'risk cup' for exposures to the chlorpyrifos oxon in drinking water after accounting for any exposures from parent compound chlorpyrifos from food and/or residential use.

Typically, for a DWLOC aggregate risk assessment, the calculated DWLOC is compared to the EDWC. When the EDWC is less than the DWLOC, there are no risk concerns for exposures to

the pesticide in drinking water. Conversely, when the EDWC is greater than the DWLOC, there may be a risk concern. For chlorpyrifos, DWLOCs were calculated for both the acute and steady state aggregate assessments for infants, children, youths and adult females. However, the acute DWLOCs were not compared to EDWCs. The steady state DWLOCs and risk estimates for drinking water will be protective of any acute aggregate exposures because the oxon concentrations expected to result in 10% ChEI are considerably higher (up to 5.7x higher) for acute exposures than for steady state. In addition, the steady state DWLOC calculations include the residential exposure component. Consequently, the calculated acute DWLOCs are much higher than those for the steady state. For example, for infants, the acute DWLOC is 24 ppb while the steady state DWLOC is 3.9 ppb (Tables 7.1 and 7.2). In fact, the lowest DWLOC calculated for any duration or population was the 3.9 ppb value (infants) and is the concentration used for comparison to the EFED-modeled EDWCs. Drinking water concentrations of chlorpyrifos oxon above 3.9 ppb may be of concern.

EFED has summarized the drinking water and aggregate assessment below (for complete details on the assumptions, results, and characterization of the screening analysis refer to EFED's DWA, (Bohaty, R. 12/23/14, D424487, *Chlorpyrifos: Updated Drinking Water Assessment for Registration Review*).

Residue Definition

A national screening level drinking water assessment was completed for the registration review of chlorpyrifos, with focus on the agricultural uses. The primary drinking water residue of concern is chlorpyrifos-oxon, the predominant chlorpyrifos transformation product formed during drinking water treatment (*e.g.*, chlorination). To illustrate the range of EDWCs, two maximum label rate application scenarios were selected to represent high and low end exposures, i.e., tart cherries at 5 applications totaling 14.5 pounds per acre per year, and bulb onions at a single application of one pound per acre per year, respectively. The application of chlorpyrifos to tart cherries resulted in concentrations in surface water that exceeded the DWLOC; whereas, chlorpyrifos applications to bulb onions result in concentrations below the DWLOC. Concentrations in groundwater are not expected to exceed the DWLOC.

To investigate whether other chlorpyrifos application scenarios may result in concentrations that exceed the DWLOC, a screen of all available surface water modeling scenarios was completed considering three different application dates and a single application at several different application rates that ranged from one to six pounds. This analysis showed that even with only one application, several chlorpyrifos uses may exceed the DWLOC at rates lower than maximum labeled rates (both single as well as yearly), including an application rate of one pound per acre per year. The analysis also showed that the DWLOC exceedances are not expected to be uniformly distributed across the country.

Further analysis was conducted to look at the spatial distribution of EDWCs at a regional level, as well as by using a drinking water intake watershed approach. This exercise demonstrated that chlorpyrifos applications will result in variable drinking water exposures that are highly localized and that the highest exposures generally occur in small hydrologic regions where there is a high percent cropped area on which chlorpyrifos use could occur.

Finally, EDWCs were compared to monitoring data. This analysis showed that when modeling scenarios are parameterized to reflect reported use and EDWCs are adjusted to reflect percent cropped area, the EDWCs are within an order of magnitude of the measured concentrations reported in the monitoring data. Therefore, although there are uncertainties associated with the model input parameters for which conservative assumptions were made (*e.g.*, one aerobic aquatic metabolism half-life value multiplied by the uncertainty factor of three, stable hydrolysis, 100% of the cropped watershed is treated on the same day, and use of the Index Reservoir as the receiving waterbody), these assumptions do not appear to lead to an overly conservative estimate of exposure. In addition, evaluation of the monitoring data further illustrates that exposures are highly localized. Additional work can be done to examine EDWCs on a regional and/or watershed scale to pinpoint community drinking water systems where exposure to chlorpyrifos-oxon as a result of chlorpyrifos applications may pose an exposure concern.

EDWCs are provided for chlorpyrifos and chlorpyrifos-oxon. Chlorpyrifos EDWCs were multiplied by 0.9541 (molecular weight correction factor) and 100% (maximum conversion during water purification) to generate chlorpyrifos-oxon EDWCs. Essentially, the concentration of chlorpyrifos and chlorpyrifos-oxon are the same. A 100% conversion factor for the oxidation of chlorpyrifos to chlorpyrifos-oxon was used as an approximation based on empirical bench scale laboratory data that indicate chlorpyrifos rapidly oxidizes to form chlorpyrifos-oxon almost quantitatively during typical water treatment (chlorination).[33] There are limited data available on the removal efficiency of chlorpyrifos prior to oxidation or the removal efficiency of chlorpyrifos-oxon during the drinking water treatment process. Based on empirical data showing that more than 75 percent of community water systems use chlorination to disinfect drinking water in the United States[34], the assumption of exposure to chlorpyrifos-oxon equivalent to 100% conversion of chlorpyrifos is not considered overly conservative. It is possible that some drinking water treatment procedures, such as granular activated carbon filtration and water softening (increased rate of chlorpyrifos-oxon hydrolysis at pH > 9) could reduce the amount of chlorpyrifos-oxon in finished drinking water; however, these treatment methods are not typical practices across the country for surface water.

While there is the potential to have both chlorpyrifos and chlorpyrifos-oxon present in finished drinking water, limited (or no) information is available to readily quantify how much of each form remains in the finished water. In the absence of available information, all the chlorpyrifos that enters a drinking water treatment facility is assumed to remain after treatment, and is converted to chlorpyrifos-oxon during treatment.

Although chlorpyrifos-oxon has a hydrolysis half-life of 5 days, the drinking water treatment simulation half-life for chlorpyrifos-oxon is approximately 12 days.[35,36,37] Therefore, once

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[33] Duirk, S. E.; Collette, T. W.; Degradation of Chlorpyrifos in Aqueous Chlorine Solutions: Pathways, Kinetics, and Modeling. *Environ. Sci. Technol.*, 2006, *40(2)*, 546-550.
[34] Community Water System Survey 2006; U.S. Environmental Protection Agency, Washington, DC 20460 **May 2009** (survey data)
[35] Tunink, A. Chlorpyrifos-oxon: Determination of hydrolysis as a function of pH, 2010 (MRID 48355201; acceptable)
[36] Wu, J.; Laird, D. A. Abiotic Transformation of Chlorpyrifos to Chlorpyrifos Oxon in Chlorinated Water. *Environ. Toxcol.Chem.*, **2003**, 22(2), 261-264.
[37] Tierney, D. P.; Christensen, B. R.; Culpepper, V. C. Chlorine Degradation of Six Organophosphate Insecticides

chlorpyrifos-oxon forms during treatment, little transformation is expected to occur before consumption (during drinking water distribution). There is a wide range of treatment processes and sequences of treatment processes employed at community water systems across the country and there are limited data available on a community water system specific basis to assess the removal or transformation of chlorpyrifos during treatment. These processes are not specifically designed to remove pesticides and pesticide transformation products including chlorpyrifos and chlorpyrifos-oxon. In general, drinking water treatment processes, with the exception of activated carbon,[38] have been shown to have little impact on removal of pesticide residues. Additional discussion of drinking water treatment can be found in the ***Drinking Water Treatment*** section of the DWA or in the preliminary DWA.

Aggregate

National Screen

The 2011 preliminary DWA, as well as the additional analyses completed as part of this assessment, indicate that exposure to chlorpyrifos-oxon in drinking water derived from surface water may pose an exposure concern. Since a large number of chlorpyrifos uses were identified in the preliminary DWA as triggering a concern, a bounding estimate of exposure was completed using a screening level national assessment approach. This was done to determine which currently registered uses could result in exposure to chlorpyrifos-oxon in drinking water that exceed the DWLOC.

Use of chlorpyrifos on tart cherries is expected to result in the highest EDWC. EDWCs for chlorpyrifos and chlorpyrifos-oxon[39] are reported below for Tier I groundwater and Tier II surface water model simulations. Because chlorpyrifos is used on a number of agricultural crops, as well as turf, a drinking water intake percent cropped area (PCA) adjustment factor of 1 was used.[40,41] While the model input values[42] have been updated since the preliminary assessment, the results presented in **7.3.1** are similar to those previously reported.

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and Four Oxons in Drinking Water Matrix. *Submitted by Syngenta Crop Protection, Inc.* **2001**.

[38] *Progress Report on Estimating Pesticide Concentrations in Drinking Water and Assessing Water Treatment Effects on Pesticide Removal and Transformation: A Consultation.* FIFRA Scientific Advisory Panel Meeting, September 29, 2000; SAP Report No. 2001-02 February 12, 2011.

[39] Chlorpyrifos EDWCs were multiplied by 0.9541 (molecular weight correction factor) and 100% (maximum conversion during water purification) to generate chlorpyrifos-oxon EDWCs. Additional details on the potential impacts on drinking water treatment on chlorpyrifos and chlorpyrifos-oxon see the **Additional Analyses** section on Drinking Water Treatment.

[40] U.S. Environmental Protection Agency Brady, D. Guidance on Development and Use of Community Water System Drinking Water Intake Percent Cropped Area Adjustment Factors for Use in Drinking Water Exposure Assessments: 2014 Updated, September 12, 2014.

[41] U.S. Environmental Protection Agency, Bohaty, R., Carleton, J., Crk, T., Echeverria, M., Ruhman, M., Thawley, M., Thurman, N., Villanueva, P., White, K., Development of Community Water System Drinking Water Intake Percent Cropped Area Adjustment Factors for Use in Drinking Water Exposure Assessments: 2014 Update, September 9, 2014.

[42] *Guidance for Selecting Input Parameters in Modeling the Environmental Fate and Transport of Pesticides*, Version 2.1, October 22, 2009.

Table 7.3.1. Estimated Drinking Water Concentrations Resulting from the Use of Chlorpyrifos

Residue	Surface Water				Groundwater
	1-in-10 Year Peak Concentration µg/L	21-day Average Concentration µg/L	1-in-10 Year Annual Average Concentration µg/L	30 Year Annual Average Concentration µg/L	SCI-GROW Tier I Concentration µg/L[a]
Michigan Tart Cherries					
Chlorpyrifos	129	83.8	39.2	29.7	0.16
Chlorpyrifos-oxon	123	80.0	37.4	28.3	0.15
Georgia Bulb Onion					
Chlorpyrifos	6.2	3.1	1.2	0.8	0.01
Chlorpyrifos-oxon	5.9	3.0	1.1	0.8	0.01

a. SCI-GROW resulted in higher EDWCs than PRZM-GW simulations.

In order to better define the extent to which other chlorpyrifos use scenarios may result in an exposure concern, the Health Effects Division developed a 21-day average DWLOC of 3.9 µg/L for chlorpyrifos-oxon[43] that could be compared to model output values. Uncertainties in this approach include potential temporal aspects of relative concentrations from day to day on AChE inhibition, geospatial distribution of exposure as a result of variability in use, environmental factors, and drinking water treatment processes.

Previous risk assessments[44] suggest that typical upper bound application rates for chlorpyrifos are similar to the maximum single application rates for a wide range of crops; however, often the number of typical applications per year is lower than the maximum number of applications currently permitted on product labels (*i.e.*, summarized in **Master Use Summary Document**). Considering this information, a screening analysis (see the DWA for details) was completed to determine which chlorpyrifos uses do not exceed the DWLOC, based on a single application of chlorpyrifos per year at 1 and 4 pounds of chlorpyrifos per acre. The results for 1 and 4 pounds per acre are reported here as a representation of the range of potential chlorpyrifos application rates, bearing in mind that chlorpyrifos can be applied at lower and higher single rates (*e.g.,* an application rate of 6 pounds per acre on citrus). This analysis showed that over a 30 year period, the current maximum application rate scenarios, as well as maximum single application rates for a wide range of chlorpyrifos use scenarios, may result in multiple 21-day average concentrations that exceed the DWLOC (Error! Reference source not found.).

[43] The average 21-day concentration of chlorpyrifos-oxon necessary to cause 10% AChE inhibition was determined by HED to be 217 ppb. This value was divided by the safety factors (50x; 4.3 ppb) and then the contribution from food (0.4 ppb) was subtracted out to give to derive a DWLOC (3.9 ppb).
[44] Dawson, J., Bohaty, R., Mallampalli, N. Evaluation of the Potential Risks from Spray Drift and the Impact of Potential Risk Reduction Measures, June 20, 2012 PC 059101 DP 399483 and 399485.

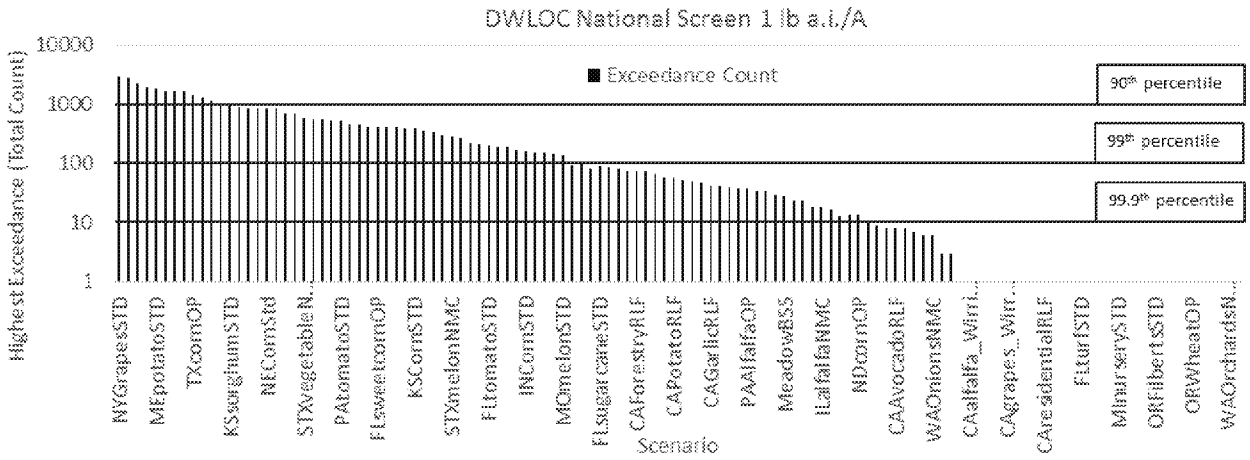

Figure 7.3.1. National Screen: Exceedance Count/Percent (one application per year)

Regional Screen

Although there are several exceedances of the DWLOC on a national basis, the incidence of high exposures is expected to be highly localized. While it is currently extremely challenging to assess exposure on a local scale due to the unavailability of data and wide range of characteristics [*i.e.*, environmental such as soil, weather, etc. or otherwise (*e.g.*, drinking water treatment process)] that affect the vulnerability of a given community drinking system to chlorpyrifos-oxon contamination, a method was developed to examine the potential geospatial concentration differences for two Hydrological Unit Code (HUC) 2 Regions – HUC 2 Region 17: Pacific Northwest and HUC 2 Region 3: South Atlantic-Gulf, in order to identify use patterns that may result in EDWCs that exceed the DWLOC on a regional basis.[45] This analysis considered all potential chlorpyrifos use sites within the HUC 2 regions based on the National Agricultural Statistics Service cropland data layers and survey data. Due to the uncertainty associated with the urban uses (e.g., wide area/general outdoor treatment) that are represented by the impervious scenarios, modeled results from the impervious scenarios are not included in this analysis. Additional clarification from the registrants is needed in order to determine if these uses pose an exposure concern.

For HUC 2 Region 17, four chlorpyrifos use patterns were identified as a potential concern based on maximum single application rates of 1 and 4 pounds per acre. However, for HUC 2 Region 3, several chlorpyrifos use scenarios were identified that could exceed the DWLOC.

Watershed Screen

The uses that exceeded the DWLOC from the regional screening exercise for HUC 2 Region 3 were further explored for one example by utilizing the drinking water intake (DWI) watershed database. This example shows an overlap of potential chlorpyrifos use sites that may result in an exceedance of the DWLOC with watersheds that supply source water for community drinking water systems. In addition, this analysis shows that exposure is not uniform within a HUC 2 Region and that some watersheds are more vulnerable than others. Watershed vulnerability is

[45] http://water.usgs.gov/GIS/huc.html

expected to be greatest for smaller watersheds with high percent cropped areas. Smaller community water systems are generally more vulnerable due to short distribution times and the reliance of chlorination to treat source surface water as well as limited access to other treatment methods such as granular activated carbon.

Monitoring Data Analysis

Water monitoring data from the USGS National Water-Quality Assessment Program (NAWQA), USEPA/USGS Pilot Reservoir Monitoring Program, USDA Pesticide Data Program (PDP), and California Department of Pesticide Regulation (CDPR) were evaluated in the preliminary DWA with reference to an acute exposure to chlorpyrifos and its degradation product chlorpyrifos-oxon. The monitoring data showed chlorpyrifos detections at low concentrations, generally not exceeding 0.5 µg/L. For example, USGS NAWQA, which contains an extensive monitoring dataset for chlorpyrifos and chlorpyrifos oxon, reports a peak chlorpyrifos detection of 0.57 µg/L in surface water with a detection frequency of approximately 15%. CDPR has detected chlorpyrifos concentrations greater than 1 µg/L in surface water on several occasions, with an observed peak chlorpyrifos concentration of 3.96 µg/L. Sampling frequencies in these monitoring programs were sporadic and generally range from once per year to twice per month.

Since the preliminary assessment, water monitoring data from Washington State Department of Ecology and Agriculture (WSDE/WSDA) Cooperative Surface Water Monitoring Program[46,47], Dow AgroSciences (MRID 44711601), and Oregon Department of Environmental Quality were evaluated and are presented as part of this update. The previously referenced data have also been re-examined to consider short-term exposure (*i.e.*, 21-day average concentrations) considering the importance of the single day exposure and the temporal relationship of exposure. A summary of all surface water monitoring data examined to date for chlorpyrifos are presented in Error! Reference source not found.. Some of the monitoring programs analyzed for chlorpyrifos-oxon; however, the number of detections as well as the concentrations were generally much lower. Since the majority of the conversion of chlorpyrifos to chlorpyrifos-oxon is assumed to occur during drinking water treatment, and not in the environment, the monitoring data presented in Error! Reference source not found. are limited to chlorpyrifos.

[46] Sargeant, D, Dugger, D. Newell, E., Anderson, P, Cowles, J. Surface Water Monitoring Program for Pesticides in Salmonid-Bearing Streams 2006-2008 Triennial Report, February 2010 (Washington State Department of Ecology and Washington State Department of Agriculture)
https://fortress.wa.gov/ecy/publications/summarypages/1003008.html;
http://agr.wa.gov/PestFert/natresources/docs/swm/2008_swm_report.pdf

[47] Sargeant, D., Newell, E., Anderson, P., Cook, A. Surface Water Monitoring Program for Pesticides in Salmonid-Bearing Streams 2009-2011 Triennial Report, February 2013 (Washington State Department of Ecology and Washington State Department of Agriculture) http://agr.wa.gov/FP/Pubs/docs/377-SWM2009-11Report.pdf

Table 7.3.1. Surface Water Monitoring Data Summary for Chlorpyrifos

Monitoring Data	Scale	Years of Sampling (number of samples)	Detection Frequency (%)	Maximum Concentration (µg/L)
USGS NAWQA	National	1991-2012 (30,542)	15	0.57
California Department of Pesticide Regulation	State	1991-2012 (13,121)	20	3.96
Washington State Department of Ecology and Agriculture Cooperative Surface Water Monitoring Program	State	2003-2013 (4,091)	8.4	0.4
USDA Pesticide Data Program	National	2004-2009 (raw water; 1,178) 2001-2009 (finished water; 2,918)	0	na
USGS-EPA Pilot Drinking Water Reservoir	National	1999-2000 (323)	5.3	0.034
Oregon Department of Environmental Quality	Watershed (Clackamas)	2005-2011 (363)	13	2.4
MRID 44711601	Watershed (Orestimba Creek)	1996-1997 (1,089)	61	2.22

In general, the monitoring data include sampling sites that represent a wide range of aquatic environments including small and large water bodies, rivers, reservoirs, and urban and agricultural locations, but are limited for some areas of the United States where chlorpyrifos use occurs. Also, the sampling sites, as well as the number of samples, vary by year. In addition, the vulnerability of the sampling site to chlorpyrifos contamination varies substantially due to use, soil characteristics, weather and agronomic practices. None of the monitoring programs examined to date were specifically designed to target chlorpyrifos use (except the Registrant Monitoring Program MRID 44711601); therefore, peak concentrations (and likely 21-day average concentrations) of chlorpyrifos and chlorpyrifos-oxon likely went undetected in these programs. See the Revised Chlorpyrifos Preliminary Registration Review Drinking Water Assessment dated June 30, 2011 for further details on the monitoring programs discussed here. http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2008-0850-0026

In general, sampling frequency needs to be approximately equal to the duration of exposure concern.[48] The chlorpyrifos monitoring data evaluated thus far also show that as sample frequency increases, so does the detection frequency. This is evident in the registrant-submitted monitoring data, as well as examination of individual sampling sites within the various datasets.

Therefore, while there are many individual samples collected and analyzed for chlorpyrifos (or chlorpyrifos-oxon) across the United States, it would not be appropriate to combine these data sources to generate exposure estimates or to use these datasets to represent exposure on a national or even regional basis. Thus, comparing the monitoring data results to the DWLOC would not be a reasonable approach for the reasons given above, including limited sample frequency, limited use information, and sampling site variability, on a national or even a regional basis. Model estimated concentrations should be considered suitable upper bound concentrations for chlorpyrifos and chlorpyrifos-oxon.

Additionally, model simulations were completed to represent two different water monitoring datasets - Washington State Department of Ecology and Agriculture (WSDE/WSDA) Cooperative Surface Water Monitoring Program and Dow AgroSciences (MRID 44711601) Orestimba Creek. For both of these water monitoring programs, enough information was available, including chlorpyrifos use information as well as the PCA, to parameterize the model. In these simulations, the modeled EDWCs were within an order of magnitude of the measured concentrations. This suggests that the modeling results are not overly conservative and supports the use of the model to estimate chlorpyrifos-oxon concentrations in drinking water.

Additional modeling can be done to pinpoint regions or watersheds where EDWCs may exceed the DWLOC. This would include completing the regional assessment presented here for all HUC 2 Regions and crop uses, as well as considering multiple applications per year. Nevertheless, based on the current analysis, concentrations of chlorpyrifos-oxon in drinking water are expected to be highly localized primarily in small watersheds with high PCA.

Summary

In summary, examination of chlorpyrifos agricultural use across the country indicates that there are a number of uses that may result in potential exposure to chlorpyrifos-oxon in finished drinking water at levels that exceed the DWLOC. The EDWCs for tart cherries and bulb onion reported here are expected to provide a reasonable bounding estimation of exposure based on maximum rates included in Master Use Summary Document. This analysis showed that the maximum use scenario for tart cherries exceeds the DWLOC, while it does not for bulb onions.

The rate used for the bulb onion simulation was 1 pound chlorpyrifos per acre; therefore, a screen of all Surface Water Concentration Calculator modeling scenarios was done using a single application of chlorpyrifos. This analysis showed that exceedances are expected even for one application of chlorpyrifos applied at 1 pound per acre per year.

[48] U.S. Environmental Protection Agency. Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel: Problem Formulation for the Reassessment of Ecological Risks from the Use of Atrazine, June 12-14, 2012, Docket Number: EPA-HQ-OPP-2012-0230.

While there are uncertainties associated with the model input parameters for which conservative assumptions were made (*e.g.*, one aerobic aquatic metabolism half-life value multiplied by the uncertainty factor of three, stable hydrolysis, 100% of the cropped watershed is treated on the same day, and use of the Index Reservoir as the receiving waterbody) these assumptions do not appear to lead to an overly conservative estimate of exposure based on a comparison of model estimates with measured concentrations. Comparison of model estimated concentrations with measured concentrations suggests that model estimates are consistent with measured concentrations when actual application rates and representative Surface Water Concentration Calculator (SWCC) scenarios are considered and a PCA adjustment factor is applied to the model estimates. This modeling/monitoring comparison suggests that when growers use maximum application rates, or even rates much lower than maximum, chlorpyrifos-oxon concentrations in drinking water could pose an exposure concern for a wide range of chlorpyrifos uses. However, these exposures are not expected to be uniformly distributed across the country. Additional analyses are needed in order to pinpoint the exact community water systems where concentrations may be of concern.

8.0 Cumulative Exposure/Risk Characterization

Section 408(b)(2)(D)(v) of the Federal Food, Drug, and Cosmetic Act (FFDCA), as amended by the Food Quality Protection Act (1996) stipulates that when determining the safety of a pesticide chemical, EPA shall base its assessment of the risk posed by the chemical on, among other things, available information concerning the cumulative effects to human health that may result from dietary, residential, or other non-occupational exposure to other substances that have a common mechanism of toxicity. The reason for consideration of other substances is due to the possibility that low-level exposures to multiple chemical substances that cause a common toxic effect by a common mechanism could lead to the same adverse health effect as would a higher level of exposure to any of the other substances individually. A person exposed to a pesticide at a level that is considered safe may in fact experience harm if that person is also exposed to other substances that cause a common toxic effect by a mechanism common with that of the subject pesticide, even if the individual exposure levels to the other substances are also considered safe.

Chlorpyrifos is a member of the organophosphate (OP) class of pesticides. Other members of this class of pesticides are numerous and include azinphos methyl, diazinon, chlorpyrifos-methyl, dichlorvos, dicrotophos, dimethoate, disulfoton, methamidophos, methidathion, monocrotophos, naled, oxydemeton-methyl, phorate, phosmet, pirimiphos-methyl, and trichlorfon to name a few. EPA considers organophosphates to express toxicity through a common biochemical interaction with cholinesterase which may lead to a myriad of cholinergic effects and, consequently the organophosphate pesticides should be considered as a group when performing cumulative risk assessments. HED published the final guidance that it now uses for identifying substances that have a common mechanism of toxicity (FR 64(24) 5796-5799, February 5, 1999) "Proposed Guidance of Cumulative Risk Assessment for Chemicals that have a Common Mechanism of Toxicity" was made available for public comment in the Federal Register (65 FR 40644, June 30, 2000). The Agency presented this approach to the FIFRA Scientific Advisory Panel in late September, 2000. The SAP reviewed revised methods used to conduct a preliminary cumulative risk assessment for organophosphate pesticides in 2002 (US EPA, 2002), found at

http://www.epa.gov/scipoly/sap/2002/index.htm.

The Agency has completed a cumulative risk assessment for OPs, (US EPA, 2001), a revised cumulative risk assessment for OPs, (US EPA, 2002), and an updated OP cumulative risk assessment (US EPA, August 2006) which can be found on the Agency's web site http://www.epa.gov/pesticides/cumulative/rra-op/. The cumulative effects of exposure to multiple OPs, including chlorpyrifos, are evaluated in those documents. Prior to the completion of registration review, OPP will update the OP CRA to incorporate any toxicity and exposure information available since 2006.

9.0 Occupational Exposure/Risk Characterization

9.1 Steady State Occupational Handler Risk

The term handlers is used to describe those individuals who are involved in the pesticide application process. There are distinct job functions or tasks related to applications and exposures can vary depending on the specifics of each task. Job requirements (amount of a chemical used in each application), the kinds of equipment used, the target being treated, and the level of protection used by a handler can cause exposure levels to differ in a manner specific to each application event. Based on the anticipated use patterns and current labeling, types of equipment and techniques that can potentially be used, occupational handler exposure is expected from chlorpyrifos use. For purpose of occupational handler assessment, the parent chlorpyrifos is the relevant compound.

The 2011 occupational exposure assessment for chlorpyrifos (W. Britton, 6/27/11, D388165, *Chlorpyrifos: Occupational and Residential Exposure Assessment*) has been updated based on a number of changes that have occurred since that time, including the following: 1) use of the PBPK-PD model for determining toxicological PoDs, 2) inclusion of use patterns based on a master use document developed for chlorpyrifos, 3) use of revised guidance for all of the currently recommended unit exposures for standard EPA occupational handler exposure scenarios[49], *Occupational Pesticide Handler Unit Exposure Surrogate Reference Table – Revised March 2013*, 4) use of the revised seed treatment policy, *Interim Guidance (SOP 15.1) Amount of Commercial Seed Treated per Day,* as was updated March 21, 2013 and 5) use of updated adult (female) body weight.

In addition, steady state durations of exposure are assumed in the updated occupational assessment. HED typically classifies exposures from 1 to 30 days as short-term and exposures 30 days to six months as intermediate-term. The steady state endpoint selection for chlorpyrifos overlaps HED's traditional short-term exposure duration endpoint selection and is considered health protective for both short- and intermediate- term exposures.

Full details regarding the updated occupational assessment can be found in D424484[7]. A summary of the assumptions and conclusions are provided below.

[49] http://www.epa.gov/opp00001/science/handler-exposure-data.html

The updated quantitative exposure/risk assessment developed for occupational handlers is based on the following scenarios:

Mixer/Loaders:

(1a) Liquids for Aerial/ Chemigation Applications
(1b) Liquids for Airblast Applications
(1c) Liquids for Groundboom Applications
(1d) Liquids for Wide Area Aerial Applications
(1e) Liquids for Wide Area Ground Applications
(2a) Wettable Powder (in WSP) for Aerial/ Chemigation Applications
(2b) Wettable Powder (in WSP) for Airblast Applications
(2c) Wettable Powder (in WSP) for Groundboom Applications
(3a) Granulars for Aerial Applications
(3b) Granulars for Tractor Drawn Spreader Applications
(3c) Granulars with SmartBox® for Aerial Applications
(3d) Granulars with SmartBox® for Tractor Drawn Spreader Applications
(4a) Water Dispersible Granulars (in WSP) for Aerial/ Chemigation Applications
(4b) Water Dispersible Granulars (in WSP) for Airblast Applications
(4c) Water Dispersible Granulars (in WSP) for Groundboom Applications

Applicators:

(5a) Aerial Liquid Applications
(5b) Wide Area Aerial Applications
(5c) Aerial Granular Applications
(6a) Airblast Applications
(6b) Wide Area Ground Applications
(7a) Groundboom Applications
(7b) Tractor Drawn Granular Applications
(7c) Tractor Drawn Granular Applications with SmartBox®
(8) Paint Brush/Roller Applications
(9) Cattle Ear Tag Applications
(10) Bait Station Applications
(11) Total Release Fogger

Mixer/Loader/Applicators:

(12a) Liquid: Manually Pressurized Handwand
(12b) Liquid: Wettable Powder (in WSP) for Manually Pressurized Handwand
(12c) Liquid: Microencapsulated for Manually Pressurized Handwand
(12d) Liquid: Backpack Sprayer
(12e) Liquid: Wettable Powder (in WSP) for Backpack Sprayer
(12f) Liquid: Microencapsulated for Backpack Sprayer
(12g) Liquid: Mechanically Pressurized Handgun
(12h) Liquid: Wettable Powder (in WSP) for Mechanically Pressurized Handgun

(12i) Liquid: Microencapsulated for Mechanically Pressurized Handgun
(13a) Granulars: Hand Dispersal
(13b) Granulars: Rotary Spreader
(13c) Granulars: Spoon
(13d) Granulars: Belly Grinder
(13e) Granulars: Belly Grinder
(14a) Water Dispersible Granular (in WSP): Manually Pressurized Handwand
(14b) Water Dispersible Granular (in WSP): Backpack Sprayer
(14c) Water Dispersible Granular (in WSP): Mechanically Pressurized Handgun

Flaggers:

(15a) Flagging for Aerial Spray Applications
(15b) Flagging for Aerial Granular Applications

Occupational Handler Exposure Data and Assumptions

A series of assumptions and exposure factors served as the basis for completing the occupational handler risk assessments.

Application Rates: In order to seek clarification of chlorpyrifos usage, the Agency compiled a master use summary document reflective of the use profile of all active product labels. The document, among other information, presents all registered uses of chlorpyrifos and corresponding maximum single application rates, equipment types, re-entry intervals (REIs), etc. HED believes that the detailed information on application rates and use patterns presented in Appendix 9 (Master Use Summary Document) will be implemented on all chlorpyrifos labels and is the basis of the occupational and residential risk assessment. If, for any reason, the final chlorpyrifos labels contain higher application rates, the actual risks posed by those products may exceed the risks estimated in this assessment.

Body weights: For adults, when an endpoint is not sex-specific a body weight of 80 kg is typically used in occupational risk assessment. However, in this case, a female-specific body weight of 69 kg was used. While the endpoint of concern, RBC AChE inhibition, is not sex-specific, the female body weight was used due to concerns for neurodevelopmental effects related to early life exposure to chlorpyrifos.

Unit Exposures: It is the policy of HED to use the best available data to assess handler exposure. Sources of generic handler data, used as surrogate data in the absence of chemical-specific data, include PHED 1.1, the AHETF database, the Outdoor Residential Exposure Task Force (ORETF) database, or other registrant-submitted occupational exposure studies. Some of these data are proprietary (e.g., AHETF data), and subject to the data protection provisions of FIFRA. The standard values recommended for use in predicting handler exposure that are used in this updated assessment, known as "unit exposures", are outlined in the "Occupational Pesticide Handler Unit Exposure Surrogate Reference Table[50]", which, along with additional information

[50] Available: http://www.epa.gov/opp00001/science/handler-exposure-table.pdf

on HED policy on use of surrogate data, including descriptions of the various sources, can be found at the Agency website.[51]

Area Treated or Amount Handled: For the updated assessment, the daily areas treated were defined for each handler scenario (in appropriate units) by determining the amount that can be reasonably treated in a single day (e.g., acres, square feet, or gallons per day). When possible, the assumptions for daily areas treated are taken from the HED Science Advisory Committee on Exposure (ExpoSAC) Policy 9.1: Standard Values for Daily Acres Treated in Agriculture, which was completed on July 5, 2000. However, standard values are not available for numerous scenarios. The area treated assumptions identified in the 2011 occupational and residential exposure assessment are largely the same as used for the updated except for the following changes:

- Paint Roller/Brush: the 2011 assessment assumed 110 gallons for treatment of sewer manhole covers, the current assumes 20 gallons for coverage of 50 manholes; 3 gallons applied for all other uses as was done previously;
- Fire Ant Mounds: the previous assessment assumed 25 mounds were treated, the current assumes that an area of 1,000 square feet is treated.

Exposure Duration: As described previously, the steady state approach is used and is appropriate for chlorpyrifos given the toxicological and exposure profile. The steady state approach accounts for the short-term exposure duration, as well as for workers that are exposed over longer periods of time (i.e., intermediate-term exposures). Long-term exposures are not expected based on the current chlorpyrifos uses.

Mitigation/Personal Protective Equipment: Estimates of dermal and inhalation exposure were calculated for various levels of personal protective equipment (PPE). The lowest tier is designated as the baseline exposure scenario (i.e., long-sleeve shirt, long pants, shoes, socks, and no respirator). If risks are of concern at baseline attire, then increasing levels of personal protective equipment or PPE (e.g., gloves, double-layer body protection, and respirators) are evaluated. If risks remain a concern with maximum PPE, then engineering controls (e.g., enclosed cabs or cockpits, water-soluble packaging, and closed mixing/loading systems) are evaluated. This approach is used to ensure that the lowest level of risk mitigation that provides adequate protection is selected, since the addition of PPE and engineering controls involves an additional expense to the user and – in the case of PPE – also involves an additional burden to the user due to decreased comfort and dexterity and increased heat stress and respiratory stress.

An assessment of handler exposure from the use of chlorpyrifos total release foggers was not performed because dermal and inhalation exposure is expected to be negligible based on the use pattern for this method of application. A total release fogger is an aerosol pesticide device designed to automatically release its total content in one operation for the purpose of creating a permeating fog within a space to deliver the pesticide throughout the space. Therefore, total release aerosols do not need any other application equipment (PR NOTICE 98-6, 1998).

Furthermore, the total release fogger product (EPA Reg. No. 499-405) directions state that once

[51] Available: http://www.epa.gov/pesticides/science/handler-exposure-data.html

cans are activated to, "leave greenhouse at once," and that, "all human occupants must be removed before treatment." In accordance with a common integrated pest management (IPM) and preventive safety practice, if multiple foggers are to be activated, the chlorpyrifos total release fogger label directs the use to "start with the can farthest away from the exit door."

The assessment of handler exposure from the use of chlorpyrifos ear tag and roach bait stations were also not conducted. Dermal exposure is expected to be negligible if gloves are worn, and inhalation exposure from these uses is not anticipated.

Exposure Data for Handler Exposure Scenarios: HED uses unit exposures to assess handler exposures to pesticides. Unit exposures are estimates of the amount of exposure to an active ingredient a handler receives while performing various handler tasks and are expressed in terms of micrograms or milligrams of active ingredient per pounds of active ingredient handled. HED has developed a series of unit exposures that are unique for each scenario typically considered in our assessments (i.e., there are different unit exposures for different types of application equipment, job functions, and levels of protection). The current assessment makes use of the document, *Occupational Pesticide Handler Unit Exposure Surrogate Reference Table – Revised March 2013*, was publically released to provide guidance for all of the currently recommended unit exposures for standard EPA occupational handler exposure scenarios.[52] A summary of all exposure data used to conduct the occupational handler risk assessment is presented in the updated occupational and residential exposure assessment.

In addition to this surrogate data, one non-chemical specific exposure study was used for the updated assessment. The exposure study was used to assess the use of SmartBox® technology for the mixing/loading of granular formulations for aerial and ground applications, and the application by ground equipment. Separate data evaluation records (DERs) were completed for the mixing/loading[53] and applicator[54] studies. A summary of the study is presented in the updated occupational and residential exposure assessment.

Summary of Occupational Handler Exposure Data Gaps: Consistent with the 2011 occupational and residential exposure assessment, no new data gaps have been identified for the assessment of occupational handler exposure/risk in addition to those identified in the Registration Eligibility Decision (RED) for chlorpyrifos (finalized 7/31/06; IRED issued 2/2002). Based on a 90-day progress report submitted (August 28, 2003) by the technical registrant, Dow AgroSciences, many of the data requirements are anticipated to be supported by the generation of data by the Agricultural Handlers Exposure Task Force (AHETF). These data requirements include exposure data for:

- seed treatment uses;
- mixing wettable powders for aerial/chemigation application;
- loading and applying granulars for aerial application;

[52] http://www.epa.gov/opp00001/science/handler-exposure-data.html
[53] L. LaSota. Evaluation of Loader Exposure Data Submitted as a Part of the Conditional Registration of Fortress 5G (Chlorethoxyfos). EPA MRID: 44180401. 1/23/1997. D232274.
[54] J. Arthur. Review of "Chlorethoxyfos Applicator Exposure Study during Application of Fortress® 5G Granular Insecticide using the Smartbox™ System during Corn Planting in the Midwest." EPA MRID: 443013-01. 7/27/1998. D238361.

- groundboom application; and
- human flaggers for aerial application.

AHETF data for groundboom application and for mixing/loading liquid formulations have been submitted and reviewed by HED and used to estimate exposure/risk for occupational handlers from the exposure scenario and, therefore, satisfy the data requirement. AHETF data for seed treatment and planting treated seed has been submitted and is currently under review by HED. The requirement for exposure data for mixing wettable powders for aerial/chemigation applications should be waived because the formulation is no longer supported by the registrant. An exposure study for loading granular formulations is being planned under AHETF. A study protocol was submitted and reviewed by the Human Studies Review Board (HSRB) November 2014. It does not appear, however, that flaggers are being supported by DAS as part of their prior response to the data requirements.

In accordance with 40CFR158, the following data are generally required for all occupational or residential pesticide uses: dislodgeable foliar residue (DFR) data are required for all occupational (e.g., crop, nursery, greenhouse use sites) or residential (e.g., ornamental and vegetable gardens, pick your own farms, retail tree farms) uses that could result in post-application exposure to foliage; and turf transferable residue (TTR) data are required for all occupational (e.g., sod farms, golf courses, parks, and recreational areas) or residential turf uses that could result in post-application exposure to turf. Adequate DFR and TTR data are available for chlorpyrifos.

Occupational Handler Non-Cancer Exposure and Risk Estimate Equations

The algorithms used to estimate non-cancer exposure and dose for occupational handlers can be found in the updated occupational and residential exposure assessment (D424484).

Combining Exposures/Risk Estimates:

Dermal and inhalation risk estimates were combined in this assessment, since the toxicological endpoint, RBC AChE inhibition, is the same for these exposure routes.

Summary of Occupational Handler Non-Cancer Exposures and Risk Estimates

Steady state exposure and risks calculated for occupational handlers (other than commercial seed treatment) for all use patterns are presented in the updated occupational and residential exposure assessment (Appendix G of D424484).

Current labels generally require that handlers use normal work clothing (i.e., long sleeved shirt and pants, shoes and socks) and coveralls, chemical resistant gloves, and dust/mist respirators. Also, some products are marketed in engineering controls such as water soluble packets. In order to determine what level of personal protection is required to alleviate risk concerns and to ascertain if label modifications are needed, steady state exposure and risk estimates were calculated for occupational handlers of chlorpyrifos for a variety of scenarios at differing levels of personal protection including engineering controls. In this assessment, a total of 285 total occupational handler exposure scenarios were assessed and 132 of them are not of concern (i.e., MOEs are $\geq$ 100) at current product label requirements. Risks of concern for 27 additional

exposure scenarios could potentially be mitigated if additional personal protective equipment or engineering controls are used. Risks of concern were estimated for 126 exposure scenarios and remain a concern regardless of the levels of personal protection and engineering controls considered. These risks could be mitigated using the highest level of personal protection available and by modification to other label requirements such as application rate or, in some cases, deleting certain types of application equipment.

Commercial Seed Treatment

Based upon the registered seed treatment uses of chlorpyrifos, occupational handler exposure is expected for individuals involved in commercial seed treatment. Post-application exposure for agricultural (field) workers is not expected based on the seed treatment use of chlorpyrifos.

The 2011 occupational commercial seed treatment assessment has been updated. All occupational exposure scenarios assessed, algorithms, and unit exposures used (taken from HED ExpoSAC Policy 14: SOPs for Seed Treatment) are the same as were used previously in the 2011 occupational and residential exposure assessment for the assessment of commercial seed treatment. However, the assessment of occupational seed treatment uses has been updated to reflect the use of the guidance document, *Interim Guidance (SOP 15.1) Amount of Commercial Seed Treated per Day,* updated March 21, 2013, as well as the updated default adult (female) body weight. Also, seed treatment risk was estimated with use of PBPK-PD modeled PoDs (dermal and inhalation) specific for occupational assessment.

The steady state approach is appropriate for the assessment of commercial seed treatment with chlorpyrifos given the toxicological and exposure profile. The steady state endpoint selection for chlorpyrifos accounts for HED's traditional short-term exposure duration endpoint selection, as well as being appropriately health protective for workers that are exposed over longer periods of time (i.e., intermediate-term exposures).

Summary of Commercial Seed Treatment Exposures and Risk Estimates

Steady state exposure and risks calculated for occupational handlers performing seed treatment activities in commercial settings and for occupational secondary handlers from planting chlorpyrifos-treated seeds are presented in the updated occupational and residential exposure assessment (Appendix H of D424484).

Of the 68 exposure scenarios assessed, 36 resulted in risk estimates which were not of concern (i.e., MOEs are $\geq$ 100) at the level of personal protection currently required by product labels. Secondary handler (seed planter) scenarios were also evaluated and none were of concern at current label requirements. The remaining 32 seed treatment scenarios are of potential concern.

9.2 Steady State Occupational Post-Application Risk

9.2.1 Dermal Post-Application Risk

HED uses the term post-application to describe exposures that occur when individuals are present in an environment that has been previously treated with a pesticide (also referred to as re-entry exposure). Such exposures may occur when workers enter previously treated areas to perform job functions, including activities related to crop production, such as scouting for pests or harvesting. Post-application exposure levels vary over time and depend on such things as the type of activity, the nature of the crop or target that was treated, the type of pesticide application, and the chemical's degradation properties. In addition, the timing of pesticide applications, relative to harvest activities, can greatly reduce the potential for post-application exposure.

The 2011 occupational post-application exposure assessment has been updated to include the following: 1) a PBPK-PD modeled dermal PoD specific for occupational assessment, 2) the master use document, 3) the updated adult (female) default body weight and 4) the changes relating to agricultural transfer coefficients (TC) as described in the *Science Advisory Council for Exposure (ExpoSAC) Policy 3 – Revised March 2013.* [55]

Occupational Post-application Dermal Exposure Data and Assumptions

A series of assumptions and exposure factors served as the basis for completing the occupational post-application risk assessments. The majority of assumptions and exposure factor detailed in the 2011 exposure assessment are the same, except for changes relating to agricultural transfer coefficients (TC) as described in the *Science Advisory Council for Exposure (ExpoSAC) Policy 3 – Revised March 2013* and the standard adult body weight assumptions.

Post-application Exposure Data: The 2011 occupational and residential exposure assessment made use of seven chemical-specific DFR studies that encompass the use of five different formulations and 12 different crops. Specifically, the DFR studies examine the use of 1) emulsifiable concentrate formulations on sugar beets, pecans, citrus, sweet corn, cotton, and turf; 2) wettable powder formulations on almonds, apples, pecans, cauliflower, tomato and turf; 3) granular formulations on sweet corn and turf; 4) a total release aerosol formulation on ornamentals; and 5) a microencapsulated formulation on ornamentals. The submitted studies were reviewed by HED. Despite limitations, HED recommended the use of all or some of the data in the studies to assess post-application risks to chlorpyrifos except for the tomato DFR data. Summaries for all DFR studies can be referenced in the updated occupational and residential exposure assessment (Appendix I of D424484).

The current assessment makes use of the same DFR data and crop pairings as was done for the 2011 occupational and residential exposure assessment. For example, DFR data for an individual crop was applied to that specific crop, as well as to crops in the same crop grouping (e.g., cauliflower data was used for cauliflower and all other cole crops). For other crops for which no crop-specific or crop group-specific data are available, the DFR data for the crop deemed the closest match were used as surrogates to calculate potential exposure (e.g., cauliflower data were also used for strawberries, cranberries, and leafy vegetables). Additionally, whenever possible, a label use was assessed using DFR data for the same

[55] http://www.epa.gov/opp00001/science/exposac-policy-3-march2013.pdf

formulation type. A full description of the criteria for selection of DFR data for assessment of post-application exposures to individual crops/crop groupings can be referenced in Section 2.4.3 of the 2011 occupational and residential exposure assessment (D388165).

For occupational post-application dermal exposures, the parent compound is the residue of concern; dermal exposure to the oxon on foliar surfaces from reentry into an outdoor environment previously treated with chlorpyrifos is not anticipated and, therefore, has not been assessed. However, based on study results (see Section 9.2.2), it may be possible that the formation of the oxon is greater and its deactivation slower in greenhouses when compared to the outdoor environment and that an assessment may be needed for exposure to the oxon in greenhouse settings. In the 2011 Preliminary Human Health Risk Assessment, additional studies to address uncertainties regarding the formation of chlorpyrifos oxon and its decay following applications in greenhouses were recommended. To date, no additional data have been submitted. Due to uncertainty regarding the formation of chlorpyrifos oxon in greenhouses, HED estimated risks for reentry into (microencapsulated and total release fogger formulation) treated greenhouses for parent chlorpyrifos only, as well as for parent chlorpyrifos plus chlorpyrifos oxon using a total toxic residue approach. This conservative approach is assumed to result in a health protective assessment of post-application exposures to chlorpyrifos oxon in greenhouses.

Summary of Occupational Post-application Exposure Data Gaps:
No new occupational data requirements have been identified for chlorpyrifos; however, as described above, additional data were recommended for the evaluation of chlorpyrifos oxon and no data have been submitted to date.

Summary of Occupational Post-application Dermal Exposure and Risk Estimates

Steady state occupational post-application dermal risk estimates are presented in the updated occupational and residential exposure assessment (Appendix J of D424484) based on the PBPK-PD model predicted dermal PoD.

Current labels require a Restricted Entry Interval (REI) of 24 hours from most crops and activities, but in some cases such as tree fruit, REIs are up to 5 days after application. All post-application worker risks have been updated in the current assessment. Results indicate that no REI increase is required for the majority of outdoor environment crops and commodities with a labeled REI (i.e., 43 of 55 total). However, for 12 crops, activities such as irrigation, hand harvesting, scouting, and thinning result in risks of concern up to as many as 10 days following application.

For the assessment of post-application exposures to parent chlorpyrifos (only) from ornamental production in greenhouses, 4 formulations were assessed: emulsifiable concentrate, microencapsulated liquid, wettable powder in WSP, and total release fogger. An REI increase of up to 5 days may be needed to alleviate risks from with use of the emulsifiable concentrate, wettable powder in WSP and total release fogger formulations. For the microencapsulated liquid formulation, the estimated REIs range from 0 to > 35 days after treatment (the completion of the monitoring period) dependent on the exposure activity considered.

9.2.2 Occupational Post-application Dermal Exposure/Risk Estimates: Chlorpyrifos Oxon

Chlorpyrifos is activated by desulfuration, reacting in bioactivation to the more toxic and potent acetyl cholinesterase (AChE) inhibitor, chlorpyrifos oxon. The oxon is highly unstable due to rapid deactivation through hydrolytic cleavage by a process called dearylation which releases TCP. Workers reentering an indoor environment (i.e., greenhouses) previously treated with chlorpyrifos could potentially be exposed to the oxon as chlorpyrifos degrades. Available exposure data indicate chlorpyrifos oxon may form in indoor environments.[56] Toxicity adjustment factors (TAFs) were used to estimate the potency of chlorpyrifos oxon relative to chlorpyrifos. HED determined the oxon to be between 11.9 (acute) and 18 (chronic) times more toxic than the parent.

Dermal exposure to the oxon on foliar surfaces from reentry into an outdoor environment (e.g., field crops and orchards) previously treated with chlorpyrifos is not anticipated and, therefore, has not been assessed. No occupational exposure studies (handler, post-application, or DFR) were identified that quantified the levels of oxon present in the environment. However, a search of open literature for the 2011 assessment resulted in 4 plant metabolism studies which measured surface residues. Three plant metabolism studies[57] measured leaf surface residues of the oxon in outdoor environments that were either well below the parent, not detectable, or detected at a level just above the level of detection (LOD). The potential for exposure to the oxon is further minimized due to rapid deactivation of the oxon to TCP. Further, the dietary exposure risk assessment[58] conducted in support of registration review concludes the following, "all residues in food are assumed to be parent chlorpyrifos since the chlorpyrifos oxon is not typically found in foods in monitoring data or crop field trials."

The 4th plant metabolism study, a tomato and green bean greenhouse metabolism study, was less definitive than the other three plant metabolism studies regarding oxon presence. HED is concerned that the formation of the oxon may be greater and its deactivation to TCP slower in greenhouses when compared to the outdoor environment. The study results indicate that oxon residue is from 9 to 14X less than the parent from fruit analyzed on the day of application in flat and asymmetric roof greenhouses. The proportion of oxon to parent is less for all days which measurable levels were observed (all but 8 and 15 days after application). The oxon was detected until day 5 with levels between 5 and 6X below that of the parent. It should be noted that residues of chlorpyrifos and oxon were measured from analysis of whole fruit samples. HED typically assesses occupational post-application exposure and risk based upon the potential for transfer from surface residues. The whole fruit samples, which include surface residues, as

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[56] J.L. Martinez Vidal, et al. 1998. Diminution of Chlorpyrifos and Chlorpyrifos Oxon in Tomatoes and Green Beans Grown in Greenhouses. J. of Agric. and Food Chem. 46 (4), 1440–1444.

[57] Iwata, Y. et al. 1983. Chlorpyrifos Applied to California Citrus: Residue Levels on Foliage and On and In Fruit. J. Agric. Food Chem. 31(3), 603-610.
H. Jin and G.R. Webster. 1997. Persistence, Penetration, and Surface Availability of Chlorpyrifos, Its Oxon, and 3,5,6-Trichloro-2-pyridinol in Elm Bark. 45(12), 4871-4876.
R. Putnam, et al. 2003. The Persistence and Degradation of Chlorthalonil and Chlorpyrifos in a Cranberry Bog. J. Agric. Food Chem. 51(1), 170-176.

[58] D. Drew. Chlorpyrifos: Acute and Steady State Dietary (Food Only) Exposure Analysis to Support Registration Review. 11/18/2014. U.S. EPA Office of Chemical Safety and Pollution Prevention. D424486.

well as residues which may have been contained within the fruit sample, may overestimate the amount of oxon on the fruit surface. Regardless, the 2011 occupational and residential exposure assessment recommended additional data to measure the chlorpyrifos and oxon residues on leaf surfaces following treatment with a liquid formulation in greenhouses in order to address these uncertainties and more accurately address the risk potential for exposure from occupational reentry into greenhouses treated with chlorpyrifos. To date, no data has been submitted to address these uncertainties. As a result, HED has conducted an assessment of occupational post-application exposures in greenhouses that uses conservative assumptions of oxon formation.

In order to account for the formation of and potential increased toxicity from exposure to chlorpyrifos oxon, a total toxic residue approach was applied which combines chlorpyrifos and chlorpyrifos oxon (expressed as toxicity equivalents). The total toxic residue approach[59] estimates the chlorpyrifos oxon equivalent residues by 1) assuming a specific fraction of the measured chlorpyrifos dislodgeable foliar residues are available as the oxon and 2) factoring in the relative potency of chlorpyrifos oxon with use of a TAF. It was conservatively assumed that 5% (0.05) of the total chlorpyrifos present as DFR in greenhouses is available for worker contact during post-application activities. This assumption is based on a review of available TTR and DFR data for other OPs where both the parent and metabolite were measured in residue samples. Five percent was found to be the high-end value for the percent of parent that metabolized during the course of the residue studies. The chronic TAF (which is appropriate for steady state assessment) of 18 was derived from BMD analysis of inhibition of RBC AChE in adult female rats (adult male rats not examined) observed in the repeated phase of the CCA study. Once predicted, these total toxic (dislodgeable foliar) residues are used to estimate exposures from post-application activities in greenhouse and risks are estimated with used of the steady state PoD for occupational exposures, 4.9 mg/kg/day.

Summary of Occupational Post-application Dermal Exposure and Risk Estimates with Use of Total Toxic Residue Approach

Due to uncertainty regarding the formation of chlorpyrifos oxon in greenhouses, HED also estimated risks for reentry into treated greenhouses (all 4 formulations) for the parent chlorpyrifos plus chlorpyrifos oxon using a total toxic residue approach. When the total toxic residue approach is used to evaluate risks from exposure to the oxon with use of the emulsifiable concentrate, wettable powder in WSP, and total release fogger formulations, results indicate that an REI increase up to 6 days may be needed to alleviate risk concerns. For the microencapsulated liquid formulation, REIs range from 3 to > 35 days after treatment (the completion of the monitoring period) dependent on the exposure activity considered.

9.2.3 Inhalation Post-application Risk

There are multiple potential sources of post-application inhalation exposure to individuals performing post-application activities in previously treated fields. These potential sources include volatilization of pesticides and resuspension of dusts and/or particulates that contain pesticides. As described in Section 6.3.2 of this document, a quantitative risk assessment is not

[59] Total DFR ($\mu g/cm^2$) = [Chlorpyrifos DFR ($\mu g/cm^2$) * TAF] + [Chlorpyrifos DFR ($\mu g/cm^2$)]

required for chlorpyrifos or chlorpyrifos oxon due to the lack of toxicity of these chemicals when in the vapor state, even at the saturation concentration.

The Worker Protection Standard for Agricultural Pesticides contains requirements for protecting workers from inhalation exposures during and after greenhouse applications through the use of ventilation requirements.[40 CFR 170.110, (3) (Restrictions associated with pesticide applications)].

A post-application inhalation exposure assessment is not required as exposure is expected to be negligible. Seed treatment assessments provide quantitative inhalation exposure assessments for seed treaters and secondary handlers (i.e., planters). It is expected that these exposure estimates would be protective of any potential low-level post-application inhalation exposure that could result from these types of applications.

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of carboxylesterase 1 and 2 in mouse and human liver. Drug Metab. Dispos. 37:1819-1825

11. List of Appendices

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Appendix 1. Evaluation of Experimental Toxicology Data

1.0. Introduction

Chlorpyrifos (*0,0*-diethyl-*0*-3,5,6-trichloro -2-pyridyl phosphorothioate) is a broad-spectrum, chlorinated organophosphate (OP) insecticide. In 2000, most residential uses were voluntarily cancelled by Dow AgroSciences but agricultural use remains. In 2011, the Agency released a preliminary human health risk assessment for chlorpyrifos. The focus of the 2011 risk assessment was on the cholinesterase (ChE) inhibiting potential of chlorpyrifos including in young animals. Like other OPs, chlorpyrifos binds to and phosphorylates the enzyme acetylcholinesterase (AChE) in both the central (brain) and peripheral nervous systems. This can lead to accumulation of acetylcholine and, ultimately, at sufficiently high doses, to clinical signs of toxicity. Consistent with the focus on ChE inhibition, in the 2011 preliminary risk assessment, EPA evaluated the extensive database of ChE data for multiple lifestages and selected points of departure based on consideration of all quality and reliable data.

In 2008 and 2012, the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) reviewed two draft science issue papers on the human health effects of chlorpyrifos which provided a preliminary review of the scientific literature on experimental toxicology and epidemiology studies available since the 2000 risk assessment. These draft issue papers considered a growing body of literature with laboratory animals (rats and mice) indicating that gestational and/or early postnatal exposure to chlorpyrifos may cause persistent effects into adulthood in addition to epidemiology studies evaluating prenatal chlorpyrifos exposure in mother-infant pairs that have reported associations with birth outcomes, childhood neurobehavioral and neurodevelopment outcomes in the offspring when evaluated in neonates, infants, and young children. In 2008 and 2012, the SAP agreed with the Agency that although the epidemiology studies were high quality with respect to design, conduct and analyses and provided information for hazard characterization, ChE inhibition remained the most robust dose response data for deriving points of departure. This appendix incorporates components from the draft SAP issues papers, comments from the SAP and from the public on those drafts and new studies since 2012[60].

In 2010, the Agency developed a draft "Framework for Incorporating Human Epidemiologic & Incident Data in Health Risk Assessment" which provides the foundation for evaluating multiple lines of scientific evidence in the context of the understanding of the adverse outcome pathway (or mode of action; (U.S. Environmental Protection Agency, 2010). The draft framework, which includes two key components: problem formulation and use of the mode of action/adverse outcome pathway frameworks, was reviewed favorably by the SAP in 2010 (FIFRA SAP, 2010). OPP's draft framework is consistent with updates to the World Health Organization/International Programme on Chemical Safety mode of action/human relevance framework which highlight the importance of problem formulation and the need to integrate information at different levels of biological organization (Meek et al, 2014). Consistent with recommendations by the NRC in its 2009 report, OPP's draft framework describes the importance of using problem formulation at the beginning of a complex scientific analysis. The problem formulation stage starts with planning dialogue with risk managers to identify goals for the analysis and possible risk management strategies. This initial dialogue provides the regulatory context for the scientific analysis and helps define the scope of such an analysis. The problem formulation stage also involves consideration of the available information regarding the pesticide use/usage, toxicological effects of concern and exposure pathways and duration along with key gaps in data or scientific information. Specific to chlorpyrifos, the 2008 and 2012 SAP reviews represent the problem formulation analyses for the weight of evidence (WOE) analysis provided in the main body of the risk assessment.

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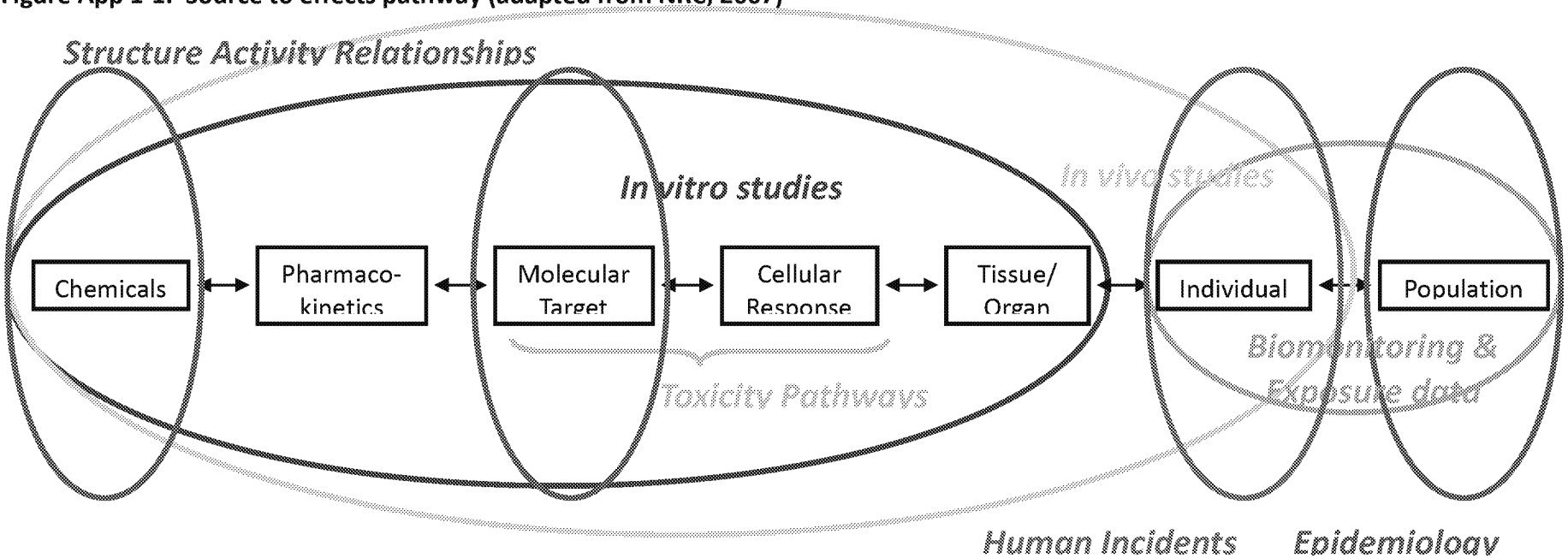
[60] The agency is aware of multiple review articles related to topics in this appendix published in the scientific literature (Prueitt et al, 2011; Goodman et al, 2013; Li et al, 2012; Li et al, 2014; Mink et al, 2014; Reiss et al, 2012). EPA has conducted an independent review of the scientific literature.

Mode of action (Boobis et al., 2006; Boobis et al., 2008; U.S. EPA, 2005; Simon et al, 2014; Meek et al, 2014) and adverse outcome pathway (Ankley et al., 2010) provide important concepts in this integrative analysis. Both a mode of action and an adverse outcome pathway are based on the premise that an adverse effect caused by exposure to a compound can be described by a series of causally linked biological key events that result in an adverse human health or ecological outcome. One of the key components of the agency's draft framework is the use the mode of action framework /adverse outcome pathway concept as a tool for organizing and integrating information from different sources to inform the causal nature of links observed in both experimental and observational studies. Specifically, the modified Bradford Hill Criteria are used to evaluate the experimental support that establishes key events within a mode of action or an adverse outcome pathway, and explicitly considers such concepts as strength, consistency, dose response, temporal concordance and biological plausibility in a weight of evidence analysis.

The draft framework is oriented around the source to outcome pathway (Figure App 1-1) discussed in the National Research Council's (NRC) report on toxicity testing in the 21st Century (National Research Council, 2007).

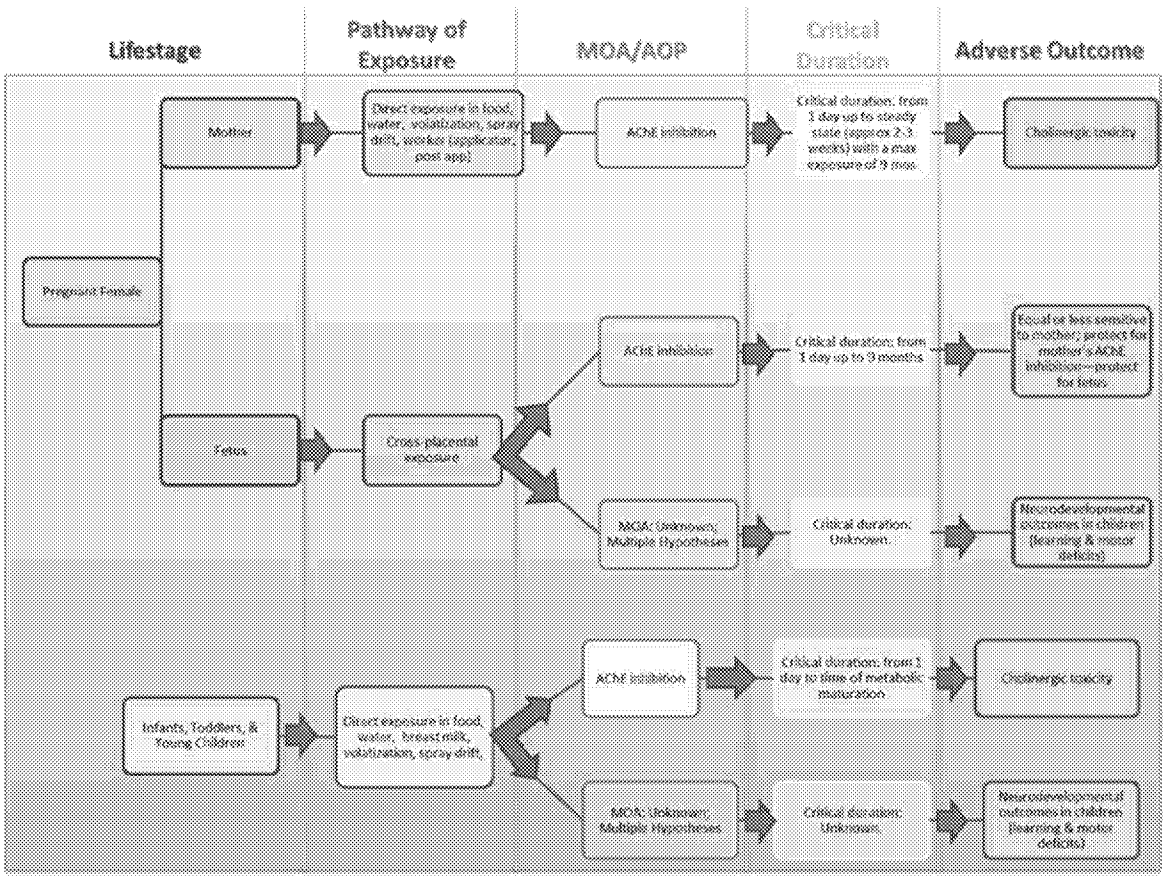
Figure App 1-1. Source to effects pathway (adapted from NRC, 2007)



2.0. Conceptual Framework

As an aid in organizing the available information and to identify the complex scientific issues being considered, the agency developed a conceptual framework. This conceptual framework (Figure App 1-2) is consistent with the source-to-outcome pathway and provides the foundation for the analysis being undertaken by the agency to delineate outcomes derived from AChE inhibition in different lifestages and from alternative modes/mechanisms (Figure App 1-1). This conceptual framework provides key information on which lifestages are thought be the most susceptible to chlorpyrifos, the exposure pathway by which these individuals are exposed, possible adverse outcome pathways and related critical duration(s) of exposure leading from exposure to adverse health outcome.

Figure App 1-2. Conceptual framework considering chlorpyrifos exposure, lifestages, critical durations, modes of action/adverse outcome pathways, and health outcomes of interest.



2.1. Adverse Outcomes

AChE inhibition is the well-established cholinergic mode of action for OPs and is typically used as the critical effect in hazard characterization for members of this class of pesticides. The 2008 and 2012 SAPs concurred with the agency that AChE/ChE data when taking into account sensitive lifestages remains the most robust dose response data for use in derivation of points of departure. However, over the last 10-15 years, experimental toxicology studies on neurotoxicological as well as epidemiology studies have been published which suggest that the developing brain of the fetus and young children may also be affected. There are a significant number of literature studies evaluating neurobehavioral outcomes in experimental animals (rats, mice). These studies vary in their study design but many involve gestational and/or early postnatal dosing with behavioral evaluation in adulthood. Epidemiological studies from three cohorts of mothers and children, funded in part by EPA and National Institutes of Environmental Health, have reported birth and neurodevelopmental outcomes associated with prenatal exposures to OPs. Two of these (Mt. Sinai and Berkeley) have focused primarily upon non-specific urinary biomarkers of OP exposure, the dialkyl phosphate metabolites (*i.e.*, DAPs), and thus present some uncertainty as to the extent these results relate to chlorpyrifos *per se*. The Columbia University investigators are studying a cohort of mothers and children in inner-city New York and have published multiple papers on associations among level of prenatal chlorpyrifos exposure and birth and neurodevelopmental outcomes at multiple time points from birth through age seven years. With respect to neurodevelopmental outcomes, multiple investigators have reported hypothesized modes/mechanisms of action. In the conceptual framework, both adverse health outcomes of interest for chlorpyrifos are being tracked—AChE inhibition and potential for neurodevelopmental effects.

2.2. Potentially Sensitive Lifestages

The agency believes that pregnant women and their fetuses, newborns, and young children represent potential sensitive lifestages for exposure to chlorpyrifos. This conclusion is based on multiple lines of evidence.

- *Pregnant Women:*
  Chlorpyrifos RBC AChE data from pregnant rats provides the most sensitive data for use in deriving a point of departure from repeated dosing studies. Although it is unclear whether metabolic changes during pregnancy are sufficient to affect metabolism at environmental exposure, some have suggested that there may be reduced ability to detoxify chlorpyrifos and/or the oxon may affect sensitivity during pregnancy.

  Metabolic activities can be altered during pregnancy (Anderson, 2006; Anger & Piquette-Miller, 2008; Bologa, et al, 1991; Carpintero et al, 1996; Czekaj et al, 2000, 2005; Dickmann et al., 2008a, b; Ejiri et al, 2005; Ferre et al., 2006; Hines, 2007; Homma et al., 2000; Howard & Sugden, 1993; Tsutsumi et al., 2001). For example, Chanda et al. (2002) showed that pregnant female rats had lower plasma, brain, and liver carboxylesterase activity compared to non pregnant females. Regarding A-esterase activity, Ferre *et al.* (2006) showed that the paraoxonase (*i.e.*, A-esterase in serum decreased from a nonpregnant background of 146 U/L to 111 U/L in late gestation, indicating 76% of normal activity in late gestation pregnant women. Carpintero et al. (1996), however,

found that phenyl acetate metabolism increased from 23.6 to 33.5 μkat/g in the third trimester, but it must be kept in mind that phenyl acetate metabolism is not necessarily a specific measure of A-esterase activity. Data in mice support the findings of Ferre et al. (2006) in humans suggesting a reduction in A-esterase activity during pregnancy. In mice, Weitman et al. (1983) found that PON1 activity after exposure to parathion was 50 nmol/min/ml in non-pregnant females, but it decreased as low as 14 nmol/min/ml during gestation (Weitman, et al., 1983). With regard to plasma butylcholinesterase (BuChE) activity, Howard et al. (1978) have shown that in six healthy pregnant women levels of plasma BuChE activity dropped by approximately 30% during the first trimester, but returned to close to pre-pregnancy levels in the third trimester. Similarly, other investigators have also reported decreases in plasma BuChE activity in pregnant women (de Peyster et al, 1994; Venkataraman et al , 1990; Whittaker, Crawford, & Lewis, 1988). Evans et al. (1988) showed that serum ChE levels in 39 of 44 pregnant women dropped after conception; in 20 of those women, the decline in ChE activity continued throughout pregnancy.

Pregnancy is a remarkably dynamic biological process in which rapid changes occurring in both the developing system (embryo/fetus) and the mother can significantly impact the pharmacokinetics of chemicals. In addition to the potential for decreased clearance of chemicals due to immature metabolic systems in the developing embryo/fetus (mentioned above), other pregnancy-related changes can also have a pronounced effect on pharmacokinetics. For a typical human pregnancy, total body weight gain is in the order of 10-30% while cardiac output can increase as much as 50% (Corley *et al*, 2003a, b; Young *et al.*, 1997a, b). A significant fraction of the body weight gain is due to increases in total body water and fat that can lead to a greater volume of distribution by simple dilution. For instance, increases in total body water dilute plasma proteins which can increase the free fraction of highly plasma protein-bound chemicals (*e.g.*, chlorpyrifos) available for distribution. Increases in fat, on the other hand, can provide a larger storage volume for lipophilic chemicals (Corley *et al.*, 2003). Additionally, significant changes take place in the maternal circulatory system to accommodate the development of placental blood flow which undergoes considerable increases throughout pregnancy along with embryo/fetus which increases in volume over a billion-fold from conception to birth (Young *et al.*, 1997).

- *Fetuses, Infants, Toddlers & Young Children:*
  With respect to distribution to the fetus during gestation, there are multiple studies on chlorpyrifos (Mattsson *et al.*, 1998, 2000; Qiao *et al.*, 2002) and other OPs (USEPA, 2006) which show that the pregnant dam exhibits similar or more AChE inhibition than the fetus at a given dose to the dam. As such, for AChE inhibition, protecting against AChE inhibition in the pregnant female is expected to be protective for AChE inhibition in the fetus. Biomonitoring data from rats and humans illustrate fetal exposure levels are similar to maternal levels which support these findings. Specifically, Whyatt *et al.*, (2003) have shown that levels of chlorpyrifos in maternal blood are similar to the levels measured in human umbilical cord blood. In Hunter *et al.*, (1999), pregnant rats were orally dosed with chlorpyrifos for 5 days during late gestation at doses of 3 or 7 mg/kg/day, and the amounts of chlorpyrifos, chlorpyrifos-oxon, and TCPy were quantified in fetal and maternal brain and liver. No chlorpyrifos or its oxon were

detected. The concentration of TCPy in the fetal brain was higher than the TCPy concentration in the maternal brain in time-course and dose-response studies but in liver the maternal levels were higher. In a study by Mattsson *et al.*, (1998, 2000), concentrations of chlorpyrifos, the oxon, and TCPy were measured in the blood of maternal and fetuses and TCPy levels in dam and fetal blood were similar and chlorpyrifos levels were approximately 2-fold higher in maternal blood than fetal blood. In another gavage gestational exposure study, Akhtar *et al.*, (2006) exposed rats to chlorpyrifos from GD 0-20 in fetal and maternal tissues on GD21 at high doses (9.6-15 mg/kg/day); while there is a high degree of variability in the Akhtar et al study, dams and fetuses showed similar levels of chlorpyrifos in liver and brain.

With respect to neurodevelopmental outcomes, numerous epidemiological investigations have observed a link between early life exposure to chlorpyrifos and adverse effects on neurodevelopment through age seven years. Epidemiology studies focused on exposure during gestation but the impact of post-natal exposure can not be ruled out. Overall, these studies are strong, well-conducted studies in which likely sources of error or bias would more likely tend to underestimate, rather than overestimate an effect size (*e.g.*, relative risk measure). In addition, there a growing body of literature with laboratory animals (rats and mice) indicating that gestational and/or early postnatal exposure to chlorpyrifos may cause persistent effects into adulthood.

Potential sensitivity, particularly observed in acute, single dose exposures, is largely derived from immature metabolic systems that have less capacity to detoxify the parent OP and its toxicologically active metabolite chlorpyrifos oxon. This sensitivity is not derived from differential inhibition of the AChE enzyme itself as supported by *in vitro* studies (Benke and Murphy, 1975; Chanda *et al.*, 1995; Mortensen *et al.*, 1996; Atterberry *et al.*, 1997). Rat fetuses and juveniles and human fetuses, infants, and young children have lower capacity to detoxify chlorpyrifos than adults. Specifically, in rats, A-esterase activity is virtually nonexistent in the fetus (Lassiter *et al.*, 1998) and increases from birth to reach adult levels around PND21 (Mortensen *et al.*, 1996; Li *et al.*, 1997). Mortenson *et al.*, (1996) showed that in the plasma level of A-esterase in 4-day old rats was 1/11 that of adult animals. The animal data regarding the role of carboxylesterase in mediating OP toxicity are also quite extensive (e.g., Clement, 1984; Fonnum *et al.*, 1985; Maxwell, 1992 a, b). Fetal rats and mice possess very little carboxylesterase activity with increasing activity as the postnatal rodent matures, reaching adult values around puberty (Lassiter *et al.*, 1998; Morgan *et al.*, 1994; Moser *et al.*, 1998; Karanth and Pope, 2000; Zhu *et al.*, 2009). The temporal pattern of A-esterase and carboxylesterase activity correlates well with studies on OP sensitivity. Several studies have shown an increased sensitivity of newborn rats to OP compounds which are detoxified via the A-esterase and/or carboxylesterase pathways (e.g., Gagne and Brodeur, 1972; Benke and Murphy, 1975; Pope *et al.*, 1991; Chambers and Carr, 1993; Padilla *et al.*, 2000; 2002; Karanth and Pope, 2000).
While there are fewer data in human tissues which could evaluate age-related maturation of carboxylesterase and A-esterase expression, there are numerous studies that present age differences in liver P-450 metabolism in humans that also play a role in OP metabolism (*e.g.*, review by Hines, 2007). Studies evaluating maturational expression and activity of liver carboxylesterases in human liver tissues show lowest levels in fetal

tissues, with increasing activity during childhood, although there are large individual differences and thus high variability (Pope *et al.*, 2005; Yang *et al.*, 2009; Zhu *et al.*, 2009). Adult tissues were approximately 10-fold and 4-fold more active in hydrolyzing certain chemicals than fetal and child (0-10 years) tissues, respectively, and enzyme expression differences were much greater (Yang *et al.*, 2009). In contrast, in Pope *et al.*, (2005), the differences in activity were relatively small (and not statistically significant) between children ages 2–24 months and adults (20–36 years); however, that youngest age evaluated in the study was 2 months old and this individual had the lowest level of carboxylesterase.
Serum A-esterase levels also appear to be very low in human infants and children compared to adults (Augustinsson and Barr, 1963; Cole *et al.*, 2003; Huen *et al.*, 2009; Mueller *et al.*, 1983; Ecobichon and Stephens, 1973; Gonzalez *et al.*, 2012; Holland *et al.*, 2006; Chen *et al.*, 2003). While some studies have shown that PON1 activity reaches adult levels by about 2 years of age (e.g., Cole *et al.*, 2003), recent papers have reported slightly lower levels as late as 9 years (Gonzalez et al., 2012). Reasons for these differences could include type of measurement (i.e., genotyping vs phenotyping), assay substrate, sample sizes, and population factors (e.g., ethnicity).

There are critical windows of vulnerability (Rice & Barone, 2000; Rodier, 2004) with regard to toxicant effects on brain development. This vulnerable period in humans spans early pregnancy to adolescence (Rice & Barone, 2000). In fact, evidence shows that synapse formation peaks quite late in human brain development at 4-8 years of age (Glantz *et al.*, 2007). Within these vulnerable periods there are key neurodevelopmental processes (*e.g.* cell division, migration, differentiation, synaptogenesis, and myleination) and each of these is region and stage specific. Consequently, the time of toxicant exposure will be a major determinate in the spectrum of neurotoxic effects

3.0. Adverse Outcome Pathways: AChE Inhibition & Plausible Pathways Leading to Neurodevelopmental Outcomes

Mode of action and adverse outcome pathways provide important concepts in the draft "Framework for Incorporating Human Epidemiologic & Incident Data in Health Risk Assessment" (U.S. EPA, 2010). As mentioned above, both a mode of action and an adverse outcome pathway (AOP) include a set of measureable key events that describe the biological processes leading to an apical effect. Figure App1-3 is a graphical presentation of a generic adverse outcome pathway (Ankley *et al.*, 2010). This figure is an extension of the source-to-outcome pathway and provides additional detail on the types of scientific information from various levels of biological organization used in establishing an adverse outcome pathway. As shown in the conceptual framework, two different adverse outcomes in laboratory animals are being evaluated: AChE/ChE inhibition and neurodevelopmental outcomes. Each of these is discussed below.

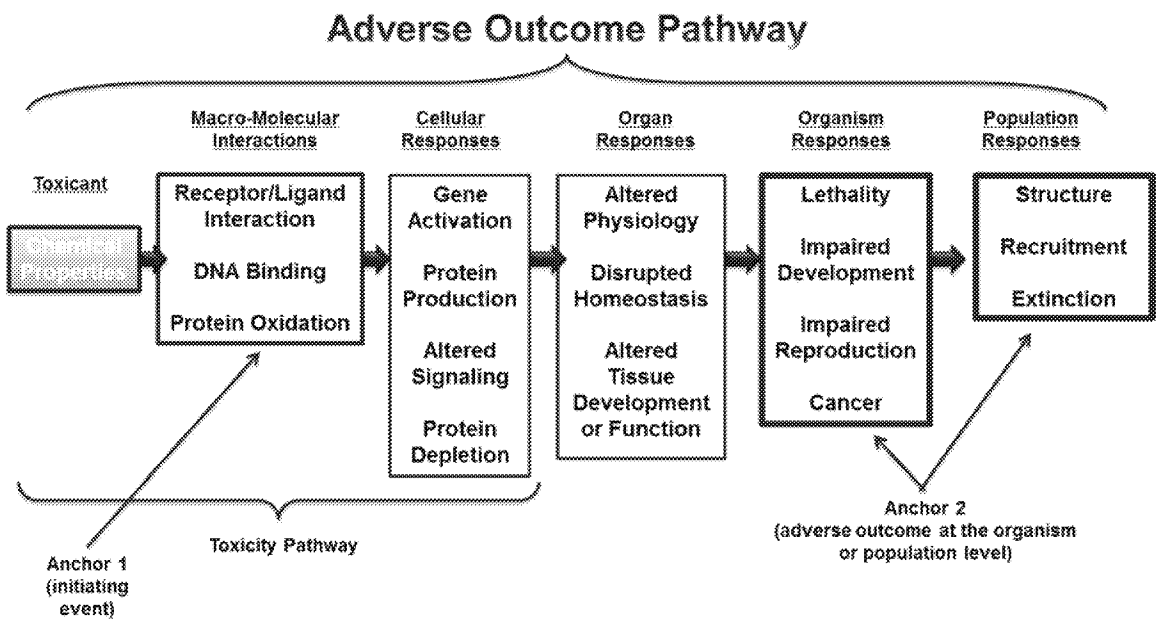


Figure App1-3. Generic Adverse Outcome Pathway (Adapted from Ankley et al., 2010)

3.1. Adverse Outcome Pathway: AChE Inhibition

3.1.1. Initiating Event & Health Outcomes

AChE inhibition is the well-established mode of action for OPs and is typically used as the critical effect in hazard characterization for members of this class of pesticides. Specifically, the initiating event for OP mediated neurotoxicity is interaction at the serine residue at the active site of the AChE. This interaction leads to accumulation of acetylcholine and ultimately to clinical signs of neurotoxicity at high doses. In experimental toxicity studies in laboratory animals, high acute doses produce signs of autonomic stimulation, ataxia, fasciculations, tremors, respiratory

difficulties, and convulsions. The Agency's recent review of human incident reporting databases (Recore & Oo, 2011) indicates that humans exposed to high levels of chlorpyrifos may complain of gastrointestinal and neurological effects consistent with acute AChE poisoning. Specifically, individuals have reported: nausea, vomiting, diarrhea, tremors, headaches, dizziness, numbness and tingling sensations, muscle spasm, shortness of breath, and seizures (Recore & Oo, 2011).

Although inhibition of AChE as the initiating event in this adverse outcome pathway has long been established, the quantitative dose response linkage between AChE inhibition and toxicity has been shown to be more variable. In other words, the amount of AChE inhibition required to elicit such responses from OP exposure is not consistent but instead varies among different OPs, different study designs, and different neurotoxic outcomes. Specific to chlorpyrifos, there are limited human data which inform the quantitative dose-response linkage between AChE inhibition and apical neurotoxic effects. There are two human deliberate dosing studies in human subjects (Nolan *et al.*, 1982)[61] which provides plasma ChE and RBC AChE data from a single oral dose (0.5 mg/kg). AChE/ChE activity (plasma and RBC) was monitored at 2, 6, 12, 24 hours, and 2, 3, 4, 8, 14, 22, 27, and 30 days post dose. Despite plasma ChE inhibition of 83-89% at the peak time of effect, no clinical signs of toxicity or inhibition of RBC ChE activity were observed. Kisicki *et al.* (1999) provides data from six human subjects exposed to chlorpyrifos in a gelatin capsule at dose levels of 0 (lactose-filled gelatin capsule, 2 control groups), 0.5, 1.0, or 2.0 mg/kg; RBC AChE activity was monitored at 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, 144, and 168 hours post dose. In the Kisicki *et al.* (1999) study RBC AChE inhibition changes were only seen in one person.

3.1.2. Dose Response Analysis for AChE Inhibition

In the 2011 preliminary risk assessment, the agency used typical reference dose and margin of exposure approaches where points of departure are derived from toxicology studies in laboratory animals and standard 10X factors for inter- and intra-species extrapolation. In the revised risk assessment, the agency is using a more sophisticated approach with the PBPK-PD model. As part of the 2008 SAP, the agency performed a comprehensive review of the literature on available AChE data across multiple lifestages, durations, and routes (FIFRA SAP, 2008b). This literature review was considered by the SAP at that time and provided part of the scientific rationale for the Panel's recommendation that the agency continue to use AChE data in the most sensitive lifestages for dose-response analysis. The literature review was updated prior to the 2011 preliminary risk assessment, the 2012 SAP review, and again for the 2014 revised risk assessment. Section 3.1.2.1 provides a summary of the benchmark dose (BMD) modeling on selected oral AChE studies conducted for the preliminary HHRA (2011). Section 3.1.2.2 provides additional dose-reponse data extracted from the 2008 draft issue paper reviewed by the FIFRA SAP.

3.1.2.1. Summary of BMD Modeling Results

[61] Nolan (1982) and Kisicki et al (1999) has been reviewed by the Human Studies Review Board (HSRB) and found to be ethically and scientifically conducted (http://www.epa.gov/osa/hsrb/files/june2009finalreport92609.pdf; http://www.epa.gov/osa/hsrb/files/meeting-materials/apr-13-14-2011/appendix1.pdf).

For the 2011 preliminary risk assessment, the Agency conducted BMD modeling on selected oral AChE studies[62]. These studies were selected based on the availability of at least two treatment groups in addition to a control group and those that showed good dose response. The agency has also focused on those studies where AChE was measured at or near the time of peak effect, typically within one to several hours after dosing. AChE data measured 24 hours or longer after dosing will underestimate the amount of AChE inhibition and are thus not appropriate for deriving points of departure. In addition, the studies used in the dose response assessment were selected as they represented a variety of ages, lifestages, and durations. The agency focused on those studies representing single and repeating dosing in post-natal exposure to rat ages (PND 10 and older); PND10 and older is considered to be approximately concordant with human postnatal exposure (Benjamins & McKhann, 1981; Dobbing & Smart, 1974). AChE data from adult laboratory animals (both pregnant and non-pregnant) from single dose and repeating dosing studies has also been collected. The focus has been on AChE data from rats as these are generally the most robust and sensitive studies. Data in fetuses for chlorpyrifos have not been evaluating using BMD techniques as these studies tend to be less sensitive than those in post-natal juveniles and adults. The agency has conducted BMD analysis on available data for blood (RBC), brain (whole or sections as appropriate), and peripheral (*i.e.*, heart) tissues.

Specifically, the agency used the decreasing exponential dose-response model similar to that used for the OP and *N*-methyl carbamate cumulative risk assessments and multiple risk assessments for individual AChE inhibiting pesticides (U.S. EPA, 2006, 2007). The use of this empirical dose-response procedure has been previously reviewed and supported by the FIFRA SAP on several occasions (2001, 2002, 2005a, 2005b, 2008bThe agency typically uses a 10% decrease in brain and peripheral ChE inhibition as the response level in deriving points of departure. This 10% response level is called the benchmark response level (BMR). This level has been shown to be a level which is protective for cholinergic outcomes for OPs and can also be reliably measured in most guideline experimental toxicology studies (U.S. EPA, 2002, 2006). The agency has calculated BMD_{10}s and $BMDL_{10}$s where the BMD_{10} is the estimated dose where AChE is inhibited by 10% compared to background and the $BMDL_{10}$ is the lower confidence bound on the BMD_{10}. As a matter of science policy, the agency uses the BMDL, not the BMD, for use as the point of departure since the BMDL accounts for variability of the data (U.S. EPA, 2012).

For the assessment of infants and children, the most robust studies are 1) the comparative cholinesterase[63] (CCA) study (MRID 48139301, also referred to as Marty *et al.*, 2012) which provides brain and RBC data in PND11 pups and 2) (Moser *et al.*, 2006) which provides data from brain and whole blood ChE data in PND17 pups. In both of these studies, the rat pups were dosed directly and ChE was measured at the time of peak effect. In addition, two studies (Timchalk *et al.*, 2006; Zheng *et al.*, 2000) provide supporting data. These two studies show AChE data in post-natal rats which are consistent with the comparative cholinesterase study and Moser *et al.* (2006). However, Timchalk et al. (2006) and Zheng *et al.* (2000) are not robust datasets for dose-response modeling (U.S. EPA, 2011). For the assessment of adults, single dosing AChE data from the comparative cholinesterase study (MRID 48139301) provide the

[62] Note: This appendix focuses on chlorpyrifos studies. See the 2011 preliminary HHRA for oxon results.
[63] Comparative cholinesterase (CCA) studies are specially designed studies in juvenile and adult rats which provide direct comparative ChE/AChE data for evaluating lifestage sensitivity. For OPs, data are typically collected from acute dosing (typically PND11, young adult) and from 11-days repeated dosing (PND11-21 and young adults).

most robust data for BMD analysis[64]. Tables App 1-1 and 1-2 provide a summary of the BMD results for the single dosing studies in post-natal rat pups and adult rats, respectively. Generally for the both the pups and the adults, the BMD and BMDL estimates for the blood measures are lower than those for the brain. In addition, the results from the pups are approximately 2-fold lower than those for adults (*i.e.*, when comparing the same compartment, administration vehicle).

Table App1-2. Results of BMD Modeling of Rat Pup Brain and Blood ChE Inhibition following a Single Oral Dose of Chlorpyrifos

Reference	Sex & Age at Dosing	Endpoint	BMD_{10} (mg/kg)	$BMDL_{10}$ (mg/kg)
Moser et al., 2006	Male PND 17 Gavage	Brain AChE	1.89	1.54
Moser et al., 2006	Male PND 17 Gavage	Whole Blood AChE	0.62	0.43
CCA Study (MRID 48139301)	Male PND 11 Gavage	Brain AChE	2.13	1.53
CCA Study (MRID 48139301)	Male PND 11 Gavage	RBC AChE	0.82	0.65
CCA Study (MRID 48139301)	Female PND 11 Gavage	Brain AChE	2.18	1.56
CCA Study (MRID 48139301)	Female PND 11 Gavage	RBC AChE	0.96	0.75
CCA Study (MRID 48139301)	Male PND 11 Milk	Brain AChE	4.4	2.4
CCA Study (MRID 48139301)	Male PND 11 Milk	RBC AChE	0.47	0.36
CCA Study (MRID 48139301)	Female PND 11 Milk	Brain AChE	1.42	0.91
CCA Study (MRID 48139301)	Female PND 11 Milk	RBC AChE	0.5	0.36

Table App1-3. Results of BMD Modeling of Adult Rat Brain and RBC AChE Inhibition following a Single Oral, Dose of Chlorpyrifos

Reference	Sex & Administration Method	Endpoint	BMD_{10} (mg/kg)	$BMDL_{10}$ (mg/kg)
CCA (MRID 48139301)	Female Gavage	Brain AChE	4.11	2.26
CCA (MRID 48139301)	Female Gavage	RBC AChE	1.9	1.2

[64] Only adult female rats were tested in MRID 48139301.

CCA (MRID 48139301)	Female 12 hr diet	Brain AChE	4.47	3.30
CCA (MRID 48139301)	Female 12 hr diet	RBC AChE	1.03	0.6

For BMD analysis of repeated dosing studies, in the preliminary risk assessment, the Agency selected the repeated dosing portions of the comparative cholinesterase study (MRID 48139301) in juvenile and non-pregnant adult rats;, the data from pregnant dams in the developmental neurotoxicity rat study (Maurissen *et al.*, 2000) (MRID 44556901) and the data from pregnant dams in the special cholinesterase study in which dams where administered chlorpyrifos from gestation day 6 through lactation day 10 (GD6 – LD10) (Mattsson *et al.*, 2000; MRID 44648101). Although the Zheng *et al.* (2000) data are not robust data for BMD modeling, the repeated dosing portion of this study provides additional supportive data for the findings from the comparative cholinesterase study in both pups and adults.

Table App1-4. Results of BMD Modeling of Pup Rat Brain and RBC AChE Inhibition following 11-days Repeated Oral, Gavage Doses of Chlorpyrifos

Reference	Sex & Age/Duration of Dosing	Endpoint	BMD_{10} (mg/kg/day)	$BMDL_{10}$ (mg/kg/day)
CCA (MRID 48139301)	Female PND 11-21 Gavage	Brain AChE	0.80	0.69
CCA (MRID 48139301)	Female PND 11-21 Gavage	RBC AChE	0.17	0.15
CCA (MRID 48139301)	Male PND 11-21 Gavage	Brain AChE	0.63	0.52
CCA (MRID 48139301)	Male PND 11-21 Gavage	RBC AChE	0.11	0.09

Table App1-5. Results of BMD Modeling of Adult, Female Rat Brain, RBC and Heart Cholinesterase Inhibition following Repeat Oral Doses of Chlorpyrifos

Reference	Sex, Age/Duration & Administration method	Endpoint	BMD_{10} (mg/kg/day)	$BMDL_{10}$ (mg/kg/day)
Dow (MRID 44556901; Maurissen et al., 2000)	Dams, GD6-20, Gavage	Brain AChE	0.65	0.54
Dow (MRID 44556901; Maurissen et al., 2000)	Dams, GD6-20, Gavage	RBC AChE	0.06[65]	0.03
Dow (MRID 44648101; Mattsson et al., 2000)	Dams, GD6-20, Gavage	Brain AChE	1.1	0.8
Dow (MRID 44648101; Mattsson et al., 2000)	Dams, GD6-20, Gavage	RBC AChE	0.14	0.08

[65] The BMD results from this dataset are not considered reliable due to high variability in the data & BMD values below the tested doses.

Dow (MRID 44648101; Mattsson et al., 2000)	Dams, GD6-20, Gavage	Heart ChE	0.85	0.22
Dow (MRID 44648101; Mattsson et al., 2000)	Dams, GD6-LD10 Gavage	Brain AChE	1.13	0.89
Dow (MRID 44648101; Mattsson et al., 2000)	Dams, GD6-LD10 Gavage	RBC AChE	0.050	0.044
Dow (MRID 44648101; Mattsson et al., 2000)	Dams, GD6-LD10 Gavage	Heart ChE	0.21	0.18
CCA (MRID 48139301)	Adult, non-pregnant 11 daysGavage	Brain AChE	1.03	0.95
CCA (MRID 48139301)	Adult, non-pregnant 11 days Gavage	RBC AChE	0.45	0.35
Subchronic Oral (MRID 40952801)	Dietary, non-pregnant female, Day 44	RBC AChE	0.180	0.117
Subchronic Oral (MRID 40952801)	Dietary, non-pregnant female, Day 91	RBC AChE	0.15	0.090

As shown in Tables App 1-3 and 1-4, there is no meaningful difference between BMD_{10}s and $BMDL_{10}$s from pups and adults in repeated dosing studies. This is not unexpected given that over the duration of the repeated dosing study, the metabolic system in pups is maturing closer to adult levels. Thus, it is not unexpected that as the metabolic system matures that the degree of sensitivity decreases. Typically, studies submitted for pesticide registration and most studies from the public literature only measure brain and/or blood ChEs. It is rare for data from peripheral tissues to be available for consideration. Chlorpyrifos is unique in that multiple studies are available which provide such peripheral data. It is notable that the BMDs and BMDLs for heart ChE are more sensitive than brain and closer in magnitude than those for RBC AChE. This finding supports the use of the blood measures for extrapolating risk.

There is good concordance in the estimates across the BMD_{10} and $BMDL_{10}$ estimates across the adult studies evaluated in this analysis. Notably, brain AChE estimates range from approximately 0.5 to 1 mg/kg/day and the RBC AChE estimates range from 0.03 to 0.35 mg/kg/day. In addition, for the OP cumulative risk assessment (U.S. EPA, 2006), the agency performed BMD modeling using a sophisticated meta-analysis approach on brain AChE data from adult, non-pregnant female and male rats from one subchronic oral toxicity study and two chronic oral toxicity studies (MRID nos. 40952801, 40952802, and 42172802). The BMD results reported in the OP cumulative risk assessment for chlorpyrifos are BMD_{10}s of 1.48 and 1.50 mg/kg/day and $BMDL_{10}$s of 1.26 and 1.27, mg/kg/day respectively in females and males. The values for brain AChE inhibition reported in the cumulative assessment from subchronic and chronic dosing are quite similar to those reported in Table 1-4 above for both pregnant and non-pregnant females and males in the selected studies and add further support to the analysis conducted for the preliminary risk assessment for chlorpyrifos.

3.1.2.2. Summary of Additional AChE/ChE Data (Extracted from USEPA, 2008)

Table App1-5. Summary of repeated studies evaluating gestational exposure to maternal rats and fetuses (Extracted from USEPA, 2008).

Study	Route (vehicle)	Time of exposure	Time of measurement post-dosing	Dose	Fetal inhibition	Maternal inhibition	Compartment
Mattsson et al., 1998, 2000	oral gavage (corn oil)	GD6-20	4 hrs	1 mg/kg/day	8% (NS)	10%/7% (p<0.02)	forebrain
					0%	12%/7% (NS)	hindbrain
					5% (NS)	87%/85% (p<0.02)	RBC
					4% (NS)	77%/60% (p<0.02)	plasma
					0%	49%/50% (p<0.02)	heart
Hoberman et al., 1998a,b; Maurissen et al., 2000	oral gavage (corn oil)	GD6-20	4-5 hrs	1 mg/kg/day	N/A	68.9%	plasma
					N/A	84.4%	RBC
					N/A	17.9%	brain
Mattsson et al., 1998, 2000	oral gavage (corn oil)	GD6-PND1	2 hrs	1 mg/kg/day	5% (NS)	6% (NS)	forebrain
					0%	6% (NS)	hindbrain
					0%	90% (p<0.02)	RBC
					5% (NS)	80% (p<0.02)	plasma
					2% (NS)	40% (p<0.02)	heart
Qiao et al., 2002	s.c. injection (DMSO)	GD 17-20	GD 21	1 mg/kg/day	3% (NS)	brainstem	N/A
					6% (NS)	forebrain	N/A

Table App1-6. Summary of acute studies evaluating post-natal exposure to juvenile rats (Extracted from USEPA, 2008).

Study	Route (vehicle)	Age	Time of measurement post-dosing[a]	Dose	Inhibition	Compartment
Dam et al. (2000)	s.c. injection (DMSO)	PND 1	2 hrs	1 mg/kg	70% (M); 25% (F)	brainstem
					80% (M); 10% (F)	cerebellum
					60% (M); 35% (F)	forebrain
Betancourt and Carr (2004)	oral gavage (corn oil)	PND 1	12 hrs	1.5 mg/kg	58%	forebrain
Timchalk et al. (2006)	oral gavage	PND 5	3 hrs	1 mg/kg	22.1%	brain
					45.7%	RBC
					62.1%	plasma
Zheng et al, 2000	oral gavage (peanut oil)	PND 7	4 hrs	1.5 mg/kg	17%	frontal cortex
					32%	RBC
					51%	plasma
Timchalk et al. (2006)	or al gavage	PND 12	6 hrs	1 mg/kg	5.2%	brain
					27.0%	RBC
					33.4%	plasma
Timchalk et al. (2006)	oral gavage	PND 17	24 hrs	1 mg/kg	2.1%	brain
					15%	RBC
					21.9%	plasma
Moser et al. 2006	oral gavage (corn oil)	PND17	4.5 hrs	0.5 mg/kg	0%	brain
				2 mg/kg	10%	
				0.5 mg/kg	10%	whole blood
				2 mg/kg	40%	

a. Reported time of peak effect

Table App1-7. Summary of repeated studies evaluating post-natal exposure to juvenile rats (Extracted from USEPA, 2008).

Study	Route (vehicle)	Time of exposure	Time of measurement post-dosing	Dose	Inhibition	Compartment
Guo-Ross et al. (2007)	oral gavage (corn oil)	PND 1-4	4 hrs	1 mg/kg/day	25%	brain
				1.5 mg/kg/day	45%	
Betancourt and Carr (2004)	oral gavage	PND 1-3	24 hrs	1.5 mg/kg/day	27%	forebrain
Song et al. (1997)	s.c. injection (DMSO)	PND 1-4	24 hrs	1 mg/kg/day	24%	brainstem
Richardson and Chambers (2005)	oral gavage (corn oil)	PND 1-6	6 hrs	1.5 mg/kg/day	49%	brain (excluding cerebellum and medulla-pons)
Betancourt and Carr (2004)	oral gavage	PND 1-6	24 hrs	1.5 mg/kg/day	28%	forebrain
Betancourt and Carr (2004)	oral gavage	PND1-11	24 hrs	1.5 mg/kg/day	None	forebrain
Richardson and Chambers (2005)	oral gavage (corn oil)	PND 1- 12	12 hrs	1.5 mg/kg/day	43%	brain (excluding cerebellum and medulla-pons)
Richardson and Chambers (2005)	oral gavage (corn oil)	PND 1-21	24 hrs	1.5 mg/kg/day	36%	brain (excluding cerebellum and medulla-pons)
Zheng et al (2000)	oral gavage (peanut oil)	PND7-20	4 hrs	1.5 mg/kg/day	42%	frontal cortex
					57%	RBC
					59%	plasma

3.2. Adverse Outcome Pathway: Neurodevelopmental Outcomes

With respect to modes of action/adverse outcome pathways leading to neurodevelopmental effects, at the present time, there is no established series of causal key events at a biological level of organization relevant to the risk assessment (*i.e.*, adverse neurodevelopmental effects from gestational and/or postnatal exposure). For the 2014 revised HHRA, the agency conducted an updated literature review on the experimental toxicology studies for chlorpyrifos (Appendix 11) for studies published since the 2012 SAP meeting. Some of the new studies since 2012 have been integrated in this section. Despite the newest studies, the agency does not believe that any of the current lines of research support a coherent set of key events and that much work remains to elucidate the modes of action and adverse outcome pathways of chlorpyrifos toxicity.

Even though a rigorous demonstration of an adverse outcome pathway for chlorpyrifos has not been constructed at this time, there are multiple studies from numerous laboratories that show that exposure to a variety of OPs (including chlorpyrifos, other pesticides, nerve gases) during the developmental period affects events in nervous system development (see below). These studies conducted to date generally report a correlation between OP exposure and a tested effect without consideration of temporal concordance (*i.e.*, the sequence of biological events leading to effects) and quantitative linkages (*i.e.*, degree of change that results in neurodevelopmental consequences). Some of this experimental work specific to chlorpyrifos is summarized below, and may be shown in the future actually to be steps in the same adverse outcome pathways and not discreet pathways. However, at this time, the experimental studies below do not provide all the information needed to demonstrate linkages among initiating event(s), subsequent events at the molecular and cellular level, and the adverse outcome of interest and are thus described separately.

3.2.1. Biologically Plausible Toxicity Pathways Leading to Neurodevelopmental Outcomes

3.2.1.1. 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, however, suggest that AChE can also serve as a morphogen, influencing the growth of cells during neurodevelopment. Alterations in the expression or structure of the AChE protein can disrupt various aspects of neuronal differentiation and growth. Because chlorpyrifos can interact with AChE, perturbation of this morphogenic activity represents a plausible adverse outcome pathway leading to developmental neurotoxicity. Additional for the plausibility of this adverse outcome pathways is found in the chlorpyrifos developmental neurotoxicity study (MRID 44556901) where PND66 pups exposed during gestation and lactation at the 1 and 5 mg/kg/day groups exhibited significant dose- and treatment-related decreases in measurements of the parietal cortex in female offspring.

One of the first indications that AChE had roles other than the termination of cholinergic

neurotransmission was the timing and distribution of its expression in the developing nervous system. In certain brain regions containing non-cholinergic neurons and few cholinergic synapses, AChE levels are still high. In addition, AChE is highly expressed throughout the brain during periods of active axonal outgrowth in the absence of other cholinergic markers and before synaptic connections are made (Bigbee *et al.*, 1999; Brimijoin & Koenigsberger, 1999). For example, AChE levels are high during early cerebellar development while there is very little evidence for the presence of cholinergic cells or cholinergic neurotransmission (Appleyard & Jahnsen, 1992; Parvari, 1983). A second line of evidence for a morphogenic role arose after the cloning and sequencing of rodent and human AChE. Surprising sequence homologies were found with a family of proteins lacking a catalytically active esterase site but pocessing a similar extacellular domain. Several of the homologs of AChE are extracellular matrix components essential to neuronal adhesion, axon guidance, and synapse formation (Brimijoin & Koenigsberger, 1999; Grisaru *et al.*, 1999). Thus, the sequence of AChE is similar to other proteins which have a morphogenic role during the development of the nervous system. Finally, experimental evidence indicates that manipulation of AChE levels or activity can influence neuronal growth *in vitro*. Studies in cultured neuroblastoma cells (Koenigsberger *et al.*, 1997) or PC12 cells (Grifman *et al.*, 1998) have shown that antisense suppression of AChE decreased neurite outgrowth, and that neurite outgrowth could be rescued or increased by transfection with sense AChE. In cultures of rat dorsal root ganglion cultures, treatment with an antibody against AChE (which did not inhibit the catalytic activity) decreased neurite outgrowth, and neurite outgrowth was restored upon removal of the antibody (Bigbee *et al.*, 1999). Expression in frog embyros of a recombinant human AChE lacking the ability to hydrolyze acetylcholine resulted in increased neurite outgrowth from cultured spinal neurons (Sternfeld *et al.*, 1998). Thus, the catalytic ability of AChE does not seem to be required for its morphogenic properties.

Studies using pharmacologic inhibition of AChE activity to alter neurite outgrowth also suggest a dissociation of the catalytic and morphogenic activities. Several laboratories using different *in vitro* systems have demonstrated that some potent AChE inhibitors including BW284c51 supress neurite outgrowth while other, equally potent AChE inhibitors like ecothiophate do not (Bigbee *et al.*, 1999; Koenigsberger, *et al.*, 1997; Layer *et al.*, 1993). These differential effects may be related to the degree of chemical binding to separate sites on the AChE enzyme. There are at least two sites that can bind inhibitors: the catalytic (active) site and the peripheral anionic site (Grisaru *et al.*, 1999). It has been suggested that inhibitors that bind directly to the peripheral anionic site, or that bind to the catalytic site in such a way that changes the conformation of the peripheral anionic site, will alter the morphogenic function of AChE (Bigbee *et al.*, 1999; Yang *et al.*, 2008). Thus inhibitors that bind only to the catalytic site (*e.g.* edrophonium, tacrine) did not affect neurite outgrowth, while ligands that bind to the peripheral anionic site (propidium, gallamine) or both sites (BW284c51) inhibited neurite outgrowth (Koenigsberger *et al.*, 1997; Munoz *et al.*, 1999). At least one study indicates that chlorpyrifos oxon can bind at both sites (Kousba *et al.*, 2004).

Data showing a potential action of chlorpyrifos on the morphogenic function of AChE are derived primarily from *in vitro* studies. Chlorpyrifos (as well as the oxon and TCPy) has been examined for effects on neurite outgrowth using a number of cell lines and in primary neuronal cultures. In some cases concurrent assessments of cholinesterase inhibition or cytotoxicity were performed. Li and Cassida (1998) examined the effect of chlorpyrifos oxon on neurite outgrowth

in the PC12 cell line. In this study cells were exposed prior to, but not during the active phase of neurite growth, which was assessed after 5 days. Chlorpyrifos oxon inhibited neurite outgrowth by 50%, but only at a relatively high concentration (200 uM) that was both cytotoxic and decreased cholinesterase activity. Flaskos *et al.* (2011) showed that neurite outgrowth in differentiating N2a cells was observed at μM concentrations of the oxon which also resulted in inhibition of AChE in the same cell line. Sachana *et al.* (2005, 2008) examined the effect of 3 uM chlorpyrifos on the initiation of neurite growth in mouse N2a neuroblastoma cells. The number of cells exhibiting neurites after 4 or 8 hours of exposure (considered as a measure of cell differentiation) was decreased by approximately 50% in the absense of cytotoxicity. Cholinesterase activity was not measured. Axelrad *et al.* (2002) exposed mouse NB2a cells to chlorpyrifos for 24 hr and directly measured neurite length. Chlorpyrifos inhibited neurite outgrowth by 50% at 25 uM. Again, cholinesterase activity was not measured. While the studies cited above suggested that chlorpyrifos and chlorpyrifos oxon could affect measures related to neuronal differentiation and neurite growth, they were not specifically designed to examine the morphogenic role of AChE.

Using PC12 cells, Das and Barone (1999) examined the concentration-related effects of chlorpyrifos, chlorpyrifos oxon, and TCPy on both neurite outgrowth and cholinesterase inhibition. Exposure to chlorpyrifos for 24 hr inhibited neurite outgrowth at a concentration (3 uM) 10-fold below that which inhibited cholinesterase activity, while chlorpyrifos oxon inhibited both measures at equivalent concentrations (1 nM). TCPy, which is inactive against AChE, inhibited neurite growth at 5 uM. Similar studies of chlorpyrifos and its metabolites were performed in a series of experiments in the Lein laboratory using primary neuronal cultures derived directly from the mammalian nervous system. Using rat sympathetic neurons, Howard *et al.* (2005) showed that 24 hr exposure to chlorpyrifos and chlorpyrifos oxon decreased axonal outgrowth at concentrations (0.001 uM and 0.001 nM, respectively) well below the concentrations that inhibited AChE activity (1 uM and 1 nM). In the same study chlorpyrifos, chlorpyrifos oxon and TCPy enhanced dendrite outgrowth. A follow-up study from the same laboratory used sensory neurons from the dorsal root ganglion, which extend only axons (Yang, et al., 2008). Similar results were observed, with both chlorpyrifos and chlorpyrifos oxon decreasing axonal outgrowth at concentrations below those that inhibited AChE activity. To establish whether the target of chlorpyrifos and chlorpyrifos oxon was AChE or some other molecule, Yang *et al.* (2008) repeated the experiments in cultures from AChE-null animals. Chlorpyrifos and chlorpyrifos oxon had no effect in the AChE-null cultures, suggesting that inhibition of neurite outgrowth required AChE. These later three studies provide the most convincing evidence for selective inhibition of the morphogenic activity of AChE. It is not yet clear whether the effects are a direct action of chlorpyrifos on AChE, or the result of *in vitro* conversion to the active oxon form. It should be mentioned that studies from the Slotkin laboratory suggest an effect of chlorpyrifos on neurite outgrowth *in vitro* (Song *et al.*, 1998). In those experiments, however, neurite outgrowth is inferred from biochemical measurements and cholinesterase activity is not assessed concurrently.

Testing the hypothesis that exposure to chlorpyrifos (or any chemical) can inhibit the morphogenic function of AChE *in vivo* and alter brain development is difficult. Neuronal differentiation and the subsequent development of axonal and dendritic networks occurs in a temporal- and region-specific manner. In the absence of prior information extensive studies

would be required to survey brain morphology during both the prenatal and postnatal period. In addition, the methods needed to assess the morphology of axonal and dendritic growth *in vivo* and detect potentially subtle chemical-induced changes are tedious and time consuming. Nevertheless, some attempts have been made to detect chlorpyrifos-induced changes in neurite growth *in vivo*. In particular, several laboratories have examined the effect of chlorpyrifos and chlorpyrifos oxon on neuronal morphogenesis in developing zebrafish. Jacobson *et al.* (2010) exposed zebrafish embryos at 3 hr post fertilization to 300 nM chlorpyrifos oxon. When examined 24 hr later (1 day post fertilization), AChE was inhibited by 50%. Gross morphology was only slightly affected and muscular development (including the neuromuscular junction) was normal; however, there was a decrease in the number of Rohon-Beard sensory neurons accompanied by abnormal extension of their axons. As a follow up to this study and to their *in vitro* work described above, Yang *et al.* (2011) exposed zebrafish embryos from 24 to 72 hr post fertilization to chlorpyrifos and chlorpyrifos oxon. Chlorpyrifos had no effect, but chlorpyrifos oxon significantly decreased AChE activity (30 nM), decreased touch-induced swimming (100 nM) and inhibited axon growth from both sensory neurons and motor neurons (100 – 1000 nM). These two studies show that exposure to chlorpyrifos oxon in an intact, developing organism can alter neurite outgrowth. However, because catalytic activity was also inhibited, it is not clear that this effect was solely due to disruption of the morphogenic role of AChE.

As noted above, neurite outgrowth has been assessed indirectly *in vitro*. This method has also been applied *in vivo* in mammals. Qiao *et al.* (2003) exposed rats to 1 or 5 mg/kg chlorpyrifos on gestational days 17-20. In a previous study from the same laboratory this exposure paradigm resulted in a non-significant 5% decrease in AChE activity after 1 mg/kg and a significant 50% decrease in AChE activity after 5 mg/kg when assessed 24 hr after the last dose (Qiao *et al.*, 2002). The ratio of membrane protein to total protein in several brain regions was assessed as a surrogate index of neurite outgrowth. The results indicated that there was no effect of treatment during early postnatal development (PND 4-21), but that both doses decreased this ratio on PND 30 and 60. While the authors interpret this biochemical measurement as a decrease in neurite growth, there has not been data presented to correlate the changes in the membrane protein to total protein ratio with actual quantification of neurite length. In addition, no effects were observed early in development when axons and dendrite growth is high.

In summary, while perturbation of the morphogenic activity of AChE is a plausible adverse outcome pathway for chlorpyrifos, a number of questions remain. There is substantial evidence for a morphogenic role of AChE in nervous system development distinct from its role as an esterase to hydrolyze acetylcholine. *In vitro* evidence suggests that AChE can regulate neurite outgrowth, and that cholinesterase inhibitors (including chlorpyrifos and its metabolites) can interfere with this process at concentrations that do not inhibit the esterase activity. 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, it has not been demonstrated that this effect is due to alteration of the morphogenic function of AChE versus other potential mechanisms.

3.2.1.2. 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 cholinesterase inhibitors can affect cholinergic signaling by inhibition of the catalytic activity of AChE and subsequent increase in acetylcholine, some, 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. Volpe *et al.* (1985) reported that nanomolar concentrations of several OPs decreased binding of quinuclidinyl benzilate (QNB), which binds equally to all five subtypes. This effect was noncompetitive and occurred at only a small fraction of the total QNB binding sites. A further study from the same laboratory proposed these receptor subtypes to be m2 and/or m3 (Katz & Marquis, 1989). Bakry *et al.* (1988) found that a number of OP nerve agents and an OP therapeutic agent inhibited QNB binding to rat brain membranes with low potency, but competitively inhibited binding of cis-methyldioxolane (CD) with high potency. In rat cardiac tissue, OPs also competitively inhibited CD binding with very high potency (Silveira *et al.*, 1990). CD is a muscarinic agonist that binds to the high affinity state of the m2 receptor in mammalian brain and heart (Closse *et al.*, 1987; Vickroy *et al.*, 1984; Watson, 1986). Subsequent research using Chinese hamster ovary (CHO) cells transfected with cDNA for the five distinct genes for muscarinic receptors (ml-m5) has identified the CD binding site as the high affinity state (GDP bound G-protein) of the m2 receptor (Huff & Abou-Donia, 1994). In light of these findings, Ward et al. (1993) examined the relationship between cholinesterase inhibition and direct binding to muscarinic receptors for a series of OPs and their active "oxon" metabolites. The results indicated a strong correlation between anticholinesterase 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.

Further studies determined whether binding of OPs to the m2 receptor had functional consequences on downstream second messenger signaling. The m2 and m4 receptors are coupled to an inhibitory G-protein (G_i) and activation inhibits adenylate cyclase and decreases cAMP formation. In contrast, m1, m3, and m5 receptors increase PI hydrolysis via the stimulatory G-protein G_p (Lameh *et al.*, 1990). Jett et al. (1991) reported that paraoxon could inhibit both CD binding and cAMP formation in rat striatum. The effects of paraoxon were similar to the classical muscarinic agonist carbachol, and were blocked by the muscarinic antagonist atropine. Similar results were observed in rat frontal cortex for paraoxon and malaoxon (Ward & Mundy, 1996). In addition, Ward and Mundy (1996) observed that the OPs had no effect on PI

hydrolysis. These data suggest that OPs can act as agonists at the m2 receptor (but not the m1, m3, or m5 receptor) and decrease cAMP formation. Huff *et al.* (1994) extended these findings to chlorpyrifos oxon, which had an IC_{50} for inhibition of cAMP of 155 nM. Unlike the other OPs, however, the effects of chlorpyrifos oxon were not completely blocked by atropine; an observation confirmed by Ward and Mundy (1996). Thus, like other OPs chlorpyrifos oxon can act directly as an agonist at the m2 receptor, but may also act at another site downstream of the receptor to inhibit adenylate cyclase.

Cholinergic receptor signaling has been shown to be involved in apoptosis, cell proliferation and neuronal differentiation (Resende & Adhikari, 2009). While the m2 receptor subtype is widely expressed in proliferating neuroepithelial cells in the ventricular zone of embryonic brain (Ma *et al.*, 2004), evidence for a specific role of in the modulation of neurodevelopment is derived primarily from *in vitro* studies. Neural precursor cells derived from embryonic rat cortex expressed the m2 receptor, and exposure to muscarinic agonists increased cell proliferation and enhanced differentiation into neurons (Ma *et al.*, 2000). Using a P19 embryonic cell line as a model for neurogenesis, Resende et al. (2008) used pharmacologic agonists and antagonists to demonstrate the ability of m2 receptors to induce cell differentiation. The m2 receptor is highly expressed in developing cells of the dorsal root ganglia, and is thought to regulate both neuronal and non neuronal cell differentiation (Biagioni *et al.*, 2000; Tata *et al.*, 2003). Another line of evidence for involvement of m2 receptor signaling in neurodevelopment is the role of cAMP in neurite outgrowth. Studies in both cell lines and primary neuronal cultures show that activation of adenylate cyclase and subsequent formation of cAMP stimulate neurite outgrowth (Kamei & Tsang, 2003; Mattson *et al.*, 1988), while inhibition of adenylate cyclase decreases neurite outgrowth (Tam *et al.*, 2006; Wong *et al.*, 1991). Thus, *in vitro* evidence supports a role of m2 receptor signaling in neurodevelopment at the cellular level. However, evidence showing that manipulation of m2 receptors can alter brain development *in vivo* is lacking.

Because they can also influence neurodevelopment, it should be noted that there are several studies demonstrating that OPs including chlorpyrifos and chlorpyrifos oxon can bind directly to and desensitize nicotinic receptors (Katz *et al.*, 1997; Smulders *et al.*, 2004). This binding, however, occurs at relatively high concentrations (5 – 30 uM) and has not been demonstrated in neuronal tissue.

In summary, *in vitro* studies have shown that chlorpyrifos oxon can bind to and activate m2 receptors at levels similar to those that inhibit AChE activity. Other work has shown that m2 receptor signaling can regulate various aspects of neurodevelopment. 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.

3.2.1.3. 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 reasoning, the EC system represents a possible adverse outcome pathway for developmental effects of chlorpyrifos.

The EC system includes inhibitory G-protein-coupled receptors (CB1 and CB2), endogenous ligands (2-arachidonoylglycerol, or 2-AG, and anadamide, or AEA), and hydrolases (monoacylglycerol, or MAG, and fatty acid amide hydrolase, or FAAH) that end the receptor actions of these ligands. CB1 receptors are predominant in certain brain regions, and the EC system modulates neuronal transmission and is involved in several physiological processes including appetite, pain sensation, mood, and cognition (Wilson & Nicoll, 2002). Emerging lines of research indicate that during development, this system plays a major role in controlling the morphological and functional specification of neurons (*e.g.*, Fride, 2008; Harkany *et al.*, 2007; Harkany *et al.*, 2008a, b; reviewed in Campolongo *et al.*, 2009). Its involvement in neurogenesis, proliferation, and axon guidance suggests a role in overall neuronal connectivity.

There are epidemiological data on neurodevelopmental outcomes following prenatal exposure to cannabinoids such as cannabis and THC through maternal drug abuse, but interpretations are often somewhat difficult given co-exposures to other neurotoxicants (*e.g.*, ethanol). Despite limitations, an overall picture has emerged of long-lasting impaired cognitive processes, including attention and problem-solving deficits, as well as anxiety and depressive symptoms in humans (*e.g.*, Campolongo *et al.*, 2009; Fried & Smith, 2001; Jutras-Aswad *et al.*, 2009; Trezza *et al.*, 2008). In addition, animal models have indicated various changes in neurotransmission of catecholaminergic and indolaminergic systems, ontogeny of motor function, and cognitive function in adults developmentally exposed to cannabis as well as other CB1 agonists (*e.g.*, WIN55,212-2) (reviewed in Campolongo *et al.*, 2011; Marco *et al.*, 2009). This supports the possibility that other chemicals, unrelated to cannabis, that also act on the EC system early in development could lead to long-term neurological dysfunction. Despite the number of studies in this area, however, the specific responsible cellular responses have not been delineated, and most studies have not evaluated basic dose-response such as correlations of the degree of receptor binding with any structural or functional outcomes.

In addition to drugs of abuse (*e.g.*, cannabis), there are several classes of chemicals that act on the EC system, including organosulfonyl fluorides and some OPs (*e.g.*, Casida & Quistad, 2004; Segall *et al.*, 2003). OPs phosphorylate and thereby block the action of the hydrolases FAAH and MAG. This inhibition of the hydrolysis of endogenous cannabinoids could prolong their actions on the receptor, which inhibits several neurotransmitter systems, including ACh. OPs also bind the CB1 receptor and block the binding of specific receptor ligands. The OPs do not bind at the agonist site, however, and may have no inherent agonist or antagonists consequences; however, this has not been adequately studied. Thus, prolongation of the ligand residence time, plus blockade of ligand binding at the receptor, could lead to downstream modulation of cholinergic activity and signaling, separate from AChE inhibition. It is unclear which actions could predominate *in vivo*.

Casida and colleagues have produced several papers relating FAAH inhibition to that of AChE and neurotoxic esterase (NTE), and considered its involvement in acute cholinergic, intermediate, and delayed toxicity syndromes (*e.g.*, Casida & Quistad, 2004; Quistad et al , 2006; Quistad et al, 2002a, b; Quistad et al 2001; Segall, et al., 2003). Several studies have included chlorpyrifos. They have reported that *in vitro* chlorpyrifos oxon inhibits FAAH and MAG with IC50s of 40 nM and 34 nM, respectively, compared to an AChE IC50 of 19 nM. Other OPs (paraoxon, dichlorvos, diazoxon) have higher hydrolase inhibitory actions *in vitro* (IC50s 540-14,000 nM). *In vitro* receptor binding assays of CB1 agonists reveal an IC50 of 14 nM for chlorpyrifos oxon, and higher IC50s (>64 nM) for other OPs (chlorpyrifos methyl oxon, diazoxon, dichlorvos).

Casida's group has also dosed adult mice with OPs and evaluated motor behavior and cholinergic signs, followed by *ex vivo* biochemical assays. These studies report inhibition of FAAH activity and CB1 binding only at highly toxic doses of chlorpyrifos and/or chlorpyrifos oxon. Chlorpyrifos dose-response differs somewhat across studies, but in general, FAAH is inhibited more than MAG and AChE, and CB1 binding is moderately inhibited, but only at doses producing cholinergic signs. Chlorpyrifos inhibition of FAAH does not correlate with the hypomotility (akinesia, rigidity) produced by the EC agonist amandamide. Furthermore, chlorpyrifos does not potentiate the effects of amandamide, suggesting that it does not appear to have functional impacts in the EC system. In contrast, treatment with other chemicals that do produce marked (>76%) FAAH inhibition does indeed potentiate anandamide effects in mice; however, chlorpyrifos doses that would be required to produce this level of FAAH inhibition are likely to be lethal. This line of research has led Casida and colleagues to conclude that chlorpyrifos actions on the EC system *in vivo* are less important to its acute toxicity than AChE inhibition.

A series of studies from the laboratories of Pope and colleagues has evaluated the influence of CB1 agonists administered *in vivo* on the signs of toxicity produced by several OPs (Baireddy et al, 2011; Nallapaneni et al, 2006, 2008; Pope et al, 2010; Wright et al, 2010). They reported that CB1 agonists and other cannabinomimetics block the acute signs of exposure to paraoxon or DFP (chlorpyrifos was not tested). Studies with chlorpyrifos show increased extracellular levels of 2-AG but not amandamide in hippocampus. Somewhat different conclusions were drawn, however, in a paper showing that the toxicity and AChE inhibition produced by an acute dose of chlorpyrifos is not altered in mice without the CB1 receptor (knockout) compared to their wild type littermates. Pope et al. also measured chlorpyrifos oxon-induced ACh release from these tissues and saw only a small difference. These somewhat contradictory results suggest that potential EC involvement in the acute toxicity of certain OPs is still uncertain.

A limited number of studies have examined chlorpyrifos effects on the EC system in developing animals. Carr et al. (2011) dosed preweanling rats for 5 days (1-5 mg/kg/day, p.o.) and took brain tissue 4 hr after the last dose. The lowest dose produced 40% inhibition of FAAH, 14% inhibition of MAG, and only 18% inhibition of AChE; in addition, the highest dose eliminated FAAH activity and produced about 52-55% inhibition of AChE. This suggests a greater sensitivity of the EC system, at least in terms of the hydrolase compared to AChE activity, in the pups. Carr et al (2013) confirmed the findings in 2011 paper and showed that FAAH recovers

more faster than brain AChE and MAGL. Another 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. Additional studies along these lines are needed.

In summary, most of the research in this area has evaluated acute responses in the EC system, even though such changes during a critical period of development could have long-term consequences. While some steps along a chlorpyrifos adverse outcome pathway for developmental neurotoxicity are plausible, there are remaining questions as to whether the EC system is sufficiently sensitive to low doses of chlorpyrifos to alter its function during development. Despite the increasing understanding of the EC system, there are no data during development on 1) inhibition of the CB1 receptor at any time during development (animal studies used only agonists); 2) inhibition of FAAH during development; 3) time-course and dose-response of OPs *in vivo* compared to AChE inhibition; and 4) whether these actions on the receptors and/or hydrolases actually change EC signaling to a point that would impact downstream nervous system development.

3.2.1.4. 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 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.

Both chlorpyrifos and chlorpyrifos oxon have been shown to induce oxidative stress in cells *in vitro* (Ojha and Srivastava, 2014; Ki et al, 2013; Chiapella et al, 2013). Lee et al (2012) exposed PC12 cells to chlorpyrifos and suggest that neuronal cell death is partly due to MAPK activation via ROS generation. In addition, a series of studies using PC12 cells were performed by the Slotkin laboratory. Crumpton et al. (2000) reported that acute exposure of undifferentiated PC12 cells to 1.5 – 150 uM chlorpyrifos for 10 min resulted in a rapid increase in ROS production. Exposure to 30 uM chlorpyrifos oxon for 10 min had no effect. Consistent with the generation of ROS, further studies demonstrated an increase in oxidative damage (lipid peroxidation) in both undifferentiated and differentiated PC12 cells after exposure to chlorpyrifos (3 – 100 uM) for 24 hr (Qiao, et al, 2005). Finally, Slotkin and Seidler (2009) used microarrays to examine the expression of genes involved in the response to oxidative stress after exposure of PC12 cells to 30 uM chlorpyrifos for 24 or 72 hr. They observed a significant effect on transcription of genes including glutathione peroxidase, glutathione-S-transferase, and superoxide dismutase, which was greater in differentiated cells compared to undifferentiated

cells. PC12 cells were also used by a separate group of researchers who reported that very high concentrations of chlorpyrifos (1.4 – 14 mM) increased ROS production during a 5 hr exposure period (Geter et al., 2008). ROS production has also been reported in other neuronal cell types. Giodano et al. (2007) examined the effects of chlorpyrifos and chlorpyrifos oxon on ROS levels and lipid peroxidation in primary cultures of cerebellar granule cells from mice. Exposure to 1 uM chlorpyrifos for 60 min increased both ROS and lipid peroxidation, and a similar response was observed with 1 uM chlorpyrifos oxon. Saulsbury et al. (2009) examined oxidative stress in oligodendrocyte progenitor cells, which are the precursors to glial cell types in the brain. Exposure to 15 – 120 uM chlorpyrifos for 3 hr resulted in a significant increase in ROS production. These *in vitro* studies suggest that exposure of cells to the micromolar concentrations of the parent compound chlorpyrifos (which is not a potent AChE inhibitor) can result in oxidative stress. The active metabolite chlorpyrifos oxon was found to be active in cerebellar granule cells but not in PC12 cells. Inhibition of AChE was not determined in these studies.

A limited number of studies have examined the oxidative stress response in rat brain after *in vivo* exposure to chlorpyrifos. Bagchi *et al.* (1995) dosed adult female rats p.o. twice with 41 mg/kg chlorpyrifos, once at 0 hr and once at 21 hr. Animals were sacrificed at 24 hr. Both ROS production and lipid peroxidation were increased in homogenates from whole brain. In a follow-up study, the same dosing paradigm resulted in an up-regulation of heat shock protein, which in some cases can be a response to oxidative stress (Bagchi *et al.*, 1996). There are two studies of oxidative stress in response to chlorpyrifos exposure in developing animals. Slotkin et al., (2005) used three dosing paradigms: s.c. exposure of pregnant rats on GD 17-20 followed by collection of the fetal brain on GD 21, s.c. exposure of rat pups on PND 1-4 followed by brain collection on PND 5, or s.c. exposure of rat pups on PND 11-14 followed by brain collection on PND 15. Lipid peroxidation was measured in brain regions including the brainstem, forebrain and cerebellum. There was a 20% increase in lipid peroxidation in the brain only after exposure to 5 mg/kg chlorpyrifos during the later postnatal period (PND 11-14) and only in males. AChE inhibition was not assessed. Ray et al. (2010) dosed 7 day old rat pups p.o. with 0.1, 0.5, 1, or 2 mg/kg chlorpyrifos and examined gene expression in the forebrain 24 hr later using microarrays. Only the highest dose inhibited AChE (25%). Gene expression changes were observed at all doses, and 'oxidative stress' was one of the six canonical pathways that was significantly affected. In a recent study, Ambali and Ayo (2012) showed that *in vivo* treatment to adult rats with vitamin C and chlorpyrifos together mediated the muscle ChE inhibition and neurobehavioral effects of chlorpyrifos following 17 weeks of gavage dosing at 10.6 mg/kg/day. Kalender *et al.* (2012) also showed that chlorpyrifos effects at a dose of 5 mg/kg/day could be modulated by co-treatment with catechin and quercetin. In recent papers (Basha and Poojary, 2012a, b; Poojary and Basha, 2012), it is hypothesized that cold temperatures induces oxidative stress which increases the toxicity from chlorpyrifos. The oral doses used by these investigators are relatively high; replication of these findings is needed by another laboratory, particularly at lower doses, to provide additional credence to the hypothesis.

Data from both *in vitro* studies with neuronal cells (including neural precursors) and *in vivo* studies in developing brain demonstrate 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. 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). Thus, while there is the potential for initiation of an adverse outcome pathway via induction of oxidative stress, it has yet to be demonstrated *in vivo* that treatments preventing the induction of oxidative stress can ameliorate the developmental neurotoxicity of chlorpyrifos.

3.2.1.5. Serotonergic system

The serotonergic system (reviewed in Rho & Storey, 2001; *Serotonin Receptors in Neurobiology*, 2007) includes 7 distinct families of serotonin receptors regulating diverse functions such as neuronal excitability, appetite control, memory, learning, circadian rhythm, sexual behavior, anxiety, aggression, and respiration. While the majority of the receptors are either negatively or positively coupled to adenylate cyclase, the 5-HT_3 serotonergic receptor is a ligand-gated ion channel. Serotonin receptors are distributed throughout the brain and body. Synthesized from L-tryptophan, the rate limiting step in serotonin biosynthesis is catalyzed by tryptophan hydroxylase. After release, the action of serotonin is primarily terminated by reuptake into the presynaptic terminal via monoamine transporters. Various drugs inhibit the reuptake of serotonin, effectively increasing the available synaptic concentrations and actions: Ecstasy, amphetamine, cocaine, tricyclic antidepressants and the newer antidepressants: SSRIs (selective serotonin reuptake inhibitors).

Beyond its classical neurotransmitter actions (described above), 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 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.

Perturbation of the development of the serotonergic system in laboratory animals causes permanent changes in the neurochemical, behavioral and structural aspects of the adult nervous system (reviewed in Borue et al, 2007; Daubert & Condron, 2010; Frederick & Stanwood, 2009;

Oberlander et al, 2009). Moreover, it is generally recognized that the development stage of serotonergic perturbation determines the developmental outcome (reviewed in Oberlander, et al., 2009; Olivier et al, 2011), meaning that the time at which the developing animal experienced the insult to the serotonergic nervous system would determine the degree and the result. The review by Daubert and Condron (2010) includes a table summarizing many laboratory studies where different aspects of the serotonergic system were manipulated (either through knock-out, conditional knock-out, or chemical treatment) during development accompanied by the behavioral and morphological results of that manipulation. For example, SERT (serotonin reuptake receptor) knock-out mice had alterations in sensory patterning, sleep pattern abnormalities, and long-term behavioral deficits (Li, 2006), and animals treated with SSRIs during development show many similarities to the SERT knock-out mice. Developmental exposure to SSRIs in mice produced permanent (adulthood) effects on behavior (Lisboa et al, 2007; Noorlander et al., 2008) and brain chemistry (Noorlander, et al., 2008) and changes in morphology in the hippocampus (Zheng et al., 2011) and somatosensory cortex (Lee, 2009).

Because of the extensive usage of SSRI antidepressants in the human population, there is a rich literature SSRI effects during pregnancy and lactation (reviewed in Alwan & Friedman, 2009; Ellfolk & Malm, 2010; Gentile, 2005; Oberlander, et al., 2009; Olivier, et al., 2011; Tuccori et al., 2009). The main effects of SSRI usage are on cardiac development, and while there is some support for concluding that actions on the serotonergic system early in development can lead to long-term neurological dysfunction, there are only few studies that have studied this relationship in humans in detail. A recent study has noted that children born to mothers who used SSRIs have increased likelihood for social-behavioral abnormalities (Klinger et al., 2011).

There are numerous studies of the effects of perinatal chlorpyrifos administration on the patency of the serotonergic system. Endpoints in various brain regions include serotonin levels (Aldridge et al, 2005a; Slotkin & Seidler, 2007b, 2007c), serotonin turnover (Aldridgeet al., 2005a; Slotkin & Seidler, 2007b, 2007c), serotonin receptor levels (Aldridge et al, 2005b; Aldridge et al, 2003; Aldridge et al, 2004; Slotkin & Seidler, 2005), serotonin reuptake receptor levels (Aldridge, et al., 2005b; Aldridge, et al., 2003; Aldridge, et al., 2004; Slotkin & Seidler, 2005), serotonin elicited second messenger activity (Aldridge et al., 2005b; Aldridge et al., 2003; Aldridge et al., 2004), gene expression of serotonin receptor and metabolism related genes (Slotkin & Seidler, 2007a; Xu et al, 2012), serotonin related behavioral assessments (Aldridge et al, 2005; Chen et al., 2011; Venerosi et al, 2010), and behavior after serotonergic drug challenge (Aldridgeet al., 2005; Venerosi, et al., 2010). Chlorpyrifos was administered during gestation (Aldridgeet al., 2005a; Aldridge et al., 2003; Aldridge et al., 2004; Slotkin & Seidler, 2007c; Venerosi et al., 2010), postnatally (Aldridge, et al., 2005; Aldridge et al., 2005a, 2005b; Aldridge et al., 2003; Aldridge et al., 2004; Slotkin & Seidler, 2005, 2007a, 2007b) or during adolescent (Chen, et al., 2011) and the animals were assessed either shortly after dosing ceased (Aldridge et al., 2003; Slotkin & Seidler, 2007a), or after a matter of weeks (Aldridgeet al., 2005b; Aldridge et al., 2003; Chen, et al., 2011; Slotkin & Seidler, 2007b, 2007c) or in adulthood (Aldridge et al., 2005a; Aldridge et al., 2004; Slotkin & Seidler, 2005; Venerosi et al., 2010). 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. For example, a group of papers found permanent effects of late gestational chlorpyrifos administration in rats (Aldridge, et al., 2005a; Aldridge et al., 2004) or

mice (Venerosi et al., 2010) on the development of the serotonergic nervous system. In rats (Aldridge et al., 2005a; Aldridge et al., 2004), chlorpyrifos was administered on GD17-20 (1 or 5 mg/kg, sc in DMSO), and the adult brain showed changes in brain regional serotonin content (5 mg/kg group), increased serotonin turnover (1 and 5 mg/kg groups), increases in 5-HT_{1A} and 5-HT_2 receptor populations (1 mg and 5 mg/kg groups), increases in serotonin reuptake receptor (1 and 5 mg/kg groups), and changes in the adenylate cyclase response to serotonin in the cerebral cortex and mid brain (1 mg/kg group)—all indicative of permanent changes in the serotonergic tone of the adult brain. In a related study in mice (Venerosi et al., 2010), chlorpyrifos was also administered during late gestation, but at a higher dose and by a different route (GD14-17; 6 mg/kg, peanut oil, gavage); the offspring were assessed upon reaching adulthood (≅ 90 days). The chlorpyrifos-treated rats showed minor differences in the behavioral tests, but when the tests were combined with a fluvoxamine (serotonin reuptake inhibitor) pharmacological challenge, there were marked differences in responses between the animals that had been exposed to chlorpyrifos during gestation and those that had not—another indication that developmental chlorpyrifos exposure had permanently altered the serotonergic tone of the adult brain.

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 cholinesterase inhibition (either brain or blood) in those same animals. In some cases, although cholinesterase activity was not assessed concurrently, a dosing regimen was used that had been characterized previously with regard to cholinesterase 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 marked inhibition of cholinesterase activity. There is, however, one of the dosing regimens [GD17-20 (daily subcutaneous dosing with chlorpyrifos in DMSO] that has been shown to produce no fetal brain cholinesterase inhibition measured 24 hours after the last dose at the 1 mg/kg , and inhibition (~20%) at 2 mg/kg (Qiao et al., 2002). As noted above, this level of gestational exposure to chlorpyrifos permanently altered the development of the serotonergic nervous system (Aldridge et al., 2005a; Aldridge et al., 2004).

In summary, with regard to development of the chlorpyrifos adverse outcome pathway for the serotonergic nervous system: the serotonergic system is intregal to mammalian brain development and function; perturbation of that system during development leads to lasting effects in mammals; and exposure to chlorpyrifos during the perinatal period can permanently change the function of serotonergic system. Therefore, as many steps in this chlorpyrifos adverse outcome pathway 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, 2007a), the actual initiating event of this potential adverse outcome pathway is unknown.

3.2.1.6. 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).

During brain development, the pattern of expression of microtubules (Bond & Farmer, 1983; Denouletet al, 1986; Meininger & Binet, 1989; Moskowitz & Oblinger, 1995), the post-translational modification of the tubulin (Fukushima, et al., 2009; Lee et al, 1990), and the spectrum of microtubule associated proteins (Fischer & Romano-Clarke, 1990; Nunez, 1986; Sánchez, et al., 2000) are all developmentally regulated. Because the phosphorylation of both microtubules and microtubule associated proteins are integral to their functional roles in the nervous system (Avila, et al., 1994; Fischer & Romano-Clarke, 1990; Fukushima, et al., 2009; Sánchez, et al., 2000), there is concern that exposure to OPs may affect the phosphorylation status and consequently the function of these molecules.

One of the shortcomings of constructing this adverse outcome pathway is that there are no studies on tubulin dynamics, microtubule associated proteins or axonal transport in developing animals, but because tubulin dynamics, microtubule associated proteins and axonal transport are so integral to the development and maintenance of the nervous system, the available adult data are first given in detail and then summarized below.

Adult rats treated for 14 days with chlorpyrifos (sc in peanut oil; 2.5, 10, 18, or 25 mg/kg; all doses produced plasma cholinesterase inhibition) show depressed anterograde and retrograde axonal transport in the sciatic nerve 6 days after cessation of treatment (Terry et al., 2003). This same laboratory followed up (Terry et al., 2007) with another, slightly different, study where axonal transport was assessed in adult rats given chlorpyrifos repeatedly (sc, 3% DMSO, 97% peanut oil vehicle, every other day for 30 days; 18 mg/kg). Depressed bidirectional axonal transport was noted after 1 dose, after the 30 days of dosing or 2 weeks after cessation of treatment. This dose produced about 30-50% brain cholinesterase inhibition 14 days after last dose. In that same year, the Terry laboratory published an *in vitro* paper (Gearhart et al, 2007) studying the effect of chlorpyrifos or chlorpyrifos oxon on kinesin-dependent microtubule motility (the mechanism of anterograde axonal transport). Using tubulin and kinesin prepared from bovine brain, they assessed kinesin-mediated movement of microtubules. This was done by either preincubating the tubulin or the kinesin fraction with chlorpyrifos or chlorpyrifos oxon and then combining the fractions and assessing movement of the microtubules. They obtained the same interesting pattern using chlorypyrifos or chlorpyrifos oxon: there was no effect if

preincubated with microtubules, but marked effect (increased detachment of the microtubules) if preincubated with kinesin. This assay contains no cholinesterase activity, thereby precluding inhibition assessment. A recently published *in vitro* paper (Middlemore-Risher et al, 2011) assessed the effect of chlorpyrifos or chlorpyrifos oxon transport of mitochondria in rat cortical neurons. They found dose-related effects of chlorpyrifos or chlorpyrifos oxon on total distance moved and number of moving mitochondria. All doses produced cholinesterase inhibition except lowest dose of chlorpyrifos (1 uM) or chlorpyrifos oxon (5 nM), and those doses still affected movement. Interestingly the toxic effect was not altered by co-treatment of either a nicotinic or muscarinic receptor blocker, an indication that the depressed transport was not mediated through cholinergic receptors. Subsequent to demonstrating that *in vitro* chlorpyrifos oxon appeared to alter tubulin dynamics (Grigoryan & Lockridge, 2009) and also covalently bound to tubulin (Grigoryan et al., 2008), the Lockridge laboratory assessed tubulin and microtubule associated proteins in the brains of mice that had been dosed *in vivo* with chlorpyrifos or chlorpyrifos oxon (Jiang et al., 2010). Repeated (14 days) dosing with chlorpyrifos (sc, 3% DMSO/97% peanut oil, 3 mg/kg) produced a transient decrease in blood AChE activity, no change in blood carboxylesterase activity and a steady decline in blood BuChE activity. Brain β-tubulin from the animals treated with chlorpyrifos had been covalently modified (diethosyphosphorylated) on tyrosine 281 (the residue also found in the *in vitro* study (Grigoryan et al., 2009). This same paper also described treating adult mice with a single dose of chlorpyrifos oxon (3 mg/kg; ip); this dose produced toxic signs and marked AChE and BuChE inhibition in the plasma. Brain tubulin was then purified, polymerized, the proteins separated by gel electrophoresis and the proteins identified by mass spectrophometry. The polymerized tubulin was also subjected to atomic force microscopy nanoimaging to assess the structure (width and height) of the microtubules. Microtubules from chlorpyrifos oxon- treated brains were missing 6 microtubule associated proteins. This observation was supported by the nanoimaging showing that microtubules from chlorpyrifos oxon-treated animals were narrower and shorter than control brain microtubules and also had fewer attached (decorated) proteins than do the microtubules from control brains. It interesting to note that an *in vitro* assessment (Prendergast et al., 2007) of the effects of chlorpyrifos oxon tubulin/microtubule associated protein dynamics in a 8 day old rat hippocampal slice preparation showed all concentrations of chlorpyrifos oxon decreased mircrotubule associated protein- staining and decreased microtubule associated protein mediated tubulin polymerization by about 45%; all chlorpyrifos-oxon doses inhibited AChE.

In summary, in the late 2000s, a number of papers were published on the *in vitro* modification of various proteins by chlorpyrifos or chlorpyrifos oxon (Grigoryan, Li, et al., 2009; Li et al., 2009), including tubulin (Grigoryan, Li, et al., 2009; Grigoryan & Lockridge, 2009; Grigoryan, Schopfer, et al., 2009; Grigoryan, et al., 2008). Although interesting and provocative, these studies were usually conducted with exceedingly high concentrations (high micromolar to millimolar) of the OP compound, making the connection to a "real world" human exposure tenuous. In 2010, however, Jiang and coworkers published an *in vivo* study in which they found covalently labeled tubulin in the brains of mice treated with chlorpyrifos or chlorpyrifos oxon, showing the real-world possibility of chlorpyrifos-induced covalent modification of brain tubulin in mammals at doses that produced minimal blood cholinesterase inhibition (Jiang, et al., 2010). Other studies have shown perturbations in tubulin dynamics, perturbations in the amount of tubulin protein or microtubule associated proteins, and changes in axonal transport elicited by treatment with chlorpyrifos or its oxon. Submicromolar concentrations of chlorpyrifos oxon

markedly inhibited tubulin polymerization *in vitro* (Prendergast *et al.*, 2007), while *in vitro* treatment with chlorpyrifos oxon (but not chlorpyrifos) reduced that amount tubulin in rat C6 glioma cells (Sachana *et al.*, 2008). Decreases in the amount of microtubule associated proteins after chlorpyrifos or chlorpyrifos oxon treatment has been reported by three separate studies: two *in vitro* studies both showed decreases after chlorpyrifos oxon treatment (Prendergast *et al.*, 2007; Sachana *et al.*, 2008), and one *in vivo* study showed extensive reduction in specific microtubule associated proteins in the brains of mice treated with chlorpyrifos oxon (Jiang *et al.*, 2010). Depressed axonal transport has been noted by many laboratories both *in vivo* (Terry *et al.*, 2007; Terry *et al.*, 2003) and *in vitro* (Gearhart *et al.*, 2007; Middlemore-Risher, et al., 2011) using both chlorpyrifos (*in vivo* and *in vitro* studies) and chlorpyrifos oxon (*in vitro* studies). In two instances (Gearhart *et al.*, 2007; Middlemore-Risher *et al.*, 2011), the effects of the chlorpyrifos and oxon were noted at concentrations of chlorpyrifos or chlorpyrifos oxon that did not inhibition cholinesterase activity (Middlemore-Risher, et al., 2011) or in systems that were so basic (purified microtubule/kinesin) that no cholinesterase was present (Gearhart *et al.*, 2007).

The construction of an adverse outcome pathway using chlorpyrifos-induced effects on tubulin and microtuble associated proteins is still in its infancy mainly because although it is thought that tubulin, microtubule associated proteins and axonal transport are integral to nervous system development and maintainence, there is no experimental evidence that perturbations of these endpoints by chlorpyrifos during development has neurotoxic outcomes.

3.2.1.7 Summary/Conclusions

There are a number of known developmentally neurotoxic chemicals with well-established relationships between exposure and neurological disorders in humans for which a definitive mode of action has not been established: for example lead, methyl mercury, and ethanol (Alfonso-Loeches & Guerri, 2011; Castoldi *et al.*, 2008; Farina *et al.*, 2011; Johansson *et al.*, 2007; Verstraeten *et al.*, 2008). Even today, despite with thousands of published papers on these three, accepted, developmental neurotoxicants, no coherent adverse outcome pathway or pathways can be constructed, because there are a multitude of initiating toxic events and cellular responses put forth, and they are not positively connected with one another. The only adverse outcome pathway available for any form of neurotoxicity has been developed for domoic acid in adult animals (Watanabe *et al.*, 2011).

As can be seen from the section above, there are several lines of evidence for actions of chlorpyrifos distinct from the classical mode of action of cholinesterase inhibition. This information has been generated from model systems representing different levels of biological organization, and provide support for molecular initiating events (binding to the morphogenic site of AChE, muscarinic receptors, or tubulin), cellular responses (alterations in neuronal proliferation, differentiation, neurite growth, or intracellular signaling) and responses at the level of the intact nervous system (serotonergic tone, axonal transport). In some cases these effects occur at chlorpyrifos exposure levels below or equivalent to those which result in cholinesterase inhibition in the same test system. When taken in aggregate, data from individual studies can be used to develop plausible hypotheses for a mode of action leading to developmental neurotoxicity (*e.g.* inhibition of the morphogenic action of AChE leading to reduced neurite outgrowth and subsequent miswiring or dysmorphology of the brain); however, as is the case for

many other developmental neurotoxicants, most of these studies have not been designed with the specific goal of construction or testing an adverse outcome pathway. Thus, there are not sufficient data available to test rigorously the causal relationship between effects of chlorpyrifos at the different levels of biological organization in the nervous system. Moreover, there is still lack of data on the toxic moiety (ies) impacting neurodevelopment (i.e., chlorpyrifos, chlorpyrifos oxon and/or TCPy) (Flaskos, 2012). This is not surprising in light of the complex, intricate, and interrelated dynamic processes ongoing in the developing brain and multiple critical windows for exposure.

3.2.2. Adverse Outcomes in Laboratory Animals: Effects on the Developing Brain

There is a considerable and growing body of literature on the effects of chlorpyrifos on the developing brain of laboratory animals (rats and mice) indicating that gestational and/or postnatal exposure may cause persistent behavioral effects into adulthood. These data provide support for the susceptibility of the developing mammalian brain to chlorpyrifos exposure. The literature was summarized and a preliminary review was provided by the Agency for the 2008 SAP with a more extensive review in 2012 and updated in 2014. The Panel agreed that exposure to doses of 1 mg/kg/d and greater, during some developmental period, produced significant and long-term effects on behavior. While behavioral changes were consistently reported, they were somewhat inconsistent, most likely due to experimental design differences. Such factors include route of exposure, developmental period of exposure, test methods, choice of dependent variable, statistical analyses, and so on, which are critical features of all developmental neurotoxicity studies.

In this section the agency reviews newer literature, *i.e.*, papers published since 2008, to build on the earlier SAP review. The new information provided is evaluated in terms of concordance with, or divergence from, the previous, pre-2008 findings. Overall, these new studies serve to strengthen the findings on which the 2008 SAP made their conclusions and further documents the scope of long-term behavioral effects resulting from chlorpyrifos exposure during development.

Text and tables in 3.2.2.2 were extracted from the 2012 draft SAP issue paper. Section 3.2.2.3 provides a summary of the newest studies published between 2012 and 2014.

Papers considered herein by the Agency as addressing long-term outcomes from developmental exposure include only those where chlorpyrifos is administered during the preweaning period (either gestational and/or postnatal) and the offspring are examined at some time after weaning. That is, papers reporting evaluations shortly after birth or during the pre-weaning period do not reflect long-term consequences and may also be confounded by AChE/ChE inhibition during concurrent or recent exposure; thus, those data are not discussed further here. In addition, the Agency focused its efforts on studies using relatively low doses, and that did not (or would not be expected to) produce a considerable degree of brain AChE inhibition and resultant cholinergic toxicity. These constraints aid in the unencumbered evaluation of longer-term effects compared to acute impacts of AChE inhibition, but it does limit the use of some papers.

At the time of the 2012 SAP meeting, the following papers were identified and evaluated by the

Agency; (Abou-Donia et al., 2006; Aldridge, Levin, et al., 2005; Billauer-Haimovitch et al., 2009; Braquenier, Quertemont, Tirelli, & Plumier, 2010; Carr et al., 2001; Chakraborti, Farrar, & Pope, 1993; Chanda & Pope, 1996; Dam et al, 2000; Haviland, Butz, & Porter, 2010; Icenogle et al., 2004; D. A. Jett, Navoa, Beckles, & McLemore, 2001; Johnson, Chambers, Nail, Givaruangsawat, & Carr, 2009; Laviola, Adriani, Gaudino, Marino, & Keller, 2006; Levin et al., 2002; Levin et al., 2001; Maurissen, et al., 2000; Muto, Lobelle, Bidanset, & Wurpel, 1992; Ricceri et al., 2003; Ricceri et al., 2006; Venerosi, Calamandrei, & Ricceri, 2006; Venerosi et al., 2008; Venerosi, et al., 2010; Venerosi, Ricceri, Scattoni, & Calamandrei, 2009; Zhang et al., 2011). It is important to appreciate the various experimental designs, encompassing species, gender, exposure scenarios, ages of assessment, and test apparatuses. Of the papers included in the 2012 analysis, four papers and one study from a fifth paper did not meet the criteria stated above, and were excluded from further consideration herein.

Specifically, these excluded studies are:

1) Muto et al. (1992) used a chlorpyrifos product that was formulated in xylene, a known developmental and reproductive toxicant, yet the vehicle control group received saline, not xylene. Thus, the findings of that study cannot be attributed to chlorpyrifos alone. Furthermore, all testing took place before weaning, no later than PND16;

2) A gestational-exposure study (GD12-19) used a dose (25 mg/kg/d sc) that produced 90% brain inhibition in the dams at the end of dosing, and ~20% brain inhibition in offspring on PND3 (Chanda & Pope, 1996). The reported motor effects are confounded with the concurrent cholinesterase inhibition on PND1 and 3, and no testing was conducted at later ages;

3) A postnatal study (Chakraborti, et al., 1993) used a very high dose (40 mg/kg/d sc) given directly to the pups postnatally (PND7, 11, 15, 19), with up to 60% brain inhibition evident four days after dosing;

4) A gestational study (Venerosi, et al., 2009) only tested pups up to PND12, reporting altered motor ontogeny and changes in ultrasonic vocalizations; and

5) The "postnatal" study described by (Jett, et al., 2001) involved dosing after weaning, while the rats were being tested in the Morris water maze. Note, part of this paper remains in consideration, since the "preweaning" study from that paper met the criteria for inclusion.

3.2.2.1 Developmental Impacts on Neurological Domains

Because many of these papers report a number of positive as well as negative findings, the agency had previously taken the approach of comparing responses that were observed following various exposures to a common dose, 1 mg/kg/d (FIFRA SAP, 2008a; U.S. EPA, 2011). A more robust approach is taken here, to include important factors such as dose-response and differences in exposure scenarios. In order to evaluate effects on domains of neurological functions, broken

down by exposure period, the papers have been summarized as such in Section 3.2.2.1.5. It is well-known that exposures during different critical periods of development can result in very different outcomes (Adams et al., 2000; Rice & Barone, 2000). Unfortunately, development of the nervous system does not occur in distinct non-overlapping temporal periods with which to attribute the apical behavioral changes observed. In the text and tables below, the papers from 2008 to 2012 are described and evaluated in terms of their contribution to and concordance with, previous papers using similar measures. The agency's evaluation follows several Bradford Hill criteria including consistency of findings across domains and dose response. The latter was considered as either graded magnitude of effects across doses or significant findings at a higher but not lower dose; unfortunately, many of the chlorpyrifos studies have evaluated only one dose.

These studies include measures that evaluate the following neurobehavioral domains: cognitive function, anxiety and emotion, social behavior and maternal interactions, and motor activity. These are discussed in greater detail below; overall, the newer data were in general agreement with previous studies.

3.2.2.1.1 Cognitive Function

A few of the new studies evaluated effects on cognitive function, using some of the same procedures used in the earlier studies (radial arm maze, Morris water maze). The earlier findings are summarized below, along with comparisons to the newer studies.

Radial Arm Maze

The radial arm maze is a spatial task requiring the subject to enter different arms for food reinforcer, which is located in only certain arms. A widely used test of learning and memory, both working errors (re-entering baited arms after taking the food) and reference errors (entering arms that are never baited) are recorded over days of training. From 2001-2005, a series of behavioral studies from a single laboratory (Duke University) described effects on radial arm maze performance in adult rats that were treated with chlorpyrifos during gestational or postnatal development. Both working and reference memory are altered in rats treated during gestation or early postnatally, and gender differences were described in all but one study. Across these studies, significant differences were detected in one or the other type of error, or both, and error selectivity was not fully consistent across studies. Following early gestational exposure (GD9-12), high-dose (5 mg/kg/d) rats of both sexes showed increased working and reference memory errors early in training (Icenogle, et al., 2004). With later gestational exposure (GD17-20), only females showed more errors; however, this was observed only at the low dose (1 mg/kg/d, not 5 mg/kg/d) (Levin, et al., 2002). With early postnatal exposure (PND1-4, 1 mg/kg/d), two separate studies showed similar results in that error rate was increased in males (worse performance) but decreased in females (better performance) (Aldridge, Levin, et al., 2005; Levin, et al., 2001). Only the late postnatal exposure (PND11-14, 5 mg/kg/d) was without effect on this measure (Levin, et al., 2001).

Several new studies have confirmed chlorpyrifos impacts on learning and memory. In a new

study using the radial arm maze and longer postnatal exposures (PND1-21), male, but not female, rats showed more working memory errors at all doses (lowest dose, 1 mg/kg/d) (Johnson, et al., 2009). This was evident later in training, indicating that the task was not learned as well as in controls. Reference memory errors showed the same gender-specific pattern as seen in two Duke studies (Aldridge, Levin, et al., 2005; Levin, et al., 2001), in that chlorpyrifos-treated females showed fewer errors during training, and males showed more. The altered reference memory pattern was only seen in the mid and high dose groups, which had incrementally increasing doses (up to 4 or 6 mg/kg/d) during the exposure period. In this study, the low-dose group had experienced about 14% brain AChE inhibition on PND20, with no inhibition evident when radial arm maze training began (PND36). On the other hand, the mid and high-dose rats showed some residual AChE inhibition at that time, making it challenging to separate the memory deficits from concurrent AChE holinesterase inhibition.

A study using mice exposed gestationally (GD17-20, 1 or 5 mg/kg/d) also used a radial arm maze, although comparisons are difficult due to differences in length of training and data presentation (Haviland, et al., 2010). Reference memory errors showed some differences across trial, dose, and sex; these are not interpreted by the authors as a meaningful effect, and may indeed be spurious. The authors used these data to compare with their novel foraging task, which used a modification of a radial arm maze and examined the rate of learning that a reward (food) is present (recognition) as well as its location (positional learning). Both of these parameters were altered by chlorpyrifos exposure. The low dose (1 mg/kg/d) females were delayed in learning to recognize the reward, but there were no statistically significant differences at that dose in positional learning (despite the authors' claim of an effect). On the other hand, low-dose males showed accelerated food recognition, and increased positional learning that was evident during only two sessions. The high-dose groups (both sexes) showed the same pattern of changes, with somewhat greater magnitude of differences, indicating dose-response. Thus, effects were observed on a spatial learning task, albeit a different apparatus and procedure. Note that the gender effect was reversed from that reported above for rats in the radial arm maze – these differences could be due to species or testing apparatus.

Morris Water Maze

The Morris water maze is a different type of spatial learning task, which can be varied to assess different types of learning and memory. In this test, the animals are trained over days to swim to the location of a submerged platform to escape from the water, and learning is evident by faster latencies and other measures of memory for the platform position. A recent paper using gestational exposure (GD9-18) in mice resulted in slower learning in the offspring, although a clear dose-response was not evident (Billauer-Haimovitch, et al., 2009). The authors report an overall effect of chlorpyrifos treatment, but state that only the lower doses (1, 3 mg/kg/d, but not 5, 10 mg/kg/d) were individually significant. While the magnitude of effect does not appear to be pronounced in the first study (visual inspection of Figure 3 in the paper), the finding was repeated, and was more obvious, in two additional studies using a single dose (3 mg/kg/d). Another recent paper from the same laboratory again reported slower learning in mice exposed during gestation (3 mg/kg/d; GD9-18) (Turgeman et al., 2011). Thus, there are internal replications of this finding across several studies, albeit in the same laboratory.

These recent findings, plus another study available only in Chinese[66], extend an earlier report of rats that showed deficits in the Morris water maze (beginning on PND23) following exposure on PND7, 11, and 15 (0.3, 7 mg/kg/d) (Jett, et al., 2001). The 2008 SAP, however, was critical of this paper, for reasons described below.

Summary

Taken together, these studies in rats and mice show altered cognitive function using well-accepted tests of spatial learning and memory (radial arm maze, Morris water maze). The direction of change may be sex-specific and dependent on the timing of exposure. Often these changes suggest impaired learning and/or memory. While enhanced function is also apparent in some studies, such changes are evidence of alterations in memory processes nonetheless. Several of these findings have recently been replicated across studies and laboratories. Effects were also reported in a spatial foraging task (Haviland, et al., 2010), but direct comparison between that and the radial arm maze is difficult. Earlier papers have reported that other cognitive tasks (spontaneous or delayed alternation, passive avoidance) are not altered, but there are no new studies using these other tasks. These outcomes are summarized in App 1-8.

[66] Another new study also evaluated Morris water maze learning, using rats exposed postnatally (PND11-14, 5 mg/kg/d, route uncertain) (Zhang et al., 2011). The abstract states that learning and memory impairments were observed, but the rest of the paper cannot be critically evaluated at this time (article is in Chinese) and so cannot be combined with other findings.

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Table App. 1-8. Summary of Outcomes on Cognitive Tests in Male (M) and Female (F) Rodents (Extracted from USEPA, 2012)

	Early gestation GD9-12; 9-18	Late gestation GD 17-20	Perinatal GD6-LD10	Early postnatal PND1-4	Late postnatal PND11-14; 7,11,15	Postnatal PND1-21
Radial Arm Maze	Cognitive deficit - rat, M&F, dose-response[6]	Cognitive deficit - rat, F not M, no dose-response[4]		Cognitive deficit in M, improved function in F - rat[3] Cognitive deficit in M, improved function in F - rat[7]	No effect – rat, M&F[3]	Cognitive deficit in M, improved function in F - rat, dose-response[9]
Morris Water Maze	Cognitive deficit - mouse, M&F, no dose-response[8] Cognitive deficit – mouse, M&F[11]				Cognitive deficit - rat, M&F, dose-response[2]	
Foraging Maze		Cognitive deficit in F, improved function in M – mouse, dose-response in F not M[10]				
T-maze Spontaneous Alternation	No effect – rat, M&F[6]	No effect – rat, M&F[4]		No effect – rat, M&F[3]	No effect – rat, M&F[3]	
Delayed Spatial Alternation			No effect – rat, M&F[1]			
Passive Avoidance				No effect – mouse, only M tested[5]	No effect – mouse, only M tested[5]	

[1] Maurissen et al., 2000

[2] Jett et al., 2001

[3] Levin et al., 2001
[4] Levin et al., 2002
[5] Ricceri et al., 2003
[6] Icenogle et al., 2004
[7] Aldridge et al., 2005
[8] Billauer-Haimovitch et al., 2009
[9] Johnson et al., 2009
[10] Haviland et al., 2010
[11] Turgeman et al., 2011

3.2.2.1.2 Anxiety/Emotion

Anxiety and despair/affect have been modeled in animals using a number of different tests, including those that measure avoidance of aversive areas, investigation of novel areas, response to forced activity, and response to preferred substances. Several new studies assessed anxiety, despair, and affect. While different procedures were used in most cases, there was some concordance in outcomes. These are described below.

Tests of Anxiety

Several studies have suggested that chlorpyrifos alters measures of anxiety. The early studies came from the laboratories of Duke University and ISS (Italy). Three newer studies, all in mice, add to the findings in this area: two were from the ISS laboratory and one came from a third laboratory (Belgium). Earlier studies employed an elevated plus maze, which has open and enclosed areas: being fearful of bright open areas, rodents tend to stay in the enclosed arms. Decreased anxiety is inferred from changes such as increased time in the open arms, decreased head dipping, and other measures. Using this paradigm, lower anxiety was reported in rats (males, not females) exposed postnatally to 1 mg/kg/d (PND1-4)(Aldridge, Levin, et al., 2005; Ricceri, et al., 2003) and in mice (females, not males) exposure postnatally to 3 mg/kg/d (PND11-14) (Ricceri, et al., 2006). On the other hand, increased anxiety was measured in mice exposed gestationally (GD15-18), but only in the low-dose (3 mg/kg/d, not 6 mg/kg/d) males (not females) (Ricceri, et al., 2006). There were no changes in anxiety behaviors in rats exposed gestationally (GD9-12, 1 or 5 mg/kg/d) (Icenogle, et al., 2004).

Three new studies have used a different apparatus, the light-dark box, to measure anxiety, but the principle is the same in that rodents typically prefer the dark chamber. In one study (Braquenier, et al., 2010), both the elevated plus maze and the light-dark box were used, providing direct comparisons between these tests. Mice were exposed both gestationally and lactationally (GD15-LD14, 0, 0.2, 1, or 5 mg/kg/d), and only female offspring were tested. When tested in the light-dark box, the middle dose group only (1 mg/kg/d) showed decreased time in the center of the light side, which was considered to reflect increased anxiety. The same dose group moved back and forth between the sides less often, which could reflect general activity levels but was also interpreted by the authors as greater anxiety. The time in the dark and light sides, however, did not differ. Littermates were tested in the elevated plus maze, and again the middle dose group showed less time spent in the open arms and fewer open arm entries, supporting a conclusion of increased anxiety, but only at the middle dose. The lack of dose-response was not addressed by the authors.

A study in which mice were treated gestationally (GD14-17, 6 mg/kg/d) (Venerosi, et al., 2010) showed no difference in time spent on either side of the light-dark box, but females (not males) spent more time in the tunnel connecting the sides. This finding, along with a few other measures that did not reach statistical significance, was interpreted by the authors as increased anxiety. Another study by this group (Venerosi, et al., 2008) exposed mice postnatally (PND11-14, 3 mg/kg/d), then bred the female offspring as adults and allowed them to deliver normally. On postpartum day 2, the dams were removed from the pups and tested in the light-dark box. They reported a decreased latency and higher proportion of mice entering the light side, indicating decreased anxiety. Time in the dark or light sides was not reported.

Despair/Affect

Only a few studies have also included measures of despair and affect, and the results are not completely consistent. Most recently, behavioral despair was measured using a forced swimming procedure (Venerosi, et al., 2010). In that study, gestational exposure to chlorpyrifos did not alter any baseline responses, and there are no other studies using similar measures with which to compare. There have been no new studies that could add to, and aid in the interpretation of, the findings reported by others: decreased preference for chocolate milk in rats, or no effect on novelty exploration in mice (Aldridge, Levin, et al., 2005; Ricceri, et al., 2003).

Summary

Taken together, these assessments suggest that, in both rats and mice, changes in anxiety are dependent on exposure period. Specifically, postnatal exposure decreases anxiety, whereas increased anxiety is observed following late gestational exposure or a longer gestational plus postnatal exposure. Some inconsistencies are evident, such as lack of dose-response in a few studies and effects on one sex or the other (same laboratory, similar dosing regimen). Thus, while the data are not fully consistent, overall there is evidence for long-term changes in anxiety behavior following chlorpyrifos exposure, as shown in Table App 1-9.

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Table App 1-9 Summary of Anxiety/Emotion Outcomes in Male (M) and Female (F) Rodents (Extracted from USEPA, 2012)

	Early gestation GD9-12	Late gestation GD14-17; GD15-18	Early postnatal PND1-4	Late postnatal PND11-14	Perinatal GD15-LD14
Elevated Plus Maze (anxiety)	No effect – rat, M&F[1]	Decreased anxiety - mouse, M not F, no dose-response[5]	Decreased anxiety - rat, M not F[2]	Decreased anxiety - mouse, F not M, dose-response[5]	Increased anxiety - mouse, only F tested, no dose-response[3]
Light-Dark box (anxiety)		Increased anxiety - mouse, F not M[7]		Decreased anxiety - mouse, dams[6]	Increased anxiety - mouse, only F tested, no dose-response[3]
Despair		No effect – mouse, M&F[7]			
Novelty/preference			Decreased anxiety - rat, M&F[2] No effect – mouse, M&F[4]	No effect – mouse, M&F[4]	

[1] Icenogle et al., 2004
[2] Aldridge et al., 2005
[3] Braquenier et al., 2010
[4] Ricceri et al., 2003
[5] Ricceri et al., 2006
[6] Venerosi et al., 2008
[7] Venerosi et al., 2010

3.2.2.1.3 Social Behaviors/Interactions

Conspecific behaviors are not typically studied in the context of developmental neurotoxicity studies, and methods for such assessments are not well-standardized (Cory-Slechta, et al., 2001). Aggressive, social, and parental behaviors have been studied following developmental exposure to chlorpyrifos, all of which were conducted in the ISS laboratory using mice. Even within this one laboratory, however, various testing methods have been used. These include: 1) social investigation of nulliparous female:female pairs; 2) social investigation and agonistic behaviors in male:male pairs; 3) maternal behaviors towards pups, induced by placing nulliparous females with foster litters; 4) maternal behaviors in lactating dams towards their own pups, and 5) agonistic behaviors in lactating dam:male pairs. In most studies, measures include social (interactive) as well as nonsocial behaviors (*e.g.*, grooming, exploration). Within each grouping, there are often many measures taken, and treatment effects have sometimes been reported on just a few, suggesting that the changes are subtle. There are few systematic comparisons of chlorpyrifos effects across these varied behaviors using similar dosing regimens.

Maternal Behavior

Two recent papers focused on maternal behavior, measuring actions of the lactating dam towards her pups, nesting activity, and agonistic behaviors towards an intruder male. In one study (Venerosi, et al., 2008), mice were exposed postnatally (PND11-14, 3 mg/kg/d), mated as adults, and the dams were tested. They reported that the treated dams showed delayed start of nesting, decreased latency to lick pups, as well as fewer defensive postures and more social investigation of an intruder male. This latter effect was replicated in that decreased aggressive behaviors were also reported for lactating dams that had been exposed in their own fetal period (GD14-17, 6 mg/kg/d) (Venerosi, et al., 2010). These findings were interpreted by the authors as impaired maternalistic behaviors.

Social/Agonistic Behavior

Evaluations of male or female same-sex social behaviors generally show no effect of postnatal (PND1-4 or PND11-14) chlorpyrifos exposures (Ricceri, et al., 2003; Venerosi, et al., 2006), a finding that was recently confirmed (Venerosi, et al., 2008; Venerosi, et al., 2010). On the other hand, increased investigation of the stranger mouse was reported following gestation exposure (GD15-18)(Venerosi, et al., 2006), and increased solicitation behaviors were observed following late postnatal exposure (PND11-14) (Ricceri, et al., 2003). Thus, there are contradictory reports of changes in female social behavior, being either increased or not altered. The only study of male social behavior showed no effect.
Earlier studies reported that male agonistic behaviors were consistently increased in mice exposed either postnatally (PND1-4, PND11-14) or gestationally (GD15-18); the lowest effective dose was 1 mg/kg/d administered during either postnatal period (Ricceri, et al., 2003; Ricceri, et al., 2006). None of the newer studies addressed male behaviors. These findings are summarized in Table 1-10.

Summary

Overall, decreased aggressive behaviors of dams that had been exposed during their development are a common finding across several exposure periods, as are increased male agonistic behaviors. Social and/or maternal behaviors in females are less consistently or convincingly altered.

Table App 1-10. Summary of Social/Interactive Behavior Outcomes in Male (M) and Female (F) Mice (Extracted from USEPA, 2012)

Exposure/effect	Late gestation GD14-17, 15-18	Early postnatal PND1-4	Late postnatal PND11-14
Female:female social	Increased investigation, dose-response[3]	No effect[1]	Increased solicitation, dose-response[1] No effect[3] No effect[4]
Male:male social	Increased aggressive postures, dose-response[2]	Increased agonistic behavior, differs across time-course, dose-response[1]	Increased solicitation, dose-response[1] Increased agonistic behavior, over time, no dose-response[1] Increased attack behaviors, dose-response[2] No effect social[4,a]
Induced maternal	No effect[2]		Increased maternalism, dose-response[2]
Natural maternal			Decreased maternalism[4]
Natural maternal:male	Decreased aggression[5]		Decreased defensiveness[4]

DR dose-response, if not mentioned, only one dose tested
[a] apparently only social, no agonistic, behaviors measured
[1] Ricceri et al., 2003
[2] Ricceri et al., 2006
[3] Venerosi et al., 2006
[4] Venerosi et al., 2008
[5] Venerosi et al., 2010

3.2.2.1.4 Motor activity

Most of the developmental studies of chlorpyrifos have evaluated motor activity of some sort; these were presented and summarized in detail in the 2008 SAP report. In general, activity levels have been recorded in terms of: 1) locomotion; 2) response latencies or other activity measures in the course of testing in various apparatuses (t-maze, radial arm maze, etc); and 3) habituation of activity over the session in activity chambers. In general, the earlier studies reported that activity is increased, decreased, or not altered, in either both or just one sex. Recent studies add to this literature, but none provide information to better explain these varied and contradictory outcomes.

Activity Devices

Evaluations of locomotor activity in open fields or other activity devices have been made in numerous studies across quite a few laboratories. Looking across developmental period and gender, there have been similar reports of increased, decreased, or no change in various measures of activity (*e.g.*, exploration, rearing, etc). Generalizations of effect could not be made in the 2008 preliminary review.

Two recent studies have used an open field to measure activity. Braquenier et al. (2010) reported no change of activity levels in female mice that had been exposed from late gestation through late lactation (GD15-LD14). While Zhang et al. (Zhang, et al., 2011) reported decreased activity in rats, those data cannot be evaluated at this time due to the language barrier.

Ancillary Activity Measures

As with activity measured specifically in activity devices, various measures of activity during other tests have shown increases, decreases, or no effect, in both or only one sex, with no obvious association to exposure period. It is important to consider, for example, that the time it takes to visit the arms of a radial arm maze is a somewhat different behavior than exploratory movement in a novel chamber. Thus, these activity measures may not be fully comparable, and the evaluations described here should be interpreted with caution.

Three recent papers reported assessments of activity during cognitive and social behavior testing. There was no change noted in postnatally exposed rats during radial arm maze testing (PND1-21) (Johnson, et al., 2009), or in gestationally exposed mice during foraging testing (GD17-20) (Haviland, et al., 2010). Mice exposed postnatally (PND11-14) showed decreased exploratory behaviors during the acclimatization phase of social testing (*i.e.*, before being exposed to another mouse); however, this was only significant in the first block of the test session (Venerosi, et al., 2008).

Habituation

Habituation, or decrease in activity level during the course of a test session, has only been specifically evaluated in the a few laboratories. As with other activity outcomes, the data are specific to exposure period and gender. Within the same laboratory, habituation is either faster (early gestation, both sexes) (Icenogle, et al., 2004), slower (late gestation, females only; late postnatal, both sexes) (Levin, et al., 2002; Levin, et al., 2001), or not altered (early postnatal) (Levin, et al., 2001). A perinatal study (GD6-LD10) also reported no change in habituation; however, the data were not statistically analyzed in the same way, so results are not comparable (Maurissen et al., 2000). This measure has not been addressed in more recent studies.

Summary

There remains to be inconsistencies in the various measures of motor activity, but it is important to note the numerous differences in procedures and apparatuses in which activity was measured. Given this, generalizable conclusions and summaries cannot be made at this time.

Table App 1-11. Summary of Motor Activity Outcomes in Male (M) and Female (F) Rodents (Extracted from USEPA, 2012)

	Early gestation GD9-12	Late gestation GD14-16; 14-17; 15-18; 17-20	Perinatal GD6-LD10; GD15-LD14	Early postnatal PND 1-4	Late postnatal PND11-14	Postnatal PND1-21
Activity chambers	No effect – rat, M&F[6]	Increased activity - mouse, only M tested, dose-response[13] No effect - mouse[8] No effect – rat, M&F[10]	No effect – rat, M&F[11] No effect – mouse, only F tested[2]	No effect - mouse, M&F[12] Decreased activity - rat, M not F[4] No effect – rat, M&F[9]	Increased activity - mouse, only M tested, dose-response[13] Increased activity - mouse, M&F, dose-response[12] Increased activity - rat, M&F[4] No effect – rat, M&F[9]	Decreased activity - rat, M&F, dose-response[3]
Activity measures	Increased activity - rat, M&F, dose-response[6]	No effect - mouse, only F tested[14] No effect - mouse, M&F[5] Increased activity - rat, M&F, DR[10] No effect - mouse, M&F[13]	No effect – mouse, only F tested[2]	Increased activity - rat, M not F[1] Increased activity - mouse, M&F[12] Decreased activity - rat, M not F[9]	Decreased activity - mouse, M&F[14] Decreased activity - rat, M not F[9] Increased activity - mouse, M&F[12] No effect – mouse, M&F[13]	No effect – rat, M&F[7]
Habituation rate	Increased habituation - rat, M&F, dose-response[6]	Decreased habituation - rat, F not M, dose-response[10] No effect – mouse[8]	No effect – rat, M&F[11]	No effect – rat, M&F[9]	Decreased habituation - rat, M&F[9]	

[1] Aldridge et al., 2005
[2] Braquenier et al., 2010
[3] Carr et al., 2001
[4] Dam et al., 2000

[5] Haviland et al., 2010
[6] Icenogle et al., 2004
[7] Johnson et al., 2009
[8] Laviola et al., 2006
[9] Levin et al., 2001
[10] Levin et al., 2002
[11] Maurissen et al., 2000
[12] Ricceri et al., 2003
[13] Ricceri et al., 2006
[14] Venerosi et al., 2008

3.2.2.1.5 Detailed information on behavioral studies (Extracted from USEPA, 2012).

Table App 1-12. *In vivo* studies of chlorpyrifos administered during gestation; effects described are only those measured after weaning. Bold indicates functional domain that showed effects

Study & laboratory	Species & strain	Dose, route, vehicle	Dosing period	AChE inhibition	Domain	Age of testing	Outcome; effective dose & gender	Notes[a]	Dose-response[b]?
Abou-Donia et al., 2006 Duke	Rat SD	1 mg/kg/d dermal in 70% ethanol	GD4-20	0-30% increase brain (regions) activity at PND90, F not M	**Neuromotor function**	PND90	Decreased grip time, M&F; Decreased performance on inclined plane, F not M;	Used 2/sex/litter, (5 litters/ treatment), did not control for litter; Cerebellar pathology	NA
Icenogle et al., 2004 Duke	Rat SD	1, 5 mg/kg/d sc in DMSO	GD9-12	Not measured	**Motor activity**	PND28-42	Faster habituation in figure-8, 5 mg/kg/d, M&F	10/sex/dose, no more than 1 of each sex from each litter; ACh system involvement	Yes LOEL=1 mg/kg/d
						PND28-56	Lower latencies (faster) in t-maze, early in testing, 1&5 mg/kg/d, M&F		
						PND>91	Increased center crosses in elevated plus maze (more active), 5 mg/kg/d, M&F		
					Cognition	PND28-56	No effect on t-maze spontaneous alternation, M&F		
						PND56-91	Increased radial arm maze working & reference memory errors, early in training, 5 mg/kg/d, M&F		
					Neuromotor function	PND>91	No effect on acoustic startle		

Study & laboratory	Species & strain	Dose, route, vehicle	Dosing period	AChE inhibition	Domain	Age of testing	Outcome, effective dose & gender	Notes[a]	Dose-response[b]?
							reflex or prepulse inhibition, M&F		
					Anxiety & Emotion	PND>91	No effect on time in sides of elevated plus maze, M&F		
Billauer-Haimovitch et al., 2009 Israel	Mouse HS/lbg	1, 3, 5, 10, 20 mg/kg/d sc in DMSO	GD9-18	Not measured	**Cognition**	PND75	Longer latencies (slower learning) in Morris water maze, 1&3 mg/kg/d, M&F	1/sex/litter; N varied,14-34/dose group; All dams dosed at 20 mg/kg/d died before delivery; Nicotine & stem cell as treatments	No LOEL=1 mg/kg/d Maximal effect at 3 mg/kg/d; Higher doses had no effect
Turgeman et al., 2011 Italy	Mouse HS/lbg	3 mg/kg/d	GD9-18	Not measured	**Cognition**	PND80	Longer latencies (slower learning) in Morris water maze, M&F	1/sex/litter; N=13-25/dose group; Stem cells as treatments	NA
Laviola et al., 2006 Italy	Mouse, wild type on C57/Bl6 background; Study included reeler homo- and heterozygous mice	Oxon 5 mg/kg/d sc in osmostic pump with DMSO	GD14-16	Not measured	Motor activity	PND>70	No difference in activity levels during habituation, before drug challenges	Only data from wild type shown here; Never mentions genders; Oxon not parent; Individual, as phenotyped, used; Total of 6 litters/treatment; ACh & DA system involvement; Also some	NA

Study & laboratory	Species & strain	Dose, route, vehicle	Dosing period	AChE inhibition	Domain	Age of testing	Outcome, effective dose & gender	Notes[a]	Dose-response[b]?
								preweaning testing	
Venerosi et al., 2010 Italy	Mouse CD-1	6 mg/kg/d po in peanut oil	GD14-17	Not measured	**Anxiety & emotion**	PND90	Increased time in tunnel between sides in light-dark box, F not M; Decreased latency to enter dark side (p=0.05) (increased anxiety), F not M;	Litter as unit; N=6-13/dose; 5HT system involvement	NA
							No effect on forced swimming task, M&F		
					Social behavior & maternal interaction	Adult F, after mating on postpartum day 8	Decreased attack behavior, increased social sniffing behavior to intruder male		
Haviland et al., 2010 UWM	Mouse Swiss Webster	1, 5 mg/kg/d sc in DMSO	GD17-20	Not measured	**Cognition**	PND60-81	Slower learning of food recognition & position, 1&5 mg/kg/d, F not M; Faster food recognition learning, 1 not 5 mg/kg/d, M not	Litter as unit; N=8/sex/dose; Thyroid involvement	Yes (F only) LOEL=1 mg/kg/d In males, 5 mg/kg/d had no effect

Study & laboratory	Species & strain	Dose, route, vehicle	Dosing period	AChE inhibition	Domain	Age of testing	Outcome, effective dose & gender	Notes[a]	Dose-response[b]?
							F;		
							Spurious changes in radial arm maze (says no effect, only 9 trials)		
					Motor activity	PND60-81	No effect on foraging rate, M&F		
Levin et al., 2002 Duke	Rat SD	1, 5 mg/kg/d sc in DMSO	GD17-20	Not measured	**Motor activity**	PND28-42	Lower latencies (faster) in t-maze, early in testing, 1&5 mg/kg/d, M&F	1/sex/dose from 10 litters	Yes LOEL=1 mg/kg/d
							Slower habituation in figure-8, 1&5 mg/kg/d, F only		
						PND56-91	Lower latencies (faster) in radial arm maze during certain blocks only, 1&5 mg/kg/d, M&F		
					Cognition	PND28-42	No effect on t-maze spontaneous alternation, M&F		
						PND56-91	Increased radial arm maze working & reference memory errors, 1 mg/kg/d only, F not M		No Radial arm maze effect at 1 mg/kg/d only

Study & laboratory	Species & strain	Dose, route, vehicle	Dosing period	AChE inhibition	Domain	Age of testing	Outcome, effective dose & gender	Notes[a]	Dose-response[b]?
Ricceri et al., 2006 Italy	Mouse CD-1	3, 6 mg/kg/d po in peanut oil Plus vehicle on PND11-14	GD15-18	Dam on GD19 (pilot study): 40% brain ChE inhibition at 6 mg/kg/d, serum inhibition at both doses Pup on GD19: no brain inhibition, serum inhibition at both doses Pup on PND15: no brain or serum inhibition	**Motor activity**	PND70	Increased crossing in open field, 6 mg/kg/d, only M tested	Litter as unit; 10 litters/ gestational dose	Yes Activity NOEL= 3 mg/kg/d
						PND120	No effect on elevated plus maze activity, M&F		
					Anxiety& emotion	PND120	Decreased head dips in elevated plus maze (decreased anxiety), 3 mg/kg/d only, M not F		No Elevated plus maze effect only at 3 mg/kg/d
					Social behavior & maternal interaction	PND75-80	Increased offensive posture behaviors, 6 mg/kg/d, only M tested; No effect on non-social behaviors		Yes Aggression NOEL=3 mg/kg/d
						PND90	No effect on induced maternal behaviors		
Venerosi et al., 2006 Italy	Mouse CD-1	3, 6 mg/kg/d sc in peanut oil Plus vehicle on PND11-14	GD15-18	Cites Ricceri 2006 findings	**Social behavior & maternal interaction**	PND120	Increased ultrasonic vocalizations, 6 mg/kg/d; only F tested Increased social investigation, 6 mg/kg/d	Litter as unit; 10 litters/ gestational dose	Yes NOEL=3 mg/kg/d

[a] Sample sizes and analyses refer only to behavioral measures
[b] Graded response across doses or else effect at higher but not lower dose(s)
M, F, Male, Female

Table App 1-13. *In vivo* studies of chlorpyrifos administered postnatally. Effects described are only those measured after weaning. Bold indicates functional domain showed effects.

Study & laboratory	Species & strain	Dose, route, vehicle	Dosing period	AChE inhibition	Domain	Age of testing	Outcome, effective dose & gender	Notes[a]	Dose-response[b]?
Dam et al., 2000 Duke	Rat SD	1 mg/kg/d sc in DMSO	PND1-4	~60% brain (regions) inhibition in M, ~20% in F; more at 2 hr than 4 hr after single dose on PND1	**Motor activity**	PND21, 30	Decreased open field activity & rearing, M not F	5-7/sex/dose; Maybe split-litter dosing but not explicit; Also some preweaning testing	NA
Levin et al., 2001 Duke	Rat SD	1 mg/kg/d sc in DMSO	PND1-4	Not measured	**Motor activity**	PND28-42	Longer latencies (slower) in t-maze, M not F	10/sex/dose; Split-litter dosing; No ACh system involvement	NA
							No effect on habituation in figure-8, M&F		
					Cognition	PND28-42	No effect on t-maze spontaneous alternation, M&F		
						PND56-91	Increased radial arm maze working & reference errors, early in training, M not F; Decreased radial arm maze working & reference errors, F not M		
Adridge et al., 2005 Duke	Rat SD	1 mg/kg/d sc in DMSO	PND1-4	Not measured	**Cognition**	PND64+	Increased radial arm maze working & reference memory errors, M not F; Decreased working memory	9/sex/dose; Probably split-litter dosing but not explicit	NA

							errors in radial arm maze, F not M		
					Anxiety & emotion	PND52-53	Increased open arm time in elevated plus maze (decreased anxiety), M not F		
						PND54	Decreased chocolate milk preference (anhedonia), M&F		
					Motor activity	PND52-53	Increased center crossings in elevated plus maze, M not F		
						PND64+	No differences in radial arm maze latencies, M&F		
Ricceri et al., 2003 Italy	Mouse CD1	1, 3 mg/kg/d sc in DMSO	PND1-4	20, 23% brain inhibition at low & high dose 1 hr after dose on PND4; no inhibition at 4, 24 hr	**Motor activity**	PND25	No effect on open field activity	Split-litter dosing; n=8-18/sex/dose; says all mice got all tests but there are unexplained differences in sample size across tests; Bonferroni-corrected post-hocs followed ANOVA main effects with p>0.05; Also some preweaning testing	No, ChE & agonistic behaviors similar at both doses, LOEL=1 mg/kg/d
						PND35-38	Increased activity at door opening in 2-chamber box, only 3 mg/kg/d tested, M&F		
					Anxiety & emotion	PND38	No effect on novelty preference in 2-chamber box, only 3 mg/kg/d tested, M&F		
					Social behavior & maternal interaction	PND45	No effect investigative, affiliative, or soliciting behaviors, M&F; Decreased self-grooming, 1&3		

							mg/kg/d, M&F		
							Increased frequency & duration agonistic behavior sum, differs across time, 1&3 mg/kg/d, M not F		
					Cognition	PND60	No effect on passive avoidance, only M tested		
Dam et al., 2000 Duke	Rat SD	5 mg/kg/d sc in DMSO	PND11-14	~20-30% brain (regions) inhibition, M&F, more at 4 hr than 2 hr after single dose on PND11	**Motor activity**	PND21, 30	Increased open field rearing & grooming, M&F	5-7/sex/dose; Maybe split-litter dosing but not explicit; Figure 3 says male only but gender differences not significant	NA
Levin et al., 2001 Duke	Rat SD	5 mg/kg/d sc in DMSO	PND11-14	Not measured	**Motor activity**	PND28-42	Longer latencies (slower) in t-maze, M not F	10/sex/dose; Split-litter dosing; ACh system involvement	NA
							Decreased habituation (slower) in figure-8, M&F		
					Cognition	PND28-42	No effect on t-maze spontaneous alternation, M&F		
						PND56-91	No effect on radial arm maze working or reference memory, M&F		
Ricceri et al., 2003 Italy	Mouse CD1	1, 3 mg/kg/d sc in DMSO	PND11-14	No brain inhibition at 1, 4, 24 hr after dose	**Motor activity**	PND25	Increased distance traveled in open field, 1&3 mg/kg/d, M&F	Split-litter dosing; n=8-18/sex/dose; says all mice got all tests but there	Yes & No Dose-response on activity, 1

						PND35-38	Increased activity following door opening in 2-chamber box, only 3 mg/kg/d tested, M&F	are unexplained differences in sample size across tests; Bonferroni-corrected post-hocs followed ANOVA main effects with p>0.05	has more effect on agonistic behaviors LOEL=1 mg/kg/d
					Anxiety & emotion	PND38	No effect on novelty preference in 2-chamber box, only 3 mg/kg/d tested, M&F		
					Social behavior & maternal interaction	PND45	No effect on investigative or affiliative behaviors, M&F; Increased soliciting behavior, 3 mg/kg/d, M&F		
							Increased frequency & duration agonistic behaviors throughout, 1&3 mg/kg/d, M not F		
					Cognition	PND60	No effect on passive avoidance, only M tested		
Ricceri et al., 2006 Italy	Mouse CD-1	1, 3 mg/kg/d sc in peanut oil Plus vehicle to dam GD15-18	PND11-14	24 hr after last dose (PND15): no brain inhibition, serum inhibition both doses	**Motor activity**	PND70	Increased crossing in open field, only 1st 5 min, 3 mg/kg/d, only M tested	Litter as unit; 10 litters/gestational dose, postnatal split-litter dosing; Says anxiety effect in both sexes but not significant in males	Yes NOEL=1 mg/kg/d (most endpoints)
						PND120	No effect on elevated plus maze activity, M&F		
					Social behavior & maternal	PND75-80	Increased attack behaviors, 3 mg/kg/d, only M		Yes maternal LOEL=1

					interaction		tested; No effect on M non-social behaviors		mg/kg/d
						PND90	Increased induced maternal behaviors, 1&3 mg/kg/d		
					Anxiety	PND120	Increased time in open arms in elevated plus maze (decreased anxiety), 3 mg/kg/d, F only		Yes NOEL=1 mg/kg/d
Venerosi et al., 2006 Italy	Mouse CD-1	1, 3 mg/kg/d sc in peanut oil Plus vehicle to dam GD15-18	PND11-14	Not measured, cites Ricceri 2006 findings	**Social behavior & maternal interaction**	PND120	No effect on ultrasonic vocalizations or social investigation, only F tested	Litter as unit 10 litters/gestational dose, postnatal split-litter dosing	Yes NOEL=3 mg/kg/d
Venerosi et al., 2008 Italy	Mouse CD-1	3 mg/kg/d sc in peanut oil	PND11-14	Not measured	**Social behavior & maternal interaction**	PND40-45	No effect on social interaction with same-sex conspecific, M&F	Litter as unit; Split-litter dosing, 2/sex/dose/litter; N=15 litters; N=9-14/dose for some maternal tests; Female offspring mated on PND60, maternal tests after giving birth	NA
						LD1-7	In lactating dams: Longer latency to build nest; Shorter latency to lick pups; Increased time digging (non-maternal behavior)		
						LD7	In lactating dams: Decreased defensive, increased social investigative, & increased digging behaviors when		

							exposed to intruder male		
					Anxiety & emotion	LD2	In lactating dams: Faster entry & more mice entering light side (decreased anxiety)		
					Motor activity	PND40-45	Decreased distance moved during acclimation before social test, 1st time block, M&F		
Johnson et al., 2009 MSU	Rat SD	Incrementing doses: low, 1 mg/kg/d; mid, 1 up to 4 mg/kg/d; hi, 1.5 up to 6 mg/kg/d Po in corn oil	PND1-21	PND20 (time after dose not given) – 14-53% brain (hippocampal) inhibition, all doses significant; PND30-40, mid & high doses still show inhibition (17-23%); No inhibition PND50; M&F same	**Cognition**	PND36-60	Increased radial arm maze working memory errors, hi dose throughout training, all doses during last week, M only; Decreased radial arm maze reference memory errors, middle to end of training, mid & hi doses, F only; Increased radial arm maze reference errors, middle week on training, mid & hi doses, M only	Split-litter dosing; n=10-14/sex/dose; Table 1 says PND20, not 21	Yes LOEL=low dose (1 mg/kg/d PND1-21)
					Motor activity	PND36-60	No effect on response time in radial arm maze		
Carr et al., 2001 MSU	Rat SD	Incrementing doses: low, 3 mg/kg/d;	PND1-21, every other day	PND25, 30 – 14-26% brain (regions)	**Motor activity**	PND25, 30	Decreased open field activity, mid & hi, M&F	Between-litter dosing; N=5 litters/dose,	Yes Activity NOEL=low

		mid, 3 up to 6 mg/kg/d; hi, 3 up to 12 mg/kg/d Po in corn oil		inhibition at low dose; all doses significant, M&F same				2/sex from each litter; Used within-litter factor in analysis; Also some preweaning testing	dose (3 mg/kg/d PND1-21)
Jett et al., 2001 JHU	Rat LE	0.3, 7 mg/kg/d sc in peanut oil	PND7, 11, 15 "preweaning study"	No brain inhibition on PND7 (3 hr after dose), 8, 16, or 28	**Cognition**	PND24-28	Longer latencies to find platform in Morris water maze, 7 mg/kg/d throughout, 0.3 mg/kg/d on day 1 (p=0.05), M&F; Less time spent in training quadrant, 7 mg/kg/d, M&F	Between-litter dosing after random culling; N=8-10/sex/dose, "2 or more litters used"; "Postweaning" study not presented	Yes NOEL or LOEL=0.3 mg/kg/d (p=0.05) Authors say both ways

[a] Sample sizes and analyses refer only to behavioral measures
[b] Graded response across doses or else effect at higher but not lower dose(s)
M, F, Male, Female

Table App 1-14. *In vivo* studies of chlorpyrifos administered during gestation and postnatally, continuous or discontinuous exposures. Effects described are only those measured after weaning. Bold indicates functional domain showed effects.

Study & laboratory	Species & strain	Dose, route, vehicle	Dosing period	AChE inhibition	Domain	Age of testing	Outcome, effective dose & gender	Notes[a]	Dose-response[b]?
Maurissen et al., 2000 Dow	Rat SD	0.3, 1, 5 mg/kg/d po in corn oil	GD6-LD10	Dam on GD20, 4-5 hr after dose, 45% blood but no brain inhibition at 0.3; greater blood and brain inhibition at higher doses	Neuromotor function	PND22	Increased auditory startle latency did not meet lower p-value, 5 mg/kg/d, M&F; "effects may have been real" – p-value =0.03	Litter as unit; 1/sex/litter; N=8/sex/dose for delayed alternation (same rats at both ages); N=20/sex/dose for startle and motor activity (same rats at both ages); alpha set at 0.02; Also some preweaning tests; Maternal toxicity at 5 mg/kg/d	No doses showed effects using higher statistical threshold
						PND61	No effect on auditory startle measures, M&F		
					Motor activity	PND21 and PND60	No effect on total counts or habituation at either age, M&F; High dose "may have had ..slight increase in activity on PNDs 17, 21, and 60"		
					Cognition	PND22-24 and PND61-91	No effect on spatial delayed alternation learning at either age, M&F		
Braquenier et al., 2010 Belgium	Mouse CD-1	0.2, 1, 5 mg/kg/d po in corn oil	GD15-LD14	Pup on PND1, 3 hr after dose to dam, 14% brain inhibition, only 5 mg/kg/d group tested	Motor activity	"Adult"	No effect on locomotor activity	1/litter for each test; only F tested; N=8-10/dose; Dunnetts post hoc after main effect p<0.10	No Effect only at 1 mg/kg/d NOEL=0.2 mg/kg/d
						PND80	No effect on elevated plus maze total arm entries		
					Anxiety & emotion	"Adult"	Decreased time in chamber center in light-dark box and decreased box switches (increased anxiety), 1mg/kg/d only;		

Study & laboratory	Species & strain	Dose, route, vehicle	Dosing period	AChE inhibition	Domain	Age of testing	Outcome, effective dose & gender	Notes[a]	Dose-response[b?]
						PND80	Decreased time & entries in elevated plus maze open arm (increased anxiety), 1 mg/kg/d only		
Ricceri et al., 2006 Italy	Mouse CD-1	3, 6 mg/kg/d po in peanut oil Plus 1, 3 mg/kg/d sc in peanut oil on PND11-14	GD15-18 "pre" plus PND11-14 "post"	Dam on GD19 (pilot study): 40% brain ChE inhibition at 6 mg/kg/d, serum inhibition at both doses Pup on GD19: no brain inhibition, serum inhibition at both doses Pup on PND15: no brain or serum inhibition	**Motor activity**	PND70	Increased crossing in open field, 6 mg/kg/d prenatal plus 1, not 3 mg/kg/d, postnatal		NA
						PND120	No pre-post interaction on elevated plus maze activity, M&F		
					Anxiety & emotion	PND120	No pre-post interaction on elevated plus maze, M&F		
					Social behavior & maternal interaction	PND75-80	No pre-post interaction on social behaviors, M&F		
						PND90	No pre-post interaction on induced maternal behaviors		
Venerosi et al., 2006 Italy	Mouse CD-1	3, 6 mg/kg/d sc in peanut oil Plus 1, 3 mg/kg/d sc in peanut oil on PND11-14	GD15-18 "pre" plus PND11-14 "post"	Cites Ricceri 2006 findings	Social behavior & maternal interaction	PND120	Increased ultrasonic vocalizations & social investigation at 6 mg/kg/d prenatally reversed by 1&3 mg/kg/d postnatally		NA

[a] Sample sizes and analyses refer only to behavioral measures
[b] Graded response across doses or else effect at higher but not lower dose(s)
M, F, Male, Female

3.2.2.2. Update on Developmental Neurotoxicity Studies of Chlorpyrifos as of August 2014

There are six new studies since early 2012, and five of these are from laboratories that have not published behavioral studies of this type before. Note, however, that most of these laboratories have published neurochemical, molecular, or *in vitro* studies with chlorpyrifos or other OPs. Most of these studies use the same dosing paradigms as earlier studies.
Of particular interest is a recent publication from the Duke laboratory (Slotkin et al., Neurotox. Teratol. 37:1-12, 2013) that reported 5-10% inhibition of fetal brain ChE activity at 2 hr after the last dose of 1 mg/kg/d sc in DMSO administered PND1-4. This dose is widely used in many of these studies, and this information clearly shows that while this dose does not induce toxic or developmental effects, it does have a small but significant effect on fetal brain ChE.

A critical review of the six papers follow:
1) Chen X-P, Chen W-Z, Wang F-S, Liu J-X. (2012) Selective cognitive impairments are related to selective hippocampus and prefrontal cortex deficits after prenatal chlorpyrifos exposure. Brain Res. 1474:19-28.
This study represents a new laboratory to the field. ICR mice were used, no information on how many pregnant females they started with. Chlorpyrifos (1, 5 mg/kg/d) was given GD13-17 sc in DMSO. The paper does not specify if litters were culled or sex distribution within litters. Littermates were randomly re-distributed across litters every 7 days, there is no information given as to whether littermates were allocated to different dams or if the litter stayed as a unit. On PND20, 8 litters were chosen for testing. It says that littermates were divided into groups, but it is not clear if all pups in a litter tested together – figure legend does say n=8 mice per group. It is also not clear how pups were sampled, whether there were equal numbers of males and females, or whether the statistics accounted for littermates.

Behavioral testing was conducted in a T-maze using an alternating procedure, i.e., for each trial the baited arm alternated. They conducted 10 trials/session, 2 sessions/ day (does not say how far apart), for 3 days for each inter-trial interval (ITI). Training used the shortest ITI (no time between trials), then used 10, 20, 40, and 60 sec ITIs. It does not give the order of presentation of the different ITIs. Win-shift failure, or errors, are described as going to the wrong arm, which triggered training that is not clearly described. Lose-shift failure, or errors, was defined as continuing to go to the wrong arm. Training began on PND45 (after 4-5 days acclimation to the apparatus) and continued 15 days.

Adult offspring showed no difference in percent correct on first choice. It reports a sex effect and then analyzes them separately, but not clear if there was a sex*trial interaction. Lose-shift errors were significant in males, showed a trend in females – they reported male effects at longer delays (40, 60 sec) and effect in females only at 10 sec. Only the high dose was significant. Procedural details are not well-explained, especially the training that was initiated after each choice error. Figure legend states 8 mice per group, which could imply one mouse from each litter, but unclear if they were still littermates. Additional uncertainties in the statistics include how littermates and sex were handled and whether there were significant dose*trial, sex*dose, or dose*sex*trial interactions. Even in controls, there is not an evident delay-retention gradient, in that 60 sec performance looks similar to 0 sec, suggesting that even 60 sec may have been too

short a delay. T-maze data are usually somewhat variable, yet the data show remarkable, and too much, consistency. This raises questions as to the actual procedure used, and whether the apparent treatment effects are real.

2) Cole TB, Fisher JC, Burbacher TM, Costa LG, Furlong CE. (2012) Neurobehavioral assessment of mice following repeated postnatal exposure to chlorpyrifos-oxon. Neurotoxicol. Teratol. 34:311-322.
This study represents another new laboratory to conduct DNT studies, although they are an experienced laboratory for studies of PON1 and OPs. This study used only the oxon in PON1-knockout mice, thus it is not exactly comparable to other chlorpyrifos studies. However, given that the knockout mice were assumed to be more sensitive, and the current interest in the oxon, the study is relevant.

This was part of a larger study with PON$^{-/-}$, $^{+/+}$, and humanized PONs, but only the PON$^{-/-}$ mice were tested behaviorally. The study was done in two cohorts and only findings that were seen in both were reported. On PND4 mice were culled to three males and three females. Pups were directly dosed (sc in DMSO; 0.15, 0.18, 0.25 mg/kg/d) on PND4-21: all mice in a litter received the same dose. One male and one female per litter were allocated to a set of tests, another male and female per litter allocated to another set of tests, designated sets "A" and "B". Before weaning, all mice were tested for ontogeny of righting reflex, cliff avoidance, and negative geotaxis. Tests for set "A" were: rotarod PND23, pre-pulse acoustic startle reflex PND25 and 50, fear conditioning PND62-64, and water radial arm maze PND77-116. Set "B" tests were: open field PND25, Morris water maze PND70-98, and served as no-shock controls in fear conditioning. All procedures were standard and appropriate for these tests. It is not clear whether sex was nested or analyzed as a within-litter variable.

Littermates of these mice were used to measure brain AChE activity on PND22 (day after last dose), and showed 10%, 16%, and 27% inhibition at the three increasing doses. Body weight was decreased at 0.25 mg/kg/d, which appeared during dosing and persisted through adulthood. Hyperkinetic responses (jumping, darting) were noted in all dose groups within 5 min of dosing beginning PND15. Of all the neurobehavioral and cognitive tests, only decreased startle latency was detected in the 0.18 and 0.25 groups on PND50. Also on that day only the 0.18 group showed an effect on pre-pulse amplitude, but no effect of the pre-pulse inhibition. The high dose of 0.25 mg/kg/d showed no behavioral effects.

The study was conducted appropriately, and generally analyzed correctly except that it appears that sex was not nested. The graphs do not show males and females separately (and it is unclear which it is, or if it's combined) but supplemental data show sex-related differences in some of the tests where it's expected (e.g., fear conditioning). The authors discuss the shorter startle latency as representing stronger synaptic connections, but do not adequately address the lack of dose response. Generally the lack of oxon effects stands in contrast to many of the studies that report effects of chlorpyrifos using the same behavioral tests.

3) Vatanparast J, Naseh M, Baniasadi M, Haghdoost-Yazdi H. (2013) Developmental exposure to chlorpyrifos and diazinon differentially affect passive avoidance performance and nitric oxide synthase-containing neurons in the basolateral complex of the amygdala. Brain Res. 1494:17-27

This paper is from another laboratory that is new to this field. Wistar rats received 1 mg/kg/d sc in DMSO either on GD15-18 or directly to pups on PND1-4. Adult offspring (PND60) were tested in an open field followed by passive avoidance. After two habituation trials, rats were subjected to shock when they crossed over. This was repeated every 2 min until they did not cross in 120 sec. At 24 hr they were retested. Note that rats that did not go into the dark side during the habituation trials were eliminated, this results in a selection bias that could have been related to dose but no information is given. Dependent variables were trials to criterion on the day of training, and step-through latency and time in dark side (directly correlated with latency) at 24 hr. Litters were culled and 1 male and 1 female per litter were tested with at least 12 litters/treatment. Analysis uses sex as a factor but not nested within litter.

There were no changes in activity, nor were there changes in trials to criterion. In the retention test, prenatally dosed rats showed decreased latency, similarly in males and females. There were no effects in postnatally treated rats.

Group means for trials to criterion are very close to 1, meaning that most rats met it in 1-2 trials; therefore, there was not much dynamic range with which to detect effects. However, the 24-hr retention data show an obvious treatment effect. Passive avoidance has not been tested previously in rats, but there were no effects in mice treated postnatally (Ricceri et al., 2013).

4) Mullen BR, Khialeeva E, Hoffman DB, Ghiani CA, Carpenter EM. (2013) Decreased reelin expression and organophosphate pesticide exposure alters mouse behaviour and brain morphology. ASN NEURO 5:27-42.

This study is from a new laboratory, and focuses on chlorpyrifos interactions in mice having decreased or normal expression of the reelin gene which has been associated with autism. Results of chlorpyrifos treatment in the reelin$^{+/+}$ mice (normal expression) are evaluated here. Osmotic minipumps were implanted on GD13.5, releasing 6 mg/ml oxon in PBS, apparently over 3 days. There was no extrapolation to administered dose. Reelin$^{+/+}$ and $^{+/-}$ were bred, resulting in litters with mixed genotypes. There were 9 oxon and 13 vehicle litters, and there were at least 6 mice per treatment/genotype group. It is unclear how many males and females tested came from the same litter. AChE was measured in fetal heads on GD16.5. On PND7, litters were separated from the dam and USVs were measured. On PND30 there was a 30-min open field session, also a social interaction test. Marble burying was measured on PND60. Measured were collected using an overhead video system. Outliers were rejected (no criterion given) and data were analyzed with t-tests and "aggregate" ANOVAs (no information where each approach was used).

AChE activity was decreased by about 15% (not stated whether this was significant). There was a decrease in the number and duration of USVs, similarly in males and females. Males only showed decreased sniffing duration in the open field. Females only showed an increased in sniffing at stranger 1 in the social interaction test, but no difference when stranger 2 was introduced. There was no change in marble burying. There were several other non-significant differences which they interpret as being effects.

The only statistically significant changes were seen with sniffing, and this was not consistent in

males vs females. It is not described what algorithm was used to define sniffing in the software using an overhead camera, and this information is not available at the manufacturer's website. Sniffing is a questionable measure of activity, and in the social interaction test, a better measure is time spent with the other mice, yet this was not altered. The data are not particularly convincing.

5) Ohishi T, Wang L, Akane H, Itahashi M, Nakamura D, Yafune A, Mitsumori K, Shibutani M. (2013). Reversible effect of maternal exposure to chlorpyrifos on the intermediate granul cell progenitors in the hippocampal dentate gyrus of rat offspring. Reprod. Toxicol. 35:125-136. This is a new laboratory to do these studies. SD rats were given chlorpyrifos in the diet from GD10-PND21, at 2.8, 14, 70 ppm. AChE was measured in dams at weaning and in male offspring on PND21 and PND77. Preweaning physical and righting reflex milestones were measured. Detailed clinical observations were noted at PND29, 48, 71. Also on PND71, rats were subject to FOB-type measures: sensory responses (click, visual approach, touch, tail pinch, pupil), righting reflex, landing foot splay, and forelimb and hindlimb grip strength. Motor activity was also measured that day in 1-hr session but it does not specify type of chamber or cage. Biel maze testing on PND45-58 looked at latencies and choice errors. Behavioral tests used 1 male and 1 female per litter, and litter was used as the unit of measure for stats. However, it does not appear that sex was considered as a factor in the analyses, and repeated measures were not used where it should have been. This study design is generally consistent with developmental neurotoxicity test guidelines, and is comparable to the Maurissen et al. (2000) paper.

Maternal intakes over exposure were calculated to be 0.36, 1.86, and 9.18 mg/kg/d. In dams (PND21), AChE was decreased in RBC (all doses, up to ~80% inhibition), plasma (14 and 70 ppm, similar magnitude), and brain (70 ppm only, similar magnitude). On PND21 in offspring, AChE was inhibited in RBC and plasma (14 and 70 ppm, ~70% inhibition) and brain (70 ppm only, ~50%). There were no treatment effects on milestones or FOB-like measures. There was increased body weight in both sexes during postnatal period but only in 14 ppm group, along with faster eye opening (2.8 and 14 ppm) and faster incisor eruption (14 ppm). Motor activity was different in 2 intervals in 14 ppm males, otherwise there were no effects. There were decreased errors in one trial in 2.8 ppm males in the Biel maze, and increased latency in the straight channel on the first trial only in the 2.8 and 14 ppm groups. The high dose of 70 ppm had no effects.

This study had no doses where AChE was not at all inhibited, but brain ChE was inhibited only at the high dose in both dams and pups. Magnitude of inhibition was quite high, but there was no mention of toxic signs, and dams gained weight normally (Note: this finding is somewhat unexpected). The pre-weaning weight and development differences at 14 ppm are in the opposite of the expected direction, and suggest differences in actual gestational age. The few significant intervals and trials were not dose-related and most likely were spurious due to the multiple tests run, instead of doing a repeated-measures ANOVA. If the appropriate statistics were used, it is doubtful there would be any significant differences.

6) Levin ED, Cauley M, Johnson JE, Cooper EM, Stapleton HM, Ferguson PL, Seidler FJ, Slotkin TA. (2014). Prenatal dexamethasone augments the neurobehavioral teratology of

chlorpyrifos: Significance for maternal stress and preterm labor. Neurotoxicol. Teratol. 41:35-42.
This laboratory published extensively in this area in the early 2000s. The focus of this study is interactive effects of prenatal dexamethasone and postnatal chlorpyrifos; however, for this review only the effects in the chlorpyrifos alone group compare to control are considered. Dams received saline sc GD17-19, then chlorpyrifos 1 mg/kg/d sc was given to pups on PND1-4. Litters were culled after birth and redistributed within treatment group every few days. Figure legend (only) states 11-12 per treatment, but never states that one male and one female came from each litter; however, that is the usual procedure used in this laboratory. The same offspring went through all behavioral tests: T-maze exploration in week 4, motor activity (figure 8 chamber) week 5, novel environment feeding in week 7, novel object recognition in week 9. There were no effects of chlorpyrifos on T-maze or the novel environment feeding, but note that while there were no significant differences, the abstract and discussion say that there were effects. Chlorpyrifos increased motor activity in males only, but the change in habituation did not reach significance (although the authors claim there is an effect). On novel object recognition, there was an increased overall exploration in males only, but no effect on differential time between novel and familiar objects.

The study was generally conducted properly, using many of the same methods from earlier publications. Effects are somewhat exaggerated in that non-significant differences are presented as effects. The increased motor activity likely accounts for increased activity in the novel object test, and there is no evidence of a cognitive effect.

The effects described in this study disagree with more than half of the findings from earlier studies in this laboratory using the same dosing paradigm. Specifically, the effects and the associated functional domains here are: 1) Activity – Increased figure-8 activity and increased exploration in novel object test, males only, and no effect on T-maze exploration, which agree with increased elevated plus maze activity, males only (Aldridge et al., 2005) but not with decreased T-maze activity in males (Levin et al., 2001) or decreased open field activity in males (Dam et al., 2000); 2) Cognition – No effect on T-maze alternation or novel object recognition, which agree with no effect on T-maze alternation (Levin et al., 2001) but disagree with deficit in radial arm maze performance in males, and improved function in females; 3) Anxiety/Emotion – No effect on novel environment feeding which disagrees with evidence of decreased anxiety in elevated plus maze and anhedonia suggested by decreased chocolate milk preference (Aldridge et al., 2005). The authors do not discuss these discrepancies.

Overall summary:

For half of the studies, the lowest dose was 1 mg/kg/d, and two studies used the oxon, making it difficult to compare dose levels. Only one study used a lower dose, 0.36 mg/kg/d, in feed, and even this level was sufficient to produce a great degree of RBC ChE inhibition. The functional effects are summarized below (See Table App 1-15).
1) Cognition – Of these studies, cognition was generally unaffected except for an effect on passive avoidance; interestingly, previous studies reported no passive avoidance effects. There were no effects on other cognitive tests that did show changes in earlier studies, as well as no effects in new tests being used for the first time. Thus, what appeared to be fairly consistent reports of cognitive changes from earlier studies is not so conclusive.

2) Anxiety & emotion – The only study which evaluated this domain reported no effect, in contrast to previous reports, including one from the same laboratory.
3) Social & interactive behavior – Only one study measured this and reported increased interaction with a stranger mouse (albeit with a questionable measure), whereas earlier studies showed mixed effects on other tests of social investigation.
4) Activity – Most of these studies report no change in activity, with only one report of increased activity and another of decreased sniffing (a questionable measure of activity). Combined with the previous literature, this continues to show inconsistent changes in motor activity levels and habituation.
5) Neuromotor development and function were generally unaltered except for one measure that did not show a dose-response. Few other studies have addressed this.

Conclusions: There continue to be inconsistencies in effects in relation to functional domains, dosing paradigms, and gender-specificity. The only studies reporting effects used doses that inhibited fetal/pup brain ChE activity to some degree, even though there were many negative effects at these same doses.

Table App 1-15. Summary of newest studies. Bolded domains indicate significance in some measure within that domain.

Study & laboratory	Species & strain	Dose, route, vehicle	Dosing period	AChE inhibition	Domain	Age of testing	Outcome, effective dose, gender	Notes	Dose-response?
Chen et al., 2012 Hangzhou China	Mouse ICR	1, 5 mg/kg/d sc in DMSO	GD13-17	Not measured	Cognition	PND45-60	Increased lose-shift errors in T-maze delayed alternation at 5 mg/kg/d M, trend F	Litter allocation and statistical treatment not clear. Control data don't show expected retention decay at longer ITIs	Yes NOEL=1 mg/kg/d
Levin et al., 2014	Rat SD	1 mg/kg/d sc in DMSO	PND1-4 (saline vehicle GD17-19)	Not measured	Cognition	PND28; PND63	No effect on T-maze alternation; No effect on differential exploration in novel object recognition	12-14/dose, no more than 1 of each sex from each litter; litters redistributed every few days Did not next sex in litter Study addresses CPF and dexamethasone interactions; Presents non-significant data as effects	NA
					Activity	PND28; PND35; PND63	No effect on T-maze alternation; Increased total activity in figure-8, M only; Increased overall activity in novel object recognition, M only		
					Anxiety & emotion	PND49	No effect on novel environment feeding		
Vatanparast et al., 2013	Rat Wistar	1 mg/kg/d sc in DMSO	GD15-18	Not measured	Activity	PND60	No effect in open field, M&F	1 male and 1 female each litter, but sex not nested in litter for analyses	NA
					Cognition	PND60-63	Decreased step-through latency at 24 hr in passive avoidance, M&F		
			PND1-4		Activity	PND60	No effect in open field, M&F		
					Cognition	PND60-63	No effect in passive avoidance, M&F		
Mullen et al., 2013	Reelin$^{+/+}$ mice	6 mg/ml oxon in PBS, with osmotic minipmp	~GD13.5-16.5	~15% inhibition in fetal heads GD16.5	**Activity**	PND30	Decreased sniffing in open field, F not M	Sampling of M and F from litters not clear, sex not nested in stats	NA
					Social	PND30	Increased sniffing of first stranger in social interaction test, F not M		
					Anxiety & emotion	PND60	No change in marble burying, M&F		

Ohishi et al., 2013	Rat SD	2.8, 14, 70 ppm in diet, giving 0.36, 1.86, 1.98 mg/kg/d	GD10-PND21	Dams on PND21: inhibition (max ~80%) in RBC (all doses), plasma (14 and 70), brain (70) Pups on PND21: inhibition (max ~70%) in RBC and plasma (14 and 70), brain (70) No inhibition on PND77	Activity	PND71	Changes in activity in two intervals only, 14 ppm, M not F, likely spurious	Did not nest sex in litter; Appears that sex was not factor in analysis and repeated measures was not used	LOEL=2.8 ppm (0.36 mg/kg/d) (RBC ChE)
					Cognition	PND56-58	Decreased errors in one trial only in Biel maze, 2.8 ppm, M not F, likely spurious		
					Neuromotor	PND56-58 Preweaning & PND29, 48, 71	Increased latency in Biel maze in straight channel, 2.8 and 14 only, M not F, likely spurious No effect on many measures of development, sensorimotor, neuromuscular function		
Cole et al., 2012	PON$^{-/-}$ mice	0.15, 0.18, 0.25 mg/kg/d oxon sc in DMSO	PND4-21	10%, 16%, 27% inhibition in littermates on PND22	Cognition	PND62-64; PND77-116; PND70-98	No effect on fear conditioning, M&F; No effect on water radial arm maze, M&F; No effect on Morris water maze, M&F		No Similar effects at 0.18, 0.25 mg/kg/d NOEL= 0.15 mg/kg/d
					Motor activity	PND25	No effect in open field		
					Neuromotor function	Preweaning; PND23; PND25, 50;	No effect on reflex ontogeny, M&F; No effect on rotarod, M&F; Decreased acoustic startle latency on PND50, 0.18 & 0.25 mg/kg/d, M&F; Altered pre-pulse startle, 0.18 mg/kg/d only, M&F		

4.0. References

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Appendix 2. Detailed Review and Synthesis of Three Children's Environmental Health Cohort Studies

NOTE: Appendix 2 contains the Detailed Review and Synthesis of the Three Children's Environmental Health Cohort Studies. This information was presented to the FIFRA Scientific Advisory Panel (SAP) in April of 2012; however, it is supplemented by both an external Federal panel review performed June –September 2012, and also the results of a on-site meeting with staff of the Columbia Children's Center for Environmental Health (CCCEH) in April 2013. The CCCEH is one of the three children's environmental health cohorts included in the EPA review. EPA has considered the FIFRA SAP input regarding the analysis and interpretation of these epidemiologic studies in the completion of the revised chlorpyrifos HHRA (April 2012 FIFRA SAP review of this information can be found at: http://www.epa.gov/scipoly/sap/meetings/2012/april/041012minutes.pdf.) Information concerning the Federal letter review of a subsequent study published by CCCEH researchers in addition to other questions can be found in the Federal docket at: http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2008-0850-0170. The Federal panel review considered the results of a Magnetic Resonance Imaging (MRI) study which detailed alterations in brain morphology in children by *in utero* chlorpyrifos exposure level. Within the Federal letter review, EPA also specifically requested feedback upon areas of child neurodevelopment testing for which the April 2012 FIFRA SAP expressed a lack of expertise to fully consider. Lastly, in April 2013, EPA scientists met with CCCEH researchers to address a series of questions generated by the April 2012 FIFRA SAP, the June 2013 Federal letter review, and EPA deliberations including the need for obtaining certain "raw data" or original analytic data files to address important uncertainties in the use of these data in the HHRA. Specifically, questions regarding whether additional information may be available through the CCCEH data collection efforts to further characterize chlorpyrifos exposure of CCCEH study participants and to better characterize other environmental exposures within the study population. The results of this meeting are encapsulated in the EPA Memorandum "Columbia Center for Children's Environmental Health (CCCEH) Epidemiology Data Acquisition ("Raw Data" Request) and is included as Appendix 6 to the revised chlorpyrifos HHRA. The input provided from the two reviews and the results of further inquiry of the CCCEH researchers by EPA is encapsulated herein. The combined force of this material informed EPA's deliberations concerning the content of the revised chlorpyrifos HHRA.

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1.0 Scope and Purpose

In September 2008, EPA presented to the FIFRA SAP its preliminary review of available epidemiologic investigations of prenatal exposure to chlorpyrifos in association with measures of fetal growth and adverse neurodevelopmental effects in three major prospective children's health cohorts in the U.S.[67] These are: 1) The Mother's and Newborn Study of North Manhattan and South Bronx performed by Columbia University researchers referred in this document as "Columbia Mother's and Newborn Study;" 2) the Mt. Sinai Inner-City Toxicants, Child Growth and Development Study or the "Mt. Sinai child growth and development study;" and, 3) Center for Health Assessment of Mother's and Children of Salinas Valley (CHAMACOS) conducted by researchers at University of California Berkeley, the "CHAMACOS study." In this document, EPA updates and expands its targeted evaluation of this important line of evidence regarding chlorpyrifos developmental neurotoxicity.

At the previous meeting, the Panel agreed with EPA's conclusions that "chlorpyrifos likely played a role in the birth and developmental outcomes noted in the three cohort studies" (pp. 37 Meeting Minutes). In support of this statement, the Panel offered that investigations performed within these three epidemiological cohorts utilized a similarly strong study design (prospective cohort); measured exposure using several different methods including specific and non-specific biomarkers of chlorpyrifos; ascertained developmental outcomes using validated assessment tools common to both clinical and research settings; and, analyzed, selected and statistically adjusted for potentially confounding variables using reasonable and appropriate methods. Overall, the Panel noted that the epidemiological database at that time presented an informative body of evidence with some notable consistencies across studies. Areas of inconsistency were also observed, and judged, in part, to be due to differing methods of measurement and evaluation, as well as dissimilar exposure profiles (*i.e.*, residential versus direct and indirect occupational (farm laborer) exposure). Importantly, the Panel at the 2008 FIFRA SAP meeting also stated that it could not conclude that chlorpyrifos was the sole contributor to these outcomes, as co-exposure to other organophosphate pesticides and mixtures of environmental exposures may also have played a part in these outcomes.

For the purpose of informing the chlorpyrifos risk assessment, the Panel concurred with the Agency with respect to the primacy of the Columbia Mother's and Newborn Study among the three birth cohort studies, although the Panel also encouraged the Agency to comprehensively consider the results of the three children's health cohorts. While the Columbia Mother's and Newborn Study researchers measured the parent compound chlorpyrifos, as opposed to non-specific organophosphate metabolites, the other cohorts examined health outcomes (the Brazelton index of neonatal development) and performed supplemental analyses (*e.g.,* effect modification by PON1 status) not reflected in the Columbia Mother's and Newborn Study database. Therefore, the Panel expected EPA could strengthen its understanding of the potential developmental neurotoxicity of chlorpyrifos by considering the three children's health cohort studies together. Additionally, the Panel in 2008 suggested supplemental statistical analyses to enhance understanding of epidemiological study results in the risk assessment context (See Attachment 1). The Panel also generally noted both strengths and limitations of these studies, and offered that random or systematic errors in the design, conduct or analysis of these studies

[67] See Meeting Minutes at: http://www.epa.gov/scipoly/sap/meetings/2008/september/sap0908report.pdf.

were unlikely to fully explain observed associations. However, the Panel also noted that absent the available toxicological and epidemiological databases was a well understood and defined mode of action as to the role of chlorpyrifos exposure in the etiology of adverse infant and child neurodevelopmental outcomes.

In the revised chlorpyrifos HHRA, EPA expands and updates its review of the available epidemiologic data concerning the effect of chlorpyrifos exposure on children's environmental health in conjunction with a review of recent experimental studies and hypothesized adverse outcome pathways (AOP). Observational studies published subsequent to the September 2008 FIFRA SAP evaluation extend the knowledge base of potential long-term sequalae of prenatal chlorpyrifos exposure. Specifically, in April 2011, researchers with each of the three prospective children's health cohort studies concurrently published results of their respective investigations of prenatal chlorpyrifos exposure and measures of intelligence among school aged children approximately 7-years (Bouchard et al., 2011; Engel et al., 2011; V. Rauh et al., 2011). Additionally, researchers with the Mt. Sinai Child Growth and Development study contributed their evaluation of organophosphate exposure and mental and psychomotor development, and authors with the CHAMACOS study published an evaluation of chlorpyrifos exposure and both fetal growth and neurodevelopment measures in young children as modified by paraoxonase-1 (*PON1*) genotype and phenotype (Eskenazi et al., 2010; Harley et al., 2011).

Researchers have also performed epidemiologic methods research which in many ways reduces uncertainties related to key measures within these studies. Within the Columbia Mothers and Newborn study, investigators published results of analyses evaluating the validity of prenatal chlorpyrifos exposure measures in the time periods immediately after the voluntary cancellation of chlorpyrifos for residential use (R. M. Whyatt et al., 2009; R. M. Whyatt et al., 2007), as well as employed innovative statistical techniques to further assess the potential confounding bias of socio-economic status (SES) in the relation between prenatal chlorpyrifos exposure and adverse neurodevelopmental health outcomes (Lovasi et al., 2011). Overall, these additions to the epidemiologic database concerning children's health effects in combination with other lines of evidence, add to the body of knowledge available to inform the ways in which chlorpyrifos exposure may be related to adverse neurodevelopment outcomes in children.

2.0 Summary of Epidemiology Findings

In this section, EPA summarizes and critically reviews epidemiologic studies of prenatal chlorpyrifos exposure and subsequent fetal growth and child development evaluated within the three prospective children's health cohorts described above. Specifically, in this section the design, conduct and methods of analysis of each cohort study are briefly presented; individual study results are summarized by type of health outcome investigated; and strengths and limitations of these investigations are discussed. Appendix 3 of the chlorpyrifos human health risk assessment (HHRA) includes detailed study reviews and critical analysis of each investigation and Appendix 4 includes extensive evidence tables summarizing details of each investigation. The following section 3.0 reflects EPA's synthesis and evaluation of the current chlorpyrifos epidemiology database from the three children's health cohorts. The results of the April 2012 FIFRA SAP review, the June 2012 Federal letter review, and the April 2013 on-site meeting with CCCEH study staff are integrated throughout the document where appropriate. In

accordance with the draft "Framework for Incorporating Epidemiology in Risk Assessment,"[68] this analysis considers the strengths and limitations reflected in each cohort and research study as well as modified Bradford Hill considerations for the synthesis of these data.

2.1 Overview of Design and Methods of Children's Health Studies

These cohorts were recruited for the purpose of studying the potential health effects of environmental exposures during pregnancy on subsequent child development: The Columbia University's Mother's and Newborn Cohort (Lovasi et al., 2011; V. Rauh et al., 2011; V. A. Rauh et al., 2006; R. M. Whyatt et al., 2009; R. M. Whyatt et al., 2007; R. M. Whyatt et al., 2004); The Mount Sinai Hospital Children's Environmental Health Cohort (Berkowitz et al., 2004; Engel et al., 2007; Engel et al., 2011); and UC Berkeley's the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) Cohort (Bouchard et al., 2011; Eskenazi et al., 2004b; Eskenazi et al., 2010; Eskenazi et al., 2007; Harley et al., 2011; Marks et al., 2010; Young et al., 2005). These studies enrolled pregnant women at baseline and prospectively assessed associations in their newborns and young children. Multiple reports have been published based on the findings in each cohort.

The three study populations reflect different exposure profiles. The Columbia Mother's and Newborn study and the Mt. Sinai Child Growth and Development study participants were likely exposed to chlorpyrifos through residential use of the pesticide for indoor pest control. In the residential setting, chlorpyrifos was among the most widely used household pesticides in the US during the time period of these epidemiologic investigations. However, pesticide companies voluntary cancelled indoor residential uses of chlorpyrifos-containing pesticide products on December 31, 2000, during the time period of these cohort investigations. In the agricultural setting, chlorpyrifos was registered for use on commonly consumed crops such as corn, almonds, apples and oranges. Therefore, these study populations were most likely additionally exposed to chlorpyrifos via the oral route through ingesting residues in the diet and from hand-to-mouth contact with in-home chlorpyrifos-contaminated surfaces, as well as possible dermal exposure through contact with treated areas in the home environment (R. M. Whyatt et al., 2003; R. M. Whyatt et al., 2009; R. M. Whyatt et al., 2007). In contrast, CHAMACOS cohort participants were employed as farm laborers or were residing in homes with farm laborers. These participants likely experienced either occupational exposure through the inhalation and dermal routes, as well as probable indirect exposure through drinking water and take-home exposures (Bradman et al., 2007). In each of the children's health cohorts, the prevalence of pesticide exposure was high; however, reported use of chlorpyrifos in the CHAMACOS region was modest (10% of total pesticide use) (Eskenazi et al., 2004a).

2.1.1. The Mother's and Newborns Study of North Manhattan and South Bronx (Columbia University)

Researchers with the Columbia Mother's and Newborn Study evaluated the association between prenatal exposure to pesticides including chlorpyrifos and developmental outcomes in children through age 7 years. In this birth cohort study, participants were recruited during early pregnancy (≤20th week) among African-American and Dominican women age 18-35 years, and registered

[68] http://www.epa.gov/scipoly/sap/meetings/2010/020210meeting.html#materials

for prenatal care and delivery at either New York Presbyterian Medical Center or Harlem City hospitals. Women who smoked, had a history of drug abuse, diabetes, hypertension, or HIV infection were excluded from participation in the study, as were women who resided in New York City for less than 1 year. The study samples represented in the reports reviewed were recruited between 1998 and 2004, a period which overlaps the voluntary cancellation of chlorpyrifos use in the residential environment.

In this cohort, authors measured chlorpyrifos exposure in several different biological and environmental matrices. These include chlorpyrifos parent compound in cord blood; the chlorpyrifos metabolite 3,5,6-trichloro-2-pyridinol (TCPy) in maternal and infant urine and meconium; and chlorpyrifos in personal and stationary air monitoring samples. In epidemiologic analyses, investigators consistently utilized cord blood measures of chlorpyrifos as the measure of prenatal exposure. Chlorpyrifos levels in umbilical cord blood samples were sampled as close to the time of delivery as possible, and within 2 days post-partum. Cord blood plasma chlorpyrifos levels were imputed from maternal blood levels for newborns for whom no cord blood sample was obtained because correlation was high (>80%). Quantification of chlorpyrifos levels in plasma were conducted by the Centers for Disease Control and Prevention (CDC). Information regarding basic demographics, socio-economic status, and pregnancy related measures, among other factors was collected through self-report questionnaire at the time of enrollment.

2.1.2. Inner-City Toxicants, Child Growth and Development Study (Mt. Sinai Hospital)

Researchers with the Mt. Sinai Children's Health and Development study conducted a prospective birth cohort study in which they enrolled primiparous women presenting for prenatal care with singleton pregnancies at the Mount Sinai prenatal clinic and two private practices and who delivered their infants at Mount Sinai Hospital in New York City between May 1998 and July 2001. Mothers were excluded if they had any of the following characteristics: an initial prenatal visit after 26 weeks of gestation, serious chronic diseases, a serious pregnancy complication that could affect fetal growth and development. Additional, participants were excluded for risky health behaviors including alcohol consumed greater than two alcoholic beverages per day or illicit drug use. Mothers and infants were also excluded if the child was born with a congenital malformation or severe prematurity.

To measure prenatal pesticide exposure, researchers implemented a self-report questionnaire to solicit information regarding pesticide usage, in-home pest pressure and other exposure characteristics. In addition, in the early third trimester, participants were asked to provide a urine sample at the time of a routine clinical blood draw. Using this biological sample, authors measured urinary concentration of pesticide metabolites including both TCPy (Berkowitz et al., 2004), and also non-specific measures of organophosphate exposure, dialkyl phosphates (DAPs) (Engel et al., 2007; Engel et al., 2011). Authors adapted analytical methods to conduct laboratory measurement of TPCy; DAPs were measured by CDC using published methods (Barr et al., 2002). Using maternal and infant (cord blood) blood sample, researchers measured PON1 enzymatic activity levels, and conducted genotyping analysis to determine prevalence of *PON1* variant alleles.

Potentially confounding variables were measured through self-report questionnaire and included in final statistical models if variables were known to be associated with either pesticide exposure or fetal growth. Authors also measured concentration of other pesticides in biological matrices including metabolites of pyrethroid exposure (PBA), pentachlorophenol (Berkowitz et al., 2004), as well as other organophosphates (malathion) and organochlorines compounds including polychlorinated biphenyls (PCBs) (Engel et al., 2007). However, authors did not present analyses concerning potential confounding effect of co-exposure to other environmental chemicals.

2.1.3 Center for Health Assessment of Mother's and Children of Salinas Valley, CHAMCOS (University of California/Berkley)

The Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) cohort is comprised by participants who live and work in the Salinas Valley, CA. The Salinas Valley is a major center of agricultural production in the United States with approximately 500,000 pounds of organophosphate pesticides applied annually (California EPA. Pesticide Use Reporting 2001 Summary Data, 2002. (www.cdpr.ca.gov/docs/pur/pur01rep/01_pur.htm)), chlorpyrifos, however, was not frequently used in agriculture in this area during the time period of this study. This cohort is comprised of low-income, predominantly Mexican-American (or Mexican immigrant) women employed as farm laborers or living with someone employed as a farm laborer. Enrollment of the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) cohort took place at regional community clinics. Women considered eligible for the study were less than 20 weeks gestation, aged 18 years or older, Medi-Cal eligible, fluent in English and/or Spanish, and planning to deliver at Natividad Medical Center. Excluded from analyses were women with gestational or preexisting diabetes, hypertension, twin births, or stillbirths.

Data collection was performed using several different tools. Authors administered self-report questionnaires to study participants to obtain information regarding demographic characteristics, work history and health behaviors. To assess exposure, participants were asked to provide biological samples (urine, blood). Specifically, maternal and fetal exposure to organophosphate pesticides was assessed by measurement of organophosphate dialkyl phosphate metabolites (DAPs) and seven different pesticide-specific metabolites, including TCPy, in maternal urine during two periods in the pregnancy. Maternal urine samples collected between 5 and 27 weeks gestation and again between 18 and 39 weeks. The post-delivery urines were collected within 1 week of delivery for 73% of the sample, with the remainder obtained up to 176 days afterwards. Total DAP, including dimethyl phosphates (DMP), and diethyl phosphates (DEP) levels were determined for each participant for each of the two pregnancy urine samples, and because the measures did not differ substantially, these values were averaged in epidemiologic analyses to estimate prenatal pesticide exposure. Quantification of organophosphate metabolites was conducted by the Centers for Disease Control and Prevention (CDC) labs (Barr et al., 2002).

In this cohort, authors also measured cholinesterase (ChE) in whole blood and butyl cholinesterase (BChE) in plasma as a surrogate for organophosphate pesticide exposure. PON1 was also measured in blood. Researchers also measured concentration of other environmental chemicals including PCBs, lead, DDT/DDE, HCB, and PBDEs; however, these compounds were

not included in final models as potential confounding variables.

2.2 Summary of Research Results

Across these three children's health cohorts, authors have assessed the relation between measures of prenatal chlorpyrifos exposure and various measures of fetal growth and infant and child neurodevelopment. In this section, research results are briefly summarized by health outcome category. A more detailed description of the study design, methods and analysis, as well as research results and a critique of individual study strengths and limitations are presented in Appendix 3. An evidence table delineating key study features is presented in Appendix 4. Table 1 briefly summarizes these results.

2.2.1 Measures of Fetal Growth

Authors with each of the three respective children's health cohorts measured prenatal chlorpyrifos exposure in association with fetal growth including birth weight, birth length, head circumference, gestational age and Ponderal index. To ascertain birth characteristics, authors linked with medical records at respective participating hospitals. Across these children's health cohorts, authors observed inconsistent evidence of an association; however, differing exposure profiles as well as dissimilar methods of prenatal chlorpyrifos exposure assessment likely played a role in this observation (Needham, 2005).

Table 1 Summary of Findings in the Columbia Mothers and Newborn Cohort, the Mount Sinai Hospital Children's Environmental Health Cohort, and the UC Berkeley Center for the Health Assessment of Mothers and Children of Salinas Studies of Prenatal Organophosphate Pesticide Exposure and Child Development

	Columbia Cohort	Mount Sinai Cohort		UC Berkeley Cohort	
Markers of Exposure:	Chlorpyrifos	TCPy	DAPS	TCPy	DAPS
Birth Length	Inverse (Null post 2000)	Null	--	Positive (NS)	Positive
Birth Weight	Inverse (Null post 2000)	Null	--	Positive (NS)	Null
Head Circumference at Birth	Null	Null (Inverse*)	--	Null	Positive (Inverse*)
Gestational Age	--	Null	--	Null	Null (Inverse*)
BNBAS Newborn (Abnormal reflexes)	--	--	Positive*	--	Positive
BNBAS Newborn (Neurodevelopment)	--	--	Null	--	Null
Bayley Scores 6 months (MDI/PDI)	--	--	--	Null/ Inverse (NS)	Inverse (NS)/ Inverse (NS)
Bayley Scores 12 months (MDI/PDI)	Null / Null	--	Inverse**/ Inverse (NS)	Inverse (NS)/ Inverse (NS)	Inverse (NS)/ Inverse (NS)
Bayley Scores 24 months (MDI/PDI)	Null / Null	--	Inverse (NS)/Null	Inverse(NS) / Inverse (NS)	Inverse/ Inverse (NS)
Bayley Scores 36 months (MDI/PDI)	Inverse/ Inverse	--	--	--	--
Pervasive Development Disorder (PDD)	Positive (36 mo.)	--	--	--	Positive (24 mo.)
Mental Development	--	--	Null	--	--

	Columbia Cohort	Mount Sinai Cohort		UC Berkeley Cohort	
Markers of Exposure:	Chlorpyrifos	TCPy	DAPS	TCPy	DAPS
(WPPSI-III, age 6 years)					
Mental Development (WISC-IV, age 7-9 years)	Inverse (Full-scale IQ and Working Memory)	--	Inverse (NS) (Full scale IQ, perceptual reasoning, working memory and processing speed)	--	Inverse (FSIQ, working memory, processing speed, verbal perceptual reasoning)
Odds of ADHD/ attention and behavior problems	Positive (36 mo.)	--	--	--	Positive (NS 3 years) Positive (5 years)

*Interaction observed between pesticide markers and PON1 activity or genotype
**Interaction observed between pesticide markers and race
NS=Not statistically significant
Inverse= Higher levels of exposure associated with adverse health outcomes (measurement value or score decreased)
Positive = Higher levels of exposure associated with adverse health outcome (measurement value or score increased)
Null = No association observed
MDI= Mental Development Index
PDI = Psychomotor Development Index

Researchers with the Columbia Mothers and Newborn study observed an association between decreased fetal growth and prenatal chlorpyrifos measures among 314 mother-infant pairs selected for this study (R. M. Whyatt et al., 2004). Controlling for potential confounders, 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). Whyatt et al. (2004) did not report an association with head circumference. Combined exposure to both chlorpyrifos and diazinon (adjusted for relative potency using US EPA cumulative risk assessment methods) were also significantly inversely associated with birth weight and length ($p < 0.05$). When births were stratified by time period prior to or after the voluntary cancellation of chlorpyrifos for residential use, researchers no longer observed evidence of an association ($p > 0.8$) among births which took place in the cancellation period (n=77). In supplementary analyses, authors replicated this analysis with additional post-cancellation era births (total n=193) and similarly did not observe evidence of an association with chlorpyrifos in the later time period (R. Whyatt & Rauh, 2011) (See Attachment 1). Overall, authors suggest that prenatal chlorpyrifos exposures may have impaired fetal growth among this inner-city, low income cohort. While suggestive of a conclusion that cessation of chlorpyrifos exposure explains the different associations with fetal growth before and after the period of the voluntary cancellation of chlorpyrifos, EPA notes that this finding may also be suggestive of a possible threshold effect, or it could also be due to a lack of statistical power to assess associations by time period.

Within the Mt. Sinai Child Growth and Development study, Berkowitz et al. (2004) assessed the association between prenatal chlorpyrifos exposure measured as urinary TCPy and subsequent risk of impaired fetal growth (Berkowitz et al., 2004). Authors also evaluated potential effect modification by *PON1* genotype and phenotype in the relation of interest. Among 404 births which took place between 1998 and 2002, authors found no statistically significant associations between fetal growth including birth weight, or birth length or head circumference and chlorpyrifos (estimated as TCPy) exposure. However, Berkowitz et al. (2004) did observe evidence of heterogeneity of effect by PON1 activity level. Specifically, researchers observed a small, but statistically significant reduction in head circumference among children of mothers with levels of chlorpyrifos above the limit of detection and also in the lowest tertile of PON1 activity (least able to metabolize exogenous exposures such as OP pesticides). In the subgroup of infants whose mothers had TCPy levels greater than the level of detection, those with low maternal PON1 had an average (SD) head circumference of 33.3cm (1.5cm) which was significantly smaller than those with medium (34.0cm (1.5cm)) and high (34.1cm (1.6cm)) maternal PON1 activity after adjusting for race/ethnicity, infant sex, and gestational age ($p = 0.014$), although the statistical interaction was not significant. Authors did not observe evidence of heterogeneity of effect by PON1 in the relation between other measures of birth outcomes including birth weight and birth length and pesticide exposure. Neither maternal *PON1* genetic polymorphisms nor infant paraoxonase levels were associated with reduced head size.

Within the CHAMACOS cohort, among 488 participants enrolled between 1999-2000 Eskenazi et al. (2004) did not observe a significant adverse relationship between fetal growth and any measure of *in utero* organophosphate pesticide exposure (Eskenazi et al., 2004b). On the contrary, investigators reported positive associations between birth length and head circumference associated with non-specific organophosphate exposure measures (DAPs). Researchers observed decreases in gestational age associated with measures of *in utero* pesticide

exposure: urinary DMP metabolites (β = –0.41 weeks per log10 unit increase; 95% CI: –0.75—0.02; p = 0.02), which reflects exposure to dimethyl organophosphate compounds such as malathion, but not chlorpyrifos. Authors did not observe an association using TCPy as a measure of chlorpyrifos exposure; however they did report an increased risk of preterm delivery with decreasing cholinesterase concentrations, a third biomarker of organophosphate exposure. To identify "critical windows" of fetal development when exposure may have a greater impact, the authors analyzed the associations of outcomes and metabolite levels measured during moving 6-week windows of pregnancy (*e.g.*, 5–10 weeks, 6–11 weeks, 7–12 weeks) using a series of multiple regression analyses. No period of greater impact was observed in this largely null study.

In a follow-up analysis within this cohort, Harley et al. (2011) performed the same analysis however evaluating the potential effect modifying role of *PON1* genotype and phenotype in the relation between prenatal pesticide and chlorpyrifos exposure (DAP, TCPy, *ChE*) and fetal growth (Harley et al., 2011). In this study, infants' (but not mother's) *PON1* genotype and PON1 activity modified the association between gestational age and head circumference. Infants with the susceptible $PON1_{-108}$ TT genotype had shorter gestational age (beta =-0.5 weeks, 95%CI: -0.9, 0.0) and smaller head circumference (beta=-0.4 cm, 95% CI: -0.7, 0.0) than those without the susceptible genotype ($PON1_{-108}$ CC genotype). Infants' arylesterase and paraoxonase activity were positively associated with gestational age. Maternal DAP concentrations were associated with shorter gestational age among infants of the susceptible $PON1_{-108}$ TT genotype, although only the interaction between $PON1_{-108}$ genotype and DEP metabolite concentrations was statistically significant (p-value for interaction = 0.09). However, maternal DAP concentrations were associated with larger birth weight (p-value for interaction = 0.06) and head circumference (p-value for interaction =0.01) in infants with non-susceptible genotypes. The authors conclude that infants with certain *PON1* genotypes (*e.g.*, $PON1_{-108}$ TT) may be more susceptible to effects of *in utero* organophosphate pesticide exposure.

2.2.2 Brazelton Neonatal Neurological Functioning

Researchers with both the Mt. Sinai Child Growth and Development study and the CHAMACOS cohort evaluated neonatal neurological functioning in association with prenatal chlorpyrifos exposure. To measure indices of abnormal neonatal behavior and/or neurological integrity authors used outcome measures derived from the Brazelton Neonatal Behavioral Assessment Scale (BNBAS). The BNBAS includes 28 behavioral items and 18 primitive reflexes which assesses the infant across several different developmental areas. This tool was administered to infants within days after birth and before they left the hospital (2-5 days post-partum). Examinations were conducted by trained neonatologists in the hospital setting using similar environmental conditions. The Mt. Sinai Child Development study and the CHAMACOS cohort evaluated this outcome measure; researchers with the Columbia Mother's and Newborn study did not measure the relation.

Among the 438 infants eligible to participate in the Mt. Sinai evaluation, Engel et al. 2007 observed an association between generic (DAPS) biomarkers of prenatal organophosphate pesticides and an increased number of abnormal primitive reflexes which are considered a critical marker of neurologic integrity. Controlling for confounding factors, subjects with prenatal total diethylphosphates levels above the median (24.7 nmol/L) delivered infants who

were 2.3 times more likely to have at least two abnormal reflexes (95% CI: 1.1, 5.0). Associations with other DAPs were also reported. Notably, no statistically significant adverse associations were found for any of the dialkylphosphate metabolites and other domains of the Brazelton assessment tool in the author's primary analyses, *e.g.*, habituation, range of state, orientation. Authors also observed evidence of potential effect modification by PON1 activity level in the relation between DAPs and neonatal neurodevelopment. Specifically, in the first tertile of paraoxonase 1 expression (slowest metabolizers), the relative risks (RR) of having abnormal reflexes were significantly related to DAP and DMP levels; the risk estimate for DEP and the number of abnormal reflexes within the lowest PON1 level was not significant. In the CHAMACOS cohort, Young et al. (2005) observed a statistically significant association between organophosphate pesticide exposure and the reflex cluster of the BNBAS (Young et al., 2005). The proportion of infants with more than three abnormal reflexes was 3-fold increased among those with prenatal DEP exposure (DEP: OR = 3.4, 95% CI = 1.2, 9.9). Similar to Engel et al. (2007), no other associations between prenatal DAPs and other aspects of the Brazelton assessment were noted (other neurodevelopmental domains) (Engel et al., 2007). No adverse associations were found between postnatal urinary metabolite levels and any of the developmental outcomes.

2.2.3. Bayley Scale of Mental and Psychomotor Development

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. As a complement to this measure of mental development and behavior, authors also used the 99-item Child Behavior Checklist (CBCL). In addition, because the quality of the home environment is a key determinant of child development, authors also used the Home Observation for Measurement of the Environment (HOME) instrument. This instrument collects data regarding the physical and interactive qualities of the child's home as a measure of mental stimulation and interactions. Results from each of these measurement tools were used to model the relation between prenatal chlorpyrifos exposure and adverse neurodevelopmental outcomes in infancy and toddlerhood (6-36 months of age).

Rauh *et al.* (2006) investigated Mental Development Index (MDI) and a Psychomotor Development Index (PDI) at 12, 24, and 36 months of age within the Columbia Mother's and Newborn Study. Children were categorized as having either high (>6.17pg/g) or low (≤6.17pg/g) prenatal exposure, using categories informed by results of the previous study on birth characteristics (R. M. Whyatt et al., 2004). Authors reported that the difference in MDI scores was "marginally significant" (p = .06) with the exposed group scoring an average of 3.3 points lower. When the same multivariate regression models were calculated regarding the PDI scores, none of the 12 or 24 month PDI scores showed significant effects, but the 36 month score was significantly related to chlorpyrifos exposure. Investigators also calculated estimates of adjusted risk for developmental delays (MDI and PDI) related to chlorpyrifos exposure and illustrated that before 36 months of age, delays were no more likely in the highly exposed group, but, 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 = .02) and motor delays were 4.9 times greater (95% CI: 1.78-13.72; p = .002) than those with lower prenatal exposure. Using general

linear modeling (GLM), authors analyzed developmental trajectories of the effects and results were consistent with the 12, 24, and 36 month milestone analyses indicating that the age effects most significantly occurred at the later phase of the 3 year period. In supplemental analyses suggested by the 2008 FIFRA SAP, authors illustrated that diazinon was a strong confounding variable in this association (correlation with chlorpyrifos 0.63), increasing the magnitude chlorpyrifos risk estimate for MDI and PDI 50-200% in the same direction (away from the null) (R. Whyatt & Rauh, 2011) (Attachment 1).

Within the Mt. Sinai Children's Environmental Health study, authors administered the BSID-II to participating children at 12 and 24 months. Among 404 women originally enrolled between May, 1998 and July, 2001 (n = 404), children of these mothers returned for neurodevelopment assessments at ages 12 months (n = 200), 24 months (n = 276) of age. Using generalized linear models, authors found that prenatal total dialkylphosphate metabolite level was associated with a decrement in mental development at 12 months among blacks and Hispanics children. The associations appeared to be strongest among children of mothers who carried the *PON1* Q192R QR/RR genotype. The authors concluded that their findings are suggestive of an association between prenatal exposure to organophosphates and decrements in cognitive development, particularly perceptual reasoning, with evidence of effects beginning at 12 months and continuing through early childhood, with *PON1* being a potentially important susceptibility factor for these deleterious effects. The authors did not observe any effect modification by PON1 enzyme activity level. In general, associations observed during the 12-month follow-up were either attenuated or non-existent at the 24-month visit.

In the CHAMACOS cohort, Eskenazi et al 2007, the authors report on the relationship between prenatal and child urinary organophosphate metabolite levels and child neurodevelopment at ages 6, 12, and 24 months of age. Controlling for indicators such as the psychometrician conducting the assessment and the location of assessment, age at assessment, sex, duration of breast-feeding, HOME score, and household income, parity and indicator of maternal intelligence (PPVT score), authors observed that prenatal DAP levels were adversely associated with MDI, while early life DAP levels were positively associated with MDI. At 24 months of age, these associations reached statistical significance (per 10-fold increase in prenatal DAPs: $\beta = -3.5$ points; 95% CI: –6.6 to –0.5; child DAPs: $\beta = 2.4$ points; 95% CI: 0.5 to 4.2). In a subsequent study, investigators did not observe evidence of effect modification by *PON1* status in the relation between prenatal DAPs and child neurodevelopment as measured by the Bayley Scale (Eskenazi et al., 2010). Neither prenatal nor child DAPs were associated with PDI or CBCL attention problems. Both prenatal and postnatal DAPs were associated with risk of pervasive developmental disorder (per 10-fold increase in prenatal DAPs: OR = 2.3, $p = 0.05$; child DAPs OR = 1.7, $p = 0.04$). TCPy as a biomarker of chlorpyrifos exposure was not significantly associated with any neurodevelopment outcome in this study.

2.2.4 Attention Problems

Also within the CHAMACOS cohort, Marks *et al.* (2010) conducted a study to investigate the association between urinary dialkyl phosphate (DAP) metabolites in pregnant women and their children, as a marker of organophosphate exposure, and attention-related outcomes among 348 children who had available data at 3.5 and/or 5 years and met inclusion criteria (Marks et al.,

2010). Attention-related health outcomes were measured through 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. To identify children whose behaviors were most suggestive of possible ADHD within the cohort, a composite ADHD variable was defined that combined the results of the maternal report (CBCL), child testing (K-CPT), and the psychometrician report (Hillside). In this study population, higher concentrations of organophosphate 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. Prenatal DAPs were non-significantly associated with maternal report of attention problems and ADHD at age 3.5 years but were significantly related at age 5 years (CBCL attention problems: $\beta = 0.7$ points; 95% CI: 0.2–1.2; ADHD: $\beta = 1.3$; 95% CI: 0.4–2.1). 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). The results of this investigation by Marks et al. (2010) in the CHAMACOS cohort are suggestive of a detrimental association between prenatal organophosphate exposure, as measured by maternal urinary metabolite levels, and attentional difficulties at age 5 years using three different measures of this neurodevelopmental outcome.

2.2.5. Intelligence Measures

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. These indices are associated with, but distinct from, overall intelligence quotient (IQ) and are sensitive to cognitive deficits related to learning and working memory. A Full-Scale IQ score combines the four composite indices. A General Ability Index score is a summary score of general intelligence, similar to Full-Scale IQ, but excludes contributions from both Working Memory Index and Processing Speed Index. WISC-IV scores are standardized against U.S. population-based norms for English and Spanish-speaking children.

Rauh et al. (2011) 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 (V. Rauh et al., 2011). 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 (4.61pg/g) there is a 1.4% reduction in Full-Scale IQ and a 2.8% reduction in Working Memory.

In the Mt. Sinai Child Growth and Development study, Engel et al. 2011 used generalized linear models to analyze the relationship between total diethylphosphates (DEPs), total

dimethylphosphates (DMPs), total dialkylphosphate metabolites (DAPs) measured in maternal urine during the third trimester, and subsequent cognitive/intelligence development evaluated at age 7 years. In this study, prenatal maternal DEP urinary metabolite concentrations were associated with slight decrements in FSIQ, Perceptual Reasoning, and Working Memory between the ages of 6 and 9 years. Among children of mothers with the susceptible *PON1* genotype, DAP and DMP urinary metabolite concentrations were associated with poorer scores on Perceptual Reasoning and FSIQ.

In the CHAMACOS cohort, Bouchard et al. (2011) observed evidence of an association between prenatal exposures to organophosphate pesticides as measured by urinary DAP metabolites in women during pregnancy, and decreased cognitive functioning in children at age 7 (Bouchard et al., 2011). Authors observed this finding using total DAP, DMP, and DEP metabolites to estimate pesticide exposure. Children in the highest quintile of maternal DAP concentrations had an average deficit of 7.0 IQ points compared with those in the lowest quintile. Authors reported the associations were linear with no threshold. Urinary DAP concentrations in childhood (postnatal) were not associated with cognitive scores in this cohort of children. Of note, the following known or suspected neurotoxicants were measured: polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), p,p'-dichlorodiphenyltrichlorethane (DDT), p,p'-dichlorodiphenyltrichlorethylene (DDE), and lead. Lead was measured in maternal blood at 26 weeks gestation, in cord blood for a subset of participants, and children's blood a 2 years of age. These other environmental exposures including blood lead concentration did not change the magnitude and direction of the associations observed between DAP/DEP and IQ measures among school-aged children.

2.2.6 MRI Imaging Study

In April 2012, CCCEH researchers published a study utilizing magnetic resonance imaging (MRI) technology to identify and contrast anatomical characteristics of the cortical region of the brain including cortical surface area and thickness in relation to prenatal pesticide exposure (V. A. Rauh et al., 2012). Between June and September 2012, EPA sought additional expertise within the Federal government as to the quality of the MRI data collection procedures used in this study as well as the appropriateness of the authors' interpretation of the MRI studies in association with chlorpyrifos exposure. EPA's critical review and analysis of this MRI study can be found in Appendices 3 and 4; the results of the Federal Panel review concerning EPA's questions about the study can be found at: http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2008-0850-0170.

In this study, authors CCCEH birth cohort study evaluated whether there were areas of morphological change in the pediatric brain in regions of the brain known to be associated with learning, cognition and social behavior in association with prenatal chlorpyrifos (CPF) exposure (V. A. Rauh et al., 2012). These are functional neurodevelopmental outcomes that have been adversely associated with chlorpyrifos exposure in previous observational research (V. A. Rauh et al., 2006; V. Rauh et al., 2011), as well as some experimental studies, as noted by the authors. Specifically, in this study, authors evaluated 1) whether morphological changes in the brain

including cortical thickness and differences in the cerebral brain surface area and volume were related to prenatal chlorpyrifos exposure (the main effect), and, 2) whether the relation between the exposure and morphological changes in the brain were modified by either full scale intelligence quotient (FSIQ) score (evidence of effect modification by IQ) and, 3) in preliminary analyses, whether changes in the normal sexual differentiation of the brain exist by exposure-level (V. A. Rauh et al., 2012).

Authors concluded that while overall brain size did not differ by prenatal chlorpyrifos exposure, certain areas of the brain were enlarged among those more highly exposed to chlorpyrifos including superior temporal, posterior middle temporal, and inferior post-central gyri, and superior frontal gyrus, gyrus rectus, cuneus, and pre-cuneus in the mesial views of the right hemisphere of the cerebrum. Further, they noted a difference exposure-response relation between cerebral surface distances and CPF exposure, by FSIQ wherein the relation between brain area enlargements and FSIQ were positively correlated in the low CPF group (greater regional brain size correlated with greater IQ), but an inverse relation among the high CPF group. In supplemental analyses, authors illustrated that areas of regional brain enlargement among those more highly exposed to chlorpyrifos were due to underlying white matter enlargements. Researchers also noted sporadic differences in cortical thickness wherein higher CPF exposure was related to reduced cortical thickness. Preliminary analyses also displayed a disruption of normal male-larger-than-female areas as well as female-larger-than-male in certain areas of the brain known to differ by child sex in normal brain. Overall, researchers concluded that differences in brain structure (regional cerebral size and thickness) exist between CPF exposure groups, and the differences (high>low CPF) in regional brain size may be due to enlargement of underlying white matter. However, authors did not speculate as to the underlying biological explanation of the effect of CPF exposure on intelligence or with morphological changes in key areas of the brain linked with learning, cognition and social behavior.

2.3. Strength and Limitations of Children's Health Cohort Studies

There are several strengths reflected in the design, conduct and analysis decisions made by investigators with each of the three children's health studies, as well as some limitations to consider. Strengths of these studies include the prospective nature of these investigations, extensive data collection including several different measures of prenatal chlorpyrifos exposure which have been validated, and use of neurodevelopmental outcome ascertainment tools which have been validated in different populations and commonly used in both clinical and research settings, among other strengths noted in this section. Key limitations reflect the difficulty in measuring multi-dimensional characteristics such as socio-economic status accurately, the challenge of conducting environmental epidemiology studies in populations exposed to several different compounds and mixtures, and the ability to assess effect modification (*e.g.*, genetic variability) with precision and accuracy. Considering both the strengths and limitations of the respective investigations, EPA puts forward epidemiologic inference at the conclusion of this section.

Study authors from each of the respective long-term studies hypothesized a role for environmental exposures, including but not limited to pesticides generally and chlorpyrifos specifically, in the etiology of adverse fetal growth and neurodevelopmental outcomes. Study

authors stated their motivation was the knowledge that these populations were highly exposed to pesticides and other environmental contaminants, and that prior research had linked *in utero* environmental exposure to adverse neurodevelopmental outcomes. Cohorts were each funded through the EPA and National Institute of Environmental Health Science (NIEHS) Children's Environmental Health and Disease Prevention Research Centers[69]. Cohort studies were comprised of several hundred mother-infant pairs (range: n=102 to n=488) and were likely sufficiently statistically powered to detect hypothesized main effects, but studies may have lacked statistical power to perform stratified analyses, *e.g.,* effect modification by genetic variant. This is especially true for the MRI imaging study performed within the CCCEH (Rauh et al. 2012). The pilot nature of the study, inclusion of only 40 child participants from the overall cohort, severely limited the evaluation of statistical interactions. Similarly, statistical methods utilized across these investigations were appropriate to the data collected and research question hypothesized, however statistical analyses were discussed in varying degrees of detail by separate study groups. EPA notes that in some instances, additional information is provided in supporting methods paper, *e.g.,* approaches to handling non-detects in large datasets (Arunajadai & Rauh, 2012). Statistical model selection was generally parsimonious in nature, and investigators performed multiple sensitivity analyses (not always shown) within each study to further explore and eliminate alternative explanations for observed results, *e.g.*, confounding by blood lead, variable transformations. Concerning the main effects hypothesized, both design and analysis decisions made by researchers enhanced the probability of identifying an association, if an association exists. For the association under study, measurement errors likely occurred due to the challenge of estimating exposure during the critical window of development.

Selection criteria were clearly defined and appropriate across the three cohorts. Investigators limited participants to individuals within a similar racial and ethnic group characteristics, income and education level, and geographic area, and who reported low prevalence of risky health behaviors (alcohol use, smoking during pregnancy), and who did not report major medical co-morbidities which may have adversely affected fetal growth and development (*e.g.*, gestational diabetes). Therefore, authors sought to control through design (restriction) variability in the sample population to isolate the effect of chlorpyrifos exposure on fetal outcomes. However, the many selection criteria applied may have affected the generalizability of study results. The selected study population is narrowly defined and dissimilar to the general U.S. population across many demographic and exposure related characteristics. However, in comparison with biomonitoring levels in the U.S. general population, exposure among these cohorts was greater, but within a similar range. In addition, the external validity of the study findings may be limited if the organophosphate pesticide exposure-fetal development association is modified by factors that are more, or less, prevalent in the study populations relative to the population(s) to which inference is being made, or to populations with a substantially different exposure range.

All three studies are prospective cohort studies in which exposure measurements were obtained prenatally and/or at delivery, and fetal growth and neurodevelopmental outcomes were assessed subsequently. This study design eliminates or reduces several potential sources of bias. This design eliminates temporal bias, *i.e.*, uncertainty as to whether exposure precedes the adverse outcome, and, and also reduces the effect of differential exposure measurement error (a type of

[69] http://epa.gov/ncer/childrenscenters/

information bias) as it is unlikely exposure measurement would be differentially assessed by (unmeasured, future) health outcome.

Researchers utilized similar tools to measure adverse health outcomes across the studies. The use of similar outcome measures across studies aids comparability of studies and assessment of the consistency of results. Neurodevelopmental outcome assessment tools utilized were common to both clinical and research settings and most have been validated in both English and Spanish speaking populations. While measures of fetal growth are somewhat objective to measure, neurodevelopmental outcomes measured within these studies are more difficult to assess. While administered consistently within these investigations, tests of mental development, cognition, psychomotor development, and intelligence tools are somewhat subjective in nature, and may be affected by child's anxiety (Bayley Scales) and influenced by cultural factors (Wechsler). However, the homogeneity of the study population may reduce the potential influence of a cultural bias or differences in assessment tool response based on external factors, and these are among the best (gold standard) of tools to assess these types of developmental issues. Authors employed appropriate quality assurance and control measures such as training of test administrators, periodic evaluation of adjudicators, consistent environments in which tests were administered, and in many instances included an indicator variable for test administrator in statistical analysis to adjust for slight differences in examiner effect. . Federal letter reviewers in September 2012 concurred that the neurodevelopmental testing utilized in these studies were reasonable and appropriate[70]. With the exception of the child behavior checklist (CBCL), errors in the measurement of health outcomes were likely non-differential in nature, leading to an attenuation of the risk estimates. Overall, across these studies researchers utilized the best available outcome measurement tools for neurodevelopment health effects, and implemented the evaluations in a consistent, standardized manner with trained health professionals and/or study staff. The results of the Federal Panel review of June-September 2012 supported this conclusion. Federal panelists agreed that these are acceptable tools and appropriate to the age range of the child participants in these cohort studies. The tools allowed researchers to address the question as to whether neurodevelopment scores differed by chlorpyrifos exposure.[71]

Across the three children's health cohorts, study authors measured parent chlorpyrifos, TCPy and diethyl phosphate (DEP) to estimate chlorpyrifos and/or organophosphate exposure. Chlorpyrifos metabolites (TCPy and DAPs) are likely more accurate and objective indicators of organophosphate pesticide exposure than other exposure ascertainment methods such as self-report, but uncertainty remains as to the extent measurement of non-specific metabolites reflects chlorpyrifos exposure. TCPy is a metabolite specific to chlorpyrifos, but can also be produced as a result of chlorpyrifos-methyl exposure or environmental exposure to TCPy itself (Morgan et al., 2005; Wilson, Chuang, Lyu, Menton, & Morgan, 2003). Urinary dialkyl phosphate (DAP) metabolites of organophosphorus insecticides are markers of exposure to organophosphate pesticides generally; chlorpyrifos and diazinon are metabolized to diethyl phosphates (DEP), while other organophosphates are metabolized to dimethyl phosphate (DMP). For risk assessment purposes, it is difficult to infer chlorpyrifos effects specifically from urinary DAPs and to a lesser extent the TCPy metabolite. Different exposure measurements may in part explain inconsistencies across study findings, as noted by others (Needham, 2005). The three birth

[70] http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2008-0850-0170.
[71] See http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2008-0850-0170.

cohorts recruited participants who likely have chlorpyrifos exposures that are higher than the general population, and the exposures in these cohorts may also be more consistent over time. Inner-city populations report frequent use of pesticides, most applied at least once per week, during the time period of these studies (R. M. Whyatt et al., 2003; R. M. Whyatt et al., 2002). In New York City, location of two of the three children's health cohort studies, housing authority reported common use of chlorpyrifos for indoor pest control during the time period of these studies, prior to the time period of the voluntary cancellation of these uses. In the CHAMACOS cohort, chlorpyrifos metabolite (TCPy) was detected in 77% of urine samples (Bradman et al., 2005; Eskenazi et al., 2004a). Despite being considered to have greater opportunities for exposure relative to the general population, the average levels of chlorpyrifos biomarkers in the three cohorts were generally comparable to the U.S. general population, which may indicate a difference between actual and measured exposure. Nevertheless, it is not clear to what extent the use of one or two biomarker measurements conducted in the reported studies reflects exposure(s) over critical windows of development during pregnancy. This remains an uncertainty within these studies. As previously mentioned, chlorpyrifos exposure was assessed by quantification of biomarkers primarily in one- or two-samples of maternal urine taken during the third trimester, and also, in one cohort, in a single maternal blood or cord blood sample obtained at the time of delivery. If the exposure is chronic, a biomarker measured at a single time point may provide a representative dosimeter, even if the toxicant has a short half-life, as is the case for chlorpyrifos and organophosphate pesticides generally. However, if pesticide exposures are sporadic or otherwise vary over short time scales, the biomarker measurement may not be representative of "usual" exposure or of the exposure during critical periods of fetal development. In the UC Berkeley CHAMACOS cohort (Eskenazi et al., 2004a), prenatal urine was collected between 5 and 27 weeks gestation and again between 18 and 39 weeks. The within-person standard deviation in the DAP metabolites was approximately three times larger than the between-person standard deviation, and concluded that a single biomarker assessment, as was conducted in the other two cohorts, "may not accurately reflect exposures over the entire pregnancy."

This potential limitation was also assessed in two validation studies conducted by Whyatt et al within the Columbia Mother's and Newborn Study. The first of these studies (R. M. Whyatt et al., 2007) assessed within- and between-home variability in indoor-air insecticides over the final 2 months of pregnancy among a subset of participants. Authors observed little within-home variability and no significant difference in air concentrations within homes over time ($p \geq 0.2$); between-home variability accounted for 92% of the variance in the indoor air levels of chlorpyrifos. Indoor and maternal personal air insecticide levels were highly correlated ($r = 0.7–0.9$, $p < 0.001$). While this study provides some evidence that assessment of chlorpyrifos exposure at a single time point may be reasonable, the study took place during a period of rapid decreasing indoor use of chlorpyrifos, and may not reflect variability in indoor air concentration prior to the period of cancellation. In addition to inhalational exposure, participants were likely also exposed by ingesting residues in the diet and from hand-to-mouth contact with surfaces contaminated with the pesticide. If true, then high correlation of chlorpyrifos air exposure over time may not indicate a true consistency in exposure levels.

The variability in exposure measures and the validity of different biomarkers of exposure was evaluated in a second study (R. M. Whyatt *et al.*, 2009) which evaluated trends over time in multiple biomarkers of prenatal chlorpyrifos exposure. Authors measured TCPy levels in repeat

prenatal urine samples and determined they were positively, but only moderately correlated (r = 0.23–0.56), and within-subject variability exceeded between-subject variability (intraclass correlation coefficient = 0.43). This indicates that variability in individual exposure over time may be considerable. Indoor air levels explained only 19% of the variance in prenatal urine TCPy (p = 0.001) in this study. However, these researchers also demonstrated the presence of TCPy in meconium; meconium is fecal matter accumulated in intestines of developing fetus from week 13 of gestation and released immediately prior to or within a few days after delivery. This matrix is considered to be an integrated measure of exogenous exposures to the fetus during the gestational period. Importantly, investigators reported a moderate and significant correlation between maternal blood and urine collected in the later part of the pregnancy period, infant cord blood collected at delivery, and meconium (R. M. Whyatt et al., 2009). As noted in earlier sections, the critical windows of effect for these specific outcomes is unknown at this time, and may span the period of early pregnancy through early childhood (Rice & Barone, 2000; Rodier, 2004). The correlation among several biomarkers of chlorpyrifos exposure reflecting different windows of exposure from week 13 through delivery (*e.g.*, correlation between meconium and infant cord blood r=0.33, p=0.01, n=56), suggests a one-time measure of exposure may accurately rank participants with respect to prenatal exposures, at least within a 2- to 3-fold level of variation as suggested by authors (R. M. Whyatt et al., 2009). Additionally, the correlation between the analytic measure of exposure and a measure reflective of exposure during a large proportion of the gestational period suggests the exposure measures may also reflect exposure during the several likely critical windows of development in the mid- to latter period of gestation. However, to the extent participants experienced "peak" exposures at key periods of development, this information would not likely be reflected in the one-time measure, and likely lead to non-differential measurement error.

Bias due to confounding occurs primarily when risk factors for the outcome are unequally distributed among exposure groups, but are not themselves caused by the exposure, and these factors are not controlled in either the design or the analysis of the study. The greatest concern is for unmeasured confounders, those variables not measured within the study that may be related to both the exposure and disease of interest but are unknown to the investigator. Residual confounding can also arise due to errors in the measurement or categorization of confounders, even after apparent adjustment for these factors in the study, *i.e.*, these are factors not measured well enough. Among the major potential unmeasured or poorly measured confounding variables in the chlorpyrifos and neurodevelopmental association are: 1) difficult to measure aspects of the social environment (*e.g.*, chronic stress, socioeconomic conditions), and 2) environmental co-exposures that may be determinants of neurodevelopment (*e.g.*, lead exposure, mercury exposure, air pollution, tobacco smoke exposure, maternal alcohol intake, and exposure to other pesticides). The selection of cohorts that are homogenous with respect to many demographic and social factors significantly reduces the potential for residual or unmeasured confounding factors related to socio-economic status to have influenced these results as they have been controlled through restriction (another method of adjustment). For example, socioeconomic status cannot confound the main association in a cohort of individuals living under the same socioeconomic condition, *i.e.*, there is little variation in the study population on this factor. However, close examination of descriptive tables in these publications indicates some degree of variability remains. Therefore, authors appropriately evaluated and included in final models several individual-level markers for socio-economic status such as education, income, and race.

Additionally, within the Columbia Mother's and Newborn study, authors performed new analyses using hierarchal regression techniques to model variability due to SES in the relation between prenatal chlorpyrifos exposure and mental and motor development (the Bayley Scale) (Lovasi et al., 2011). These techniques utilize individual- as well as group-level variables to model SES. In this research, authors did not observe a significant difference in risk estimates, indicating that the role of SES as a major confounder in the Columbia cohort is appropriately adjusted. However, others argue that the multi-dimensional nature of a characteristic such as SES can never be fully captured through commonly used variables, and suggest within a term such as SES there could be components that act as both positive and negative confounding variables (Bellinger, 2011). However, at this time, there is no compelling evidence that lack of control of the confounding influence of SES significantly biased the reported risk estimates.

Additionally, authors have measured several other environmental chemicals and in some instances considered these other exposures as potential confounding variables (not always shown). Researchers collected information from participants regarding biomarkers of other organophosphates, environmental tobacco smoke (ETS), blood lead, PAH, methylmercury (R. Whyatt & Rauh, 2011) (See Attachment 1), pyrethroids/pyrethrins, organochlorines such as DDT/DDE and hexachlorobenzene, PBDE, and polychlorinated biphenyls. While authors evaluated these environmental exposures in relation to both chlorpyrifos and outcomes, none were included as confounders in final models due to lack of statistical evidence of confounding. Within the Columbia Mothers and Newborn study, authors performed additional analyses suggested by the 2008 FIFRA SAP to evaluate the role of other organophosphate exposures, specifically diazinon and propoxur, in the relation between chlorpyrifos and both birth characteristics and mental and motor delays (Bayley scores) (R. Whyatt & Rauh, 2011) (See Attachment 1). Chlorpyrifos and diazinon biomarkers were highly correlated in this cohort (r=63%). While the effect sizes reported for the reported relation between both birth length and also birth weight remained unchanged from published reports when diazinon was added to the model, significant differences were observed in the relation with neurodevelopmental functions (mental (MDI) an PDI (motor) scores). Specifically, authors reported a 50-200% increase in effect sizes with MDI and PDI, respectively, *i.e.*, observed adverse effects became more pronounced with diazinon added to the model (See Attachment 1). Limited evidence of confounding by propoxur was also observed, but the correlation between chlorpyrifos and propoxur was moderate (23%), the confounding effect was not significant. In addition, neither pre- nor post-natal blood lead levels or methyl mercury levels measured in cord blood were significantly correlated with chlorpyrifos, and was therefore not considered a confounding variable in the association of interest within the Columbia cohort (V. A. Rauh et al., 2006; R. Whyatt & Rauh, 2011).

The appropriate measurement and controlling of potentially confounding variables was a major line of inquiry in the EPA initiated Federal Panel review in 2012. On this point, reviewers agreed with EPA that factors relating to socio-economic status were adequately measured and characterized by authors. In addition, reviewers did not note other major potential confounders inadequately measured or controlled in analysis. With respect to blood lead, reviewers of the MRI study indicated the sample size may have been inadequate to fully control for the effect of low-levels of lead exposure. As a result of this comment, EPA contacted researchers with the CCCEH to gain additional information as to the association between chlorpyrifos and blood lead

in the cohort (additional data collection beyond the published study data). In further written and oral communication with the CCCEH staff, EPA learned that when the correlation between the two environmental exposures was assessed among a larger sub-set of the cohort than previously published (n=300), the correlation between chlorpyrifos and cord blood lead remained extremely low (See Appendix 6 to the revised chlorpyrifos HHRA). This information reinforces EPA's understanding that lead exposure is not likely a confounding variable in this study.

However, uncertainties remain as to the role of other (unmeasured) environmental compounds as potential confounding variables in this association. None of the authors evaluated the possible effect modifying role of other environmental chemicals in the relation between chlorpyrifos and neurodevelopment, nor were studies suited to examining the effect of mixtures (simultaneous combined exposures at potentially relevant critical windows of development). New investigations are needed to address these hypotheses, if relevant. While the possibility of unknown, unmeasured positive confounding bias can never be completely excluded, given the evidence available it is unlikely this potential bias may entirely explain observed associations.

Selection bias occurs when study participants are either selected or lost to observation as a result of a third, unmeasured factor that is associated with both the exposure and outcome of interest. In these prospective cohort studies, selection bias is most likely induced not by selection into the study, but by selection out of the study, *i.e.* due to attrition of study participants and missing data. Because data are often not obtained on those that are lost to follow-up or for whom data is otherwise missing, it is difficult to determine whether or not, and to what extent, the observed associations are biased due these factors. The remedy is simple, in theory – follow the entire cohort and obtain all relevant data – but often difficult in practice, particularly for long-term cohort studies such as those reviewed here. Across these studies, the amount of missing data varied but was great in some studies. To address missingness in data, authors imputed values in several instances, and performed sensitivity analyses with and without imputed data to illustrate comparability in reported results. In addition, authors were able to illustrate differences between those included and excluded were comparable on several major characteristics, but some were not evaluated such as blood lead levels. Therefore, while it is difficult to ascertain the degree to which selection bias due to missing data may have influenced these study results, authors employed appropriate analytic tools to address missingness in data to the extent possible. Regarding external validity, the inclusion and exclusion criteria employed may limit the generalizability of study results. This is particularly true of the MRI imaging study, which was a pilot effort.

Information bias arises due to misclassification or error in the measurement of either the exposure or outcome of interest, or the accurate measurement of confounding variables. Qualities of the outcome ascertainment method, the exposure assessment method and the measurement of confounding variables were reviewed above. In summary, regarding exposure measurement error the true relevant exposure in these studies is prenatal chlorpyrifos to the developing fetus. Sources of exposure measurement errors include 1) the single measurement chlorpyrifos or its metabolites during the third trimester, 2) error arising from differences between measured biomarker levels and actual chlorpyrifos or chlorpyrifos oxon exposure, even at the one time point, 3) unmeasured time- and space- dependent patterns of chlorpyrifos exposure, 4) uncertainty regarding the critical period for chlorpyrifos effects on development, 5)

missing exposure data, 6) laboratory errors, and 7) imputation of missing exposure levels. Measurement errors in the ascertainment of chlorpyrifos exposure are likely to have occurred in the studies reviewed. However, because of the prospective study designs employed, the errors are unlikely to result in falsely positive findings, because the probability and magnitude of these errors are likely to be independent of the outcome status of participants. Measurement errors in the ascertainment of the health outcomes were also likely to have occurred, perhaps more for the neurocognitive outcomes, than for the indicators of fetal growth and development, and also are unlikely to have spuriously positive findings. These measurement errors are more likely to have resulted in false negative findings, if a causal association truly exists.

While the MRI study by Rauh et al. 2012 follows upon both previous epidemiological research and experimental toxicology studies that suggest an association between chlorpyrifos exposure in the pre-natal and early life periods with adverse neurodevelopmental outcomes observed in children through school age, EPA believes that the use of these data to inform specific underlying biological mechanisms or pathways of the potential toxic action of CPF is limited; additional and more sophisticated imaging would have to be performed to determine links to the very specific types of neurodevelopmental effects posited by authors in the Rauh et al. (2012) study.

2.3.1. Epidemiologic Inference of Combined Children's Health Cohorts

As stated earlier, the three children's health cohorts considered herein have several strengths as well as limitations to consider in the interpretation of these studies. Within these studies, there are several factors that would tend to under-estimate the actual association (possibly leading to false negative association), as well as some characteristics that may lead to over-estimation (possibly leading to a false positive association). However, it must also be noted that methodological research and supplemental analyses (primarily within the Columbia Mothers and Newborn study) performed subsequent to the 2008 FIFRA SAP deliberations concerning these children's health cohort studies have reduced and further characterized important sources of uncertainty. As noted in the Introduction to this paper, currently scientists cannot determine with accuracy the critical window of exposure for these outcomes (early gestation through early childhood). Across the children's health cohorts, researchers used one or two measure(s) of exposure to estimate gestational exposure during the critical window of development, and investigators assessed the main exposure using non-specific biomarkers of chlorpyrifos. Undoubtedly, exposure measurement error occurred. However, exposure validation studies illustrate some degree of correlation across exposure measures made at different periods of gestation (*e.g.,* meconium, week 13 through delivery; maternal urine during last 8-weeks of pregnancy; and cord blood at delivery). Additionally, given the prospective nature of these studies, it is unlikely the measurement error was differential by outcome, *i.e.,* non-differential exposure misclassification leading to biased estimates toward the null is anticipated. Finally, as noted above the degree of missingness in some key variables across these studies may have resulted in a form of selection bias which may have also lead to an under-estimation of effects. However, given the data are missing, it is difficult to assess the magnitude and direction of this error on study results. Low sample size may have limited the ability of researcher to identify sources of effect modification or perform stratified analyses with accuracy.

Conversely, there may have been factors at play which led to an inflated estimate of the true association. Factor that may lead to false positive associations include unmeasured or poorly measured confounding factors which are positively associated with both chlorpyrifos use and adverse neurodevelopmental outcomes (positive confounding variables). The measurement of socio-economic factors and other environmental exposures experienced either pre- or post-natal environment are most likely among these potential factors, although others may exist. However, when additional adjustment was made for these factors using both individual- and group-level variables, the magnitude and direction of the associations remained stable (diazinon, propoxur, blood lead, methyl mercury, SES, post-natal exposure) (Lovasi et al., 2011; V. A. Rauh et al., 2006; R. M. Whyatt et al., 2004; R. Whyatt & Rauh, 2011) (See Attachment 1). In the instances in which authors assessed the role of early childhood exposure to chlorpyrifos or other environmental contaminants as potentially confounding variables in the relation between prenatal exposure and neurodevelopmental outcomes, confounding bias was not observed. However, not all investigations evaluated the role of post-natal pesticide exposure, and authors note the limitation. Selection bias due to missing or drop outs from the study could also have influenced the observation of a false positive association, and this is again difficult to assess in the absence of data. However, researchers uniformly discussed and described the comparison of those included and excluded from individual analyses based in past of missing data using several important factors, and in many instances reported a level of comparability which is reassuring. The observation of positive associations as a result of multiple comparisons may also be a factor to consider; however the *a priori* identification of research questions of interest and the consistency of findings across several neurodevelopmental domains argues against a large role for multiple statistical testing to explain positive findings.

Overall, these are well performed studies which are shielded from several major sources of bias in the interpretation of results due to the strong design, conduct and analyses utilized in these investigations. While factors are present across these studies which may have led to either false positive or negative associations, it is notable that positive associations were observed as EPA believes the possibility of under-estimation of effect size is more likely than factors that would lead to over-estimation of effect size. Authors have taken significant steps to address major sources of uncertainty that may lead to over-estimation of effects, *i.e.*, positive confounding bias as a result of poorly measured factors related to socio-economic status and other environmental chemical exposures.

3.0. Chlorpyrifos Epidemiology Synthesis and Evaluation

In this chapter, EPA reviewed the results of epidemiological investigations of the association between prenatal chlorpyrifos exposure and adverse effects upon fetal growth and neonatal and early childhood neurodevelopmental outcomes across three major prospective children's health cohort studies in the U.S. In accordance with the OPP's draft "Framework for the Incorporation of Epidemiology into Regulatory Risk Assessment"[72], including the use of the modified Bradford Hill considerations in judging the potential causal nature of observed associations, in this section EPA considers the totality of the epidemiological evidence from these cohorts. To perform this analysis, EPA considered the strength of the associations observed and the presence of exposure-response trends, the temporality of the observed associations, and the degree to

[72] http://www.epa.gov/scipoly/sap/meetings/2010/020210meeting.html#materials

which alternative explanations have been considered and eliminated as explanatory factors, among other considerations. Issues of biological plausibility and specific mechanisms of action which may explain a causal role for chlorpyrifos in adverse neurodevelopmental outcomes, while broadly considered within the context of these investigations, are discussed in depth in the revised chlorpyrifos HHRA and supporting documents. EPA notes that while the pilot MRI study by the CCCEH researchers supports other findings from these cohorts, additional and more sophisticated imaging would have to be performed to determine links to the very specific types of neurodevelopmental effects posited by authors in the Rauh et al. (2012) study.

As noted previously, each investigation is a prospective cohort study in which prenatal exposures occurred prior to the developmental of either fetal growth anomalies or neonatal or early childhood neurological delays. Therefore, in each study exposure preceded the health effect, risk may be directly calculated, and measurement error is more likely non-differential in nature, *i.e.*, likely under-estimation rather than over-estimation of risk effect. Table 1 summarizes the associations observed for chlorpyrifos markers and neurodevelopmental outcomes in the three cohorts. Within this database, moderately strong associations have been observed, and in many instances measured with precision. The relation between both neonatal neurological development as measured using the Brazelton index (number of abnormal reflexes) and also mental and psychomotor development among toddlers (Bayley Scale for 24-36 months) with prenatal chlorpyrifos exposure is elevated approximately 2- to 4- fold among those more highly exposed. This observation was consistent across cohorts with respect to the neonatal period, but not across measures using the Bayley Scale. Considering adverse effect in the 24-36 months period of development, the effects became more pronounced over time in one cohort (the Columbia Mother's and Newborn study), and became less pronounced over time in the Mt. Sinai Child Development study using non-specific biomarker of chlorpyrifos exposure (DAPs). However, it is notable the significant effects were seen when chlorpyrifos parent compound was measured directly; and, the effect size became significantly greater when diazinon was considered in the final statistical model. Notable decrements in intelligence measures were consistently observed across all three cohorts. While effect sizes measured as beta-coefficients are reflective of average change in intelligence score per unit change in chlorpyrifos exposure, and may appear modest in effect size, EPA notes that according to these models, the adverse influence at the extremes of the chlorpyrifos exposure distribution, if truly present in nature, would be more deleterious. Strong, consistent evidence of a positive association between prenatal chlorpyrifos and attentional problems, pervasive developmental disorder, and ADHD-like symptoms was observed in these studies, although not always measured with precision.

An association is consistent when a similar magnitude and direction of results are replicated in studies in different settings using different methods. If a relationship is causal, one may expect to observe it consistently in different studies and among different populations. Several consistencies were noted across these studies. Higher levels of the chlorpyrifos-specific metabolite TCPy were associated with prevalence of abnormal reflexes among newborns, as assessed using the Brazelton Neonatal Behavioral Assessment Scale (BNBAS), in both the Mount Sinai Hospital and UC Berkeley (CHAMACOS) Cohorts. The BNBAS assessment was not conducted in the Columbia Mothers and Newborns cohort. TCPy levels were consistently not associated with BNBAS indicators of newborn neurodevelopment such as behavioral domains other than those observed for number of abnormal reflexes. In addition, associations between pesticide markers

and the Mental Development Index (MDI) portion of the Bayley Scores at 12, 24, and 36 months varied between small, largely non-statistically significant decrements, and null. However, in supplemental (post-publication) analyses, authors illustrated that adjustment for other organophosphate pesticides in the relation between prenatal chlorpyrifos and MDI strengthened the magnitude of the effect significantly at the 36 months time point (R. Whyatt & Rauh, 2011) (See Attachment 1). Associations between early life organophosphate pesticide exposure biomarkers and decrements in mental development in childhood (age 7-9 years) were observed in all three studies, although the relationships were not consistently statistically significant.

Some notable inconsistencies were also observed. These may be explained by differing exposure biomarkers, timing of biomarker collection, or other limitations of study design and measurement, *e.g.,* the magnitude of non-differential exposure misclassification. However, inconsistency in results may also argue against a true causal association. Associations between markers of prenatal pesticide exposure and fetal growth were not consistent across the three studies, as noted in the FIFRA SAP 2008 meeting. In the Mount Sinai Child Growth and Development cohort, no statistically significant associations were observed in primary analyses, although a modest decrease in average head circumference with increasing TCPy was noted among those with low PON1 activity. In the Columbia Mothers and Newborn Study (R. M. Whyatt et al., 2004), chlorpyrifos levels in maternal blood were modestly associated with *decreased* fetal growth. In contrast, among the UC Berkeley CHAMACOS study participants, modest *increased* birth weight and head circumference were associated with *in utero* DAP concentrations (Eskenazi et al., 2004; Harley et al., 2011). In fact, stratification by *PON1* status seemed to enhance these positive associations, such that in the non-susceptible groups (*i.e.*, $PON1_{-108}$ CC, $PON1_{192}$ RR, and high arylesterase activity), increasing DAP concentrations were significantly associated with increased birth weight and head circumference. The pesticide biomarkers differed between the two studies; cord blood chlorpyrifos levels were assessed in the Columbia Mothers and Newborn study, while DAPs were assessed in the UC Berkeley CHAMCOS cohort. It is possible that high urinary DAP concentrations may be an indication of rapid detoxification and excretion of organophosphate pesticides, rather than a marker of high exposure, particularly among the participants that are less potentially less susceptible to pesticide effects due to their *PON1* genotype or PON activity level, as suggested by study authors.

Although consistency of associations across studies set in different populations, and employing different methods has been used as a criterion for causality, it is not the only compelling factor. In fact, heterogeneity across studies is expected to occur between studies in the presence of effect modification, if the prevalence of the effect modifier varies between the populations being assessed. Apparent inconsistencies could also be due to chance differences, and due to the presence of biases operating in the studies under consideration.

There was evidence for an exposure-response relationship in several of the studies reviewed, whereby increasing levels of exposure were associated with increasingly large decrements in measures of neurodevelopment (V. Rauh et al., 2011; V. A. Rauh et al., 2006). For the analyses in which continuous distributions of exposure markers were used, the levels were often, but not always, log transformed prior to entry in statistical models. The estimated association between the biomarker level and the outcome in these cases is not linear; rather, the association between the log-transformed exposure and the outcome is linear. In such cases, results are interpretable

as changes in mean level/risk of the outcome of interest for a given percentage increase in the exposure. Examination of departures from linearity of exposure-response relationships was reported in only a small subset of the reviewed articles. For example, Rauh et al. (2011) reported that, in the Columbia cohort, "the dose-effect relationships between CPF [chlorpyrifos] exposure and log-transformed Working Memory Index and Full-Scale IQ scores are linear across the range of exposures in the study population, with no evidence for a threshold" (V. Rauh et al., 2011). Departures from linearity were not statistically significant. Two possible explanations for this finding are that 1) the exposure response is linear on the scale assessed, or that 2) the studies did not have sufficient power to detect departures from linearity in the shape exposure-outcome relationship. The smoothed exposure-response curve superimposed upon the scatter plot of log-transformed working memory appears to be monotonically decreasing; however, a similar curve superimposed upon the scatter plot of log-transformed IQ score and chlorpyrifos levels appears flat if not slightly positive, before turning negative beyond an inflection point at about 5 pg/gram of chlorpyrifos. As such, it may be that both explanations for the null finding regarding departures from linearity are operating in this study. As another example, Bouchard et al used cubic splines to evaluate the shape of dose-response curves, test the linearity assumption, and investigate potential thresholds (Bouchard et al., 2011). Again, no statistically significant departures from linearity were observed.

Due to the voluntary cancellation by registrants of indoor residential uses of chlorpyrifos-containing pesticide products, children born after the year 2000 were likely exposed to far lower levels of chlorpyrifos, on average, than children born prior to 2001. In their report, Whyatt et al. (2004) took advantage of this "natural experiment" by stratifying their analyses on birth date prior to, or after, January 1, 2001. Although reported household use of pesticides in general did not change over the same time period in this study, maternal chlorpyrifos in maternal air samples were significantly lower after the voluntary cancellation (4.9 ng/m^3), relative to those taken before 2001 (8 ng/m^3; p<0001). Associations between birth outcomes and organophosphate insecticide levels in maternal personal air samples were not statistically significant in both un-stratified analyses and among subgroups stratified by birth prior to or after January, 2001. However, the associations between birth weight and length and cord plasma chlorpyrifos were highly significant ($p \leq 0.007$) among newborns born before the 2001 cancellation of registrants. Among newborns born after January 2001, no association with fetal growth was observed ($p > 0.8$) (R. M. Whyatt et al., 2004). Supplemental analysis suggested this observation persisted among additional number of infants born after the period of the voluntary cancellation (n=193) (R. Whyatt & Rauh, 2011) (Attachment 1). This finding by Whyatt et al provides evidence that the association "can be altered (prevented or ameliorated) by an appropriate experimental regimen," as advocated by AB Hill in the assessment of causality in observational studies. Changes in exposure were reflected in decreasing chlorpyrifos levels, and appear to have been associated with changes in developmental outcomes. However, as noted by the 2008 FIFRA SAP Panel in their report, this study was not designed to test this hypothesis, specifically.

It must also be considered that given the many selection criteria applied within each of the three children's health cohorts, the generalizability of study results may have been affected. These studies reflect a somewhat narrowly defined sub-population who likely experienced a greater range of exposures and higher peak exposures than the general U.S. population; a comparison of maternal urinary concentration of TCPy in the three children's health cohorts with the general

population (NHANES) illustrates higher exposures occurred among the cohort participants. The exception is with the urinary concentration data collected by Whyatt et al. (2009) which were lower than the U.S. general population; however, these data were collected 2001-2004, a period after the voluntary cancellation of indoor residential uses of chlorpyrifos. In addition, the external validity of the study findings may be limited if the organophosphate pesticide exposure-fetal development association is modified by factors that are more, or less, prevalent in the study populations relative to the population(s) to which inference is being made, or to populations with a substantially different exposure range. Generalizability may also be affected by low sample size such as in the brain imaging (MRI) study. In the studies reviewed, the exploration of gene-by-environment, and phenotype-by-environment interactions is a strength of many of the analyses, although as noted above the studies are uniformly underpowered to assess such interactions. There was limited assessment of interaction between chlorpyrifos and other environmental co-exposures conducted in these investigations including blood lead levels.

4.0 Conclusions

The FIFRA SAP panel convened in September 2008 concluded that given the strengths of these studies, the effect of biases likely present, and rigorous statistical analyses performed, the observation of associations across these studies can be interpreted as a conclusion that chlorpyrifos likely played a role in the neurodevelopmental outcomes observed among children more highly exposed to chlorpyrifos in the *in utero* environment. Since that time, researchers with each of the three children's health cohorts have published several new etiologic and methodological studies and performed supplemental analyses as suggested by the 2008 Panel which extend the knowledge base, and reduce several major sources of uncertainty present in the 2008 database (*i.e.*, confounding by SES, exposure validation in the Columbia Mothers and Newborn study, further evaluation of *PON1* genotype and PON activity level, pilot MRI imaging study). In the current document, EPA utilized the draft "Framework for Incorporating Epidemiology into Risk Assessment"[73], including use of modified Bradford Hill considerations, to assess the strengths and limitations of the studies in this database on the association of interest, and to clarify the epidemiologic evidence in support of a causal role for chlorpyrifos in infant and child adverse neurodevelopmental outcomes.

In EPA's analysis of the strengths and weaknesses of these studies, the chance that positive associations observed are false positives due to systematic errors in the studies cannot be excluded; however, it is more likely that error present in these studies would lead to the under-estimation of the true association. Therefore, while alternative explanations for positive association can be hypothesized (*e.g.*, additional unmeasured or poorly measured positive confounding variables), these explanations are judged to be less plausible than the alternative that associations have been missed or under-estimated due to non-differential measurement error and low sample size across exposure strata. In occupational settings, exposure measurement error has been shown to more greatly influence epidemiology study results than unknown or unmeasured confounding variables (Blair et al., 2011). Additionally, the elimination of temporal bias due to the prospective study design employed within each of the three children's health cohorts assures that prenatal exposures preceded neurodevelopmental outcomes measured at birth, and in early and later childhood through age 7 years. The strength of the associations

[73] http://www.epa.gov/scipoly/sap/meetings/2010/020210meeting.html#materials

measured in some studies was notably strong. However, associations in many instances were weak to moderate, possibly due to measurement error. Associations with neurodevelopmental outcomes were consistently identified with respect to the number of abnormal reflexes in the neonatal period, the presence of mental and behavioral issues as well as gross motor delays were pronounced especially in later toddler years of 24-36 months, and the observation of intelligence decrements were seen across the three cohorts using different measures of prenatal chlorpyrifos exposure, although not consistently statistically significant. EPA notes that other organophosphates may be involved as well as the CHAMACOS and Mt. Sinai Child Development study utilized dialkyl phosphates which measure exposure to several different organophosphates, and that use of chlorpyrifos in the CHAMACOS study region was low at the time of the study. However, links with diethyl phosphate which result from either chlorpyrifos or diazinon exposure were observed as well.

In summary, while the strengths and limitations of the studies would be more likely to lead to an under-estimation of the true effect, the possibility of false positive associations cannot be entirely ruled out. The temporal association, strength of the statistical associations observed and presence of exposure response across some (but not all) of the investigations, as well as some notable consistencies in findings within this epidemiologic database tend to support a role for chlorpyrifos in this relation. It is true that the Columbia Mothers and Newborn study is the only cohort to have measured chlorpyrifos parent compound directly; replication in other cohorts that use this exposure metric would aid causal inference. The presence of factors which may have over-estimated effects, the lack of consistency in many domains (*e.g.*, fetal growth), and the lack of a clear mechanism of action may argue against a true association. Overall, the current database supports the conclusion from 2008 Panel that "chlorpyrifos likely played a role in the birth and developmental outcomes noted in the three cohort studies." Additionally, the subsequent studies made available since the time of the 2008 evaluation helps to clarify the potential role for chlorpyrifos in adverse neurodevelopmental health effects.

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Appendix 2. Attachment 1

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

Date: June 30, 2011

SUBJECT: **Chlorpyrifos Correspondence with Columbia Researchers: (1) Responses to Scientific Advisory Panel (SAP) comments (Whyatt and Rauh 2010), and (2) Responses to Dow AgroSciences inquiries (Whyatt 2010).**

PC Code: 059101
Decision No.: NA
Petition No.: NA
Risk Assessment Type: Single Chemical Aggregate
TXR No.: 0056066
MRID No.: NA

DP Barcode: D391257
Registration No.: NA
Regulatory Action: NA
Case No.: NA
CAS No.: NA
40 CFR: NA

FROM: Deborah Smegal, MPH,
Immediate Office (IO)
Health Effects Division (7509P)

TO: Danette Drew, Risk Assessor
RABV
Health Effects Division (7509P)

Backrgound
On September 16-19, 2008, the Agency held a FIFRA Scientific Advisory Panel (SAP) meeting (SAP 2008) to discuss the Agency's Evaluation of the Toxicity Profile of Chlorpyrifos, which includes more recent and extensive research on various aspects of chlorpyrifos including its neurological effects in animals and humans following gestational and post-natal exposures, its pharmacokinetics, and mechanism of action. Details can be found at the Scientific Advisory Panel website (http://www.epa.gov/scipoly/sap/meetings/2008/091608_mtg.htm), and in the Chlorpyrifos Final SAP report (http://www.epa.gov/scipoly/sap/meetings/2008/september/sap0908report.pdf.)

Attached is a response to the SAP comments on the "Epidemiologic Appendix: Columbia Study" that Deborah Smegal summarized and sent to Robin Whyatt and Virginia Rauh, dated 1/28/2010. In addition, the Columbia researchers prepared responses to questions raised by Dow AgroSciences.

Columbia Response to the SAP Queries
2/19/2010

The following is a response to the Scientific Advisory Panel comments on the "Epidemiologic Appendix: Columbia Study" that Deborah Smegal summarized and sent to Robin Whyatt and Virginia Rauh, dated 1/28/2010.

1. **SAP comment: EPA should continue its collaboration with Columbia researchers in analyzing the epidemiologic data ...[and] should seek clarification of the reported exposure levels . . . EPA should use the epidemiological cohort data for bounding exposure levels and in conjunction with PBPK models, address current or potential human exposures and to determine the final RfD/RfC . . . PBPK models enable estimation of exposure dose metric for multiple sources (e.g., air, food, water etc). EPA should pursue simpler PBPK model in short-term.**

Response: We are happy to continue the collaboration. In addition, as described in our letter, Dr. Dale Hattis at Clark University is conducting PBPK modeling of the Columbia data under a U.S. EPA Star Grant. The grant is entitled "Use of Biomarkers and Physiologically Based Pharmacokinetic [PBPK] Modeling in Risk Analysis for Developmental Effects of Chlorpyrifos". We believe that the PBPK model being developed by Dr. Hattis will prove extremely helpful to EPA in addressing the SAP comments and in clarifying the exposure concentrations that correspond to the chlorpyrifos levels in umbilical cord blood at which we begin to see statistically significant adverse effects on fetal growth and child neurocognitive development. Thus this data should be helpful in bounding the exposure data as recommended by the SAP. We recommend that EPA contact Dr. Hattis.

2. **SAP comment: Determine if the chlorpyrifos effect on birth weight would result in more infants in "low birth weight" category.**

Response: We are not able to address this question as our cohort was predominantly full term by design. This is because we did not conduct our environmental monitoring until the 3rd trimester of pregnancy and completion of the environmental monitoring was a prerequisite for enrollment. For example, of the subjects in our 2004 analyses (Whyatt et al., EHP, 112:1125-1132, 2004), only 12/314 (3.8%) of the women delivered prior to 37 weeks gestation and only 12/314 (3.8%) of the infants were <2500 grams at birth. We, therefore, do not have adequate statistical power to evaluate the relationship between prenatal chlorpyrifos exposure and low birth weight.

3. **SAP comment: Ascertain whether there are statistically significant differences in neurodevelopmental outcomes at 3 yrs between chlorpyrifos levels in cord blood below LOD and those with chlorpyrifos >6.17 pg/g . . . Verify/clarify there is no suggestion of an effect as the most highly exposed group and the group with values below the LOD had lower MDI and PDI scores than did the middle levels.**

Response: Table 1 below provide the same type of information as Table 2 in the 2006 Pediatrics paper (Rauh et al., Pediatrics, 118:e1845-e1859, 2006) with respect to the % of subjects whose tests scores indicated developmental delay with respect to the PDI and the MDI, as a function of

chlorpyrifos exposure. However, here we provide the % delayed for all four exposure groups, rather than just the 'high' and 'low' exposure groups shown in the paper. For both the PDI and MDI the rate of delay is clearly highest in the high chlorpyrifos group (Group 4).For the 36 month PDI, the rate of developmental delay is relatively low in all 3 low-exposure groups, and is about three times higher in the highest exposure group. And for the 36 month MDI, while the <LOD group experienced a higher rate of delay than group 2 and 3, the very highest rate of delay is among the most exposed children. In univariate analyses, the difference in % delay between the highest exposure (>6.17pg/g) group versus the lowest exposure (<LOD) group, was statistically significant for PDI (chi-square p=.018, 1 sided; p=.031 two-sided), but not significant for MDI. However, importantly, there was no significant difference in the rate of delay between groups 1, 2 and 3 for either the PDI (chi-squared= 0.97, p=0.61) or MDI (chi-squared= 4.4, p=0.11). And the rate of developmental delay in terms of both PDI and MDI in group four was significantly greater than in groups 1-3 combined, as reported in our paper.

Table 1. The proportion of children at age 36 month who were at risk of delay (score < 85) on the Psychomotor Development Index (PDI) or Mental Development Index (MDI) of the Bayley Scales of Infant Development by chlorpyrifos exposure group

	Group 1 (<LOD)	Group 2 (1st tertile > LOD)	Group 3 (2nd tertile > LOD)	Group 4 (3rd tertile > LOD)
PDI	7/80 (8.8%)	3/65 (4.6%)	3/38 (7.95)	11/45 (24.4%)
MDI	30/80 (37.5%)	14/65 (21.5%)	11/39 (28.2%)	20/44 (45.5%)

4. **SAP comment: Conduct an analysis of exposure levels as dependent variable and year (an ordinal variable) as the primary independent variable, while adjusting for any factors associated with both exposure levels and year of study.**

Response: We have already published on changes in indoor and personal air levels, as well as in multiple biomarker levels by year of monitoring and have enclosed the most relevant articles (Whyatt et al., 2007; Whyatt et al., 2009). In addition, Dr. Hattis has also recently completed analyses looking at changes in chlorpyrifos levels by year in the context of other factors such as season of monitoring. As stated above, we recommend that EPA contact Dr. Hattis to review his results.

5. **SAP comment: EPA should request additional details on the loss to follow-up in each of the 4 exposure groups and provide additional details on how the exposure groups were combined.**

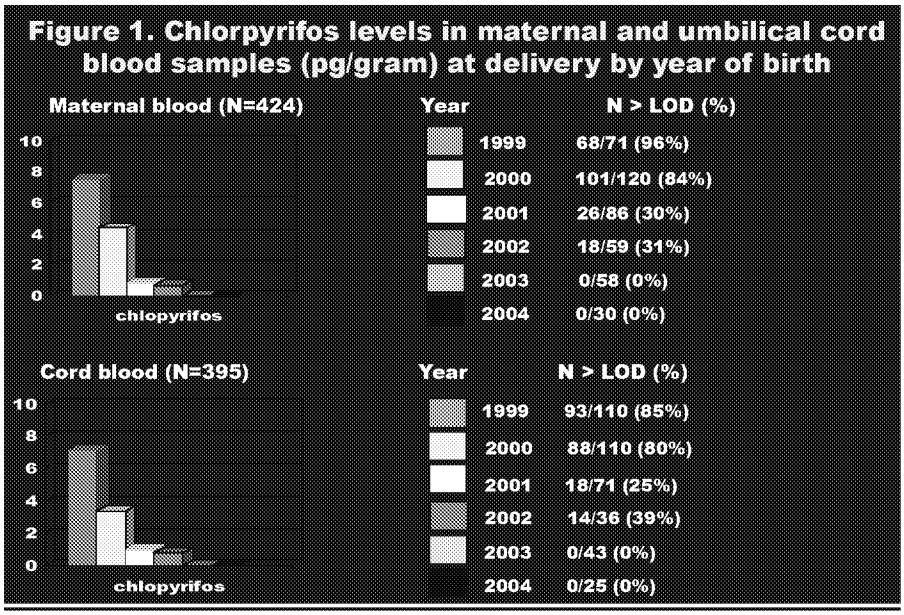
Response: Subjects were eligible for inclusion in the Pediatric paper if they had been born by 7//12/02 and thus had reached age three years by the time of the analyses and also had: (1) measures of chlorpyrifos in blood at birth; (2) neurologic assessments at least once between ages 12-36 months and (3) data on model covairates. A total of 381 subjects in the cohort had been born by 7/12/02 and had birth chlorpyrifos blood measure; of these 254 (67%) met the above criteria for inclusion, including 228 who had developmental outcomes at age 36 months. The remaining subjects had either been lost to follow-up (accounting for approximately 14%) or were missing outcome and/or covariate data. Table 2 below shows the 254 subjects included in the analyses over all 381 subjects by the chlorpyrifos groups. (The numbers per group just for the 228 subjects with outcome data at 36 months are given in the Pediatric manuscript.) Group 1 includes all subjects

with chlorpyrifos blood levels below LOD; the remaining subjects were ranked into tertiles based on ascending levels (Groups 2-4). However, as describe in the Pediatric manuscript, this grouping was done on subjects born prior to 1/1/01 to be consistent with our prior publication (see Whyatt et al. 2004). This was essential in order to compare results across analyses moving forward, given that chlorpyrifos concentrations were changing rapidly following the ban and thus we needed consistent cutpoints. Groups 1-3 were combined and compared with group 4 for the analyses presented in the Pediatrics manuscript.

Table 2. Subjects included in the Pediatric paper over all cohort subjects born at or before 7/12/02 who had chlorpyrifos measured in cord blood at birth

Group 1 (<LOD)	Group 2 (1st tertile > LOD)	Group 3 (2nd tertile > LOD)	Group 4 (3rd tertile > LOD)
88/121 (72%)	72/127 (56.7%)	44/63 (69.8%)	50/70 (71.4%)

6. **SAP comment: Post ban declines in effects are based on 1 subject being classified as highly exposed, which lacks statistical power and may be sensitive to the cutoff value chosen to classify subjects as highly exposed. Can an analysis be conducted to look at relationships between more equally sized exposure groups that separate between the lower exposure levels?**



Response: We believe the SAP has misunderstood that our post ban declines were based not on the change in the number of subjects that fell into the highly exposure group but on the decrease we had seen in chlorpyrifos concentrations in both maternal and cord blood among all cohort subjects by year of birth (see Figure 1). As can be seen in the above figure, chlorpyrifos levels had dropped dramatically by 2001. Our 2004 study categorized the full cohort available at that time (most born prior to1/1/01) into four groups based on ascending levels of chlorpyrifos in umbilical cord blood. The only significant difference we saw was between infants in the highest exposure group (group 4) compared to those with levels below the limit of detection (group 1). By contrast, there was no significant difference in birth outcomes between subjects in the intermediate

exposure groups (2 or 3) compared to group 1 (see Whyatt et al. EHP, 112: 1125-1132. 2004). At the time we conducted the analyses for our 2004 paper, only n=77 infants were born after 1/1/01, which limited our power to determine whether or not there was any continuing effect of prenatal chlorpyrifos exposure on birth outcomes after2001. In April 23, 2008, we presented undated analyses to EPA that included more infants (n=193) born after 1/1/01. Results are shown in Table 3 below and are consistent with our 2004 publication. Specifically, we saw a highly significant decrease in birth weight for each log unit increase in umbilical cord

Table 3. Change in birth weight for each log unit increase inchlorpyrifos concentrations in umbilical cord plasma among babies born before and after 1/1/01

	B (95% CI)	**P-value**
Born before 1/1/01 (n=233)	-71/4 (-121/1, -21.8	P=0.005
Born after 1/1/01 (n=193)	32.7 (-59.3, 124.7)	P=0.48

chlorpyrifos concentrations among babies born before 1/1/01, but no association between chlorpyrifos and birth outcomes among infants born after 1/1/01. Of those infants born after 1/1/01, only 16% had chlorpyrifos levels in cord blood above the LOD and only one of these was in the previously defined 'high exposure' group. It is obvious that this marked reduction in chlorpyrifos exposure, and the virtual elimination of high exposure levels after 1/1/01, is due to the chlorpyrifos ban , . To address the SAP comments further, we have conducted analyses that categorize infants born after 1/1/01 into those with chlorpyrifos above and below the LOD. Keeping in mind that there was only one child with high (group 4) chlorpyrifos exposure in this post-ban sample, it is not surprising that there was no significant difference in birth weight between the <lod group and the small group with some exposure during this post-ban period. These findings thus support the hypothesis that chlorpyrifos exposures following the ban had dropped below concentrations that have adverse effects on fetal growth. However, it is also possible that our sample size is simply too small to detect relatively small but real negative effects on birth outcomes associated with the much reduced exposure levels after 1/1/01.

7. SAP comment: The panel felt it might be helpful to have a longitudinal component to the study to determine if the adverse effect persisted in children who exhibited poor birth outcomes or neurobehavioral deficits.

Response: As described in the attached letter, we have just completed statistical analyses on the relationship between prenatal chlorpyrifos exposure and outcomes on the Wechsler Intelligence Scale for Children (Fourth Edition) and the Child Behavior Check List, which have been administered to cohort children at age 7 years. We anticipate that a manuscript on these findings will be completed and ready for submission within several months.

8. SAP comment: It would be useful to examine the results of a statistical analysis that includes all three AChE-inhibiting insecticides in the analysis model as dichotomous variables (above or below the LOD) in combination with continuous measurements for these variables. This is not likely to change the results but could be helpful in illustrating threshold or dose response effects . . . Are all 3 AChE inhibiting insecticides correlated (if lack of correlation—less likely would confound cyp and neuro outcomes) . . . Conduct additional

analysis controlling for multiple exposures examining additive effects to elucidate the role of other Ops. This is important for AChEI to be considered as mode of action.

***Response*:** It should be noted that our 2004 paper (Whyatt et al., EHP, 112: 1125-1132) looked at the additive effects of chlorpyrifos and diazinon on birth outcomes using the U. S. EPA guidelines for conducting cumulative risk assessments for the organophosphates. However, in response to the SAP comments, we have now conducted a number of additional analyses both to evaluate the correlations among the three AChE inhibiting insecticides (chlorpyrifos, diazinon and propoxur) detected in our cohort and to look at the effects of chlorpyrifos on both birth outcomes and child neurocognitive development after controlling for diazinon and propoxur. Specifically we have devloped 4 additional models for birth weight and birth length and 4 models for the psychomotor development index (PDI) and mental development index (MDI) on the Bayley Scales of Infant Development at age 36 months. All models controlled for the same covariates as in our 2004 and 2006 publications. Model 1 includes only chlorpyrifos as the independent variable (i.e. is identical to the data in our initial publications); model 2 includes chlorpyrifos and diazinon; model 3 includes chlorpyrifos and 2-isopropoxyphenol (the chemical-specific metabolite of propoxur); and model 4 includes all three acetylcholinesterase inhibiting insecticidess Results are presented below:

Correlations among pesticides: For infants born prior to the chlorpyrifos phase out (the relevant time period), there was a modest correlation between 2-isopropoxyphenol (the chemical specific metabolite of propoxur) and both chlorpyrifos ($r=0.25$, $p<0.001$, $n=222$) and diazinon ($r=0.21$, $p=0.002$, $p=227$) in umbilical cord blood samples. However, there was a higher correlation between chlorpyrifos and diazinon ($r=0.63$, $p<0.0001$, $n=222$), incdicating that approximately 33% shared variabilitybetween these two pesticides. Given this relationship between chlorpyrifos and diazinon, the question raised by the SAP—whether the chlorpyrifos effects we report could be due to confounding by diazinon—is therefore very appropriate. This question is addressed in the new analyses reported below.

Results on birth outcomes. Results are presented in Tables 4 and 5 below. As can be seen, chlorpyrifos remained inversely associated with birth weight and length in all models after controlling for diazinon and 2-isopropoxyphenol, and the magnitude of the significant negative effect of chlorpyrifos on infant size remained relatively constant across the models, indicating that neither of these two other acetylcholinesterase inhibitors was confounding the relationship between chlorpyrifos and birth outcomes. Further neither diazinon nor 2-isopropoxyphenol, either individually or in combination, was associated with birth weight, although the inverse association reported previously between 2-isopropoxyphenol and birth length (see Whyatt et al., 2004) remains of borderline significant ($p=0.05$). Consistent with our prior findings, birth weight decrease significantly by 198 grams among infants in the highest compared to the lowest chlorpyrifos groups (Group 4 versus Group 1) after controlling for both diazinon and 2-isopropoxyphenol, as well as the other model covariates ($p<0.05$). These analyses thus indicate that it is primarily prenatal chlorpyrifos and not the other two acetylcholinesterase-inhibiting insecticides that is having an adverse effect on fetal growth. Further, the findings suggest that the effect of chlorpyrifos on fetal growth may well be mediated by mechanisms other than acetylcholinesterase inhibition, given the lack of an association between the other two acetylchlolinesterase inhibitors and birth weight.

Table 4. Decrease in birth weight for each log-unit increase in insecticide levels in umbilical cord blood at birth among infants born prior to 1/01/01. Values shown are regression coefficient (B), 95% confidence interval, and p-value

	Model 1	Model 2	Model 3	Model 4
(ln)Chlorpyrifos	-67.3 (-17.8, -116.8) p=0.008	-65.9 (-5.7, -126) p=0.03	-60.9 (-10.0, -111.8) p=0.02	-60.2 (0.9, -121.3) p=0.054
(ln)Diazinon		-4.7 (107.7, -117.1) p=0.94		-2.5 (110, -114) p=0.96
(ln)2-isopropoxyphenol			-50.9 (46.1, -147.8) p=0.30	-50.8 (46.5, -114.9) p=0.30

All models controlled for gestational age, parity, maternal pre-pregnancy weight and net weight gain during pregnancy, maternal self-reported environmental tobacco smoke in the home, gender of the newborn and season of delivery.

Table 5. Decrease in birth length for each log-unit increase in insecticide levels in umbilical cord blood at birth among infants born prior to 1/01/01. Values shown are regression coefficient (B), 95% confidence interval, and p-value

	Model 1	Model 2	Model 3	Model 4
(ln)Chlorpyrifos	-0.43 (-0.14,-0.73) p=0.004	-0.46 (-0.1, -0.82) p=0.01	-0.36 (-0.06,, -0.66) p=0.02	-0.40 (-0.75, -0.35) p=0.03
(ln)Diazinon		0.09 (0.76, -0.58) p=0.80		0.12 (-0.78, 055) p=0.73
(ln)2-isopropoxyphenol			-0.57 (0.002, -1.14) p=0.051	-0.58 (-.001, -1.15) p=0.05

All models controlled for gestational age, parity, maternal pre-pregnancy weight and net weight gain during pregnancy, maternal self-reported environmental tobacco smoke in the home, gender of the newborn and season of delivery.

Results on PDI and MDI are presented in Table 6 and 7 below. The findings were quite surprising. There was no significant association between 2-isopropoxyphenol and delay on either the PDI or MDI. However, when diazinon was added to the model, the negative effect of chlorpyrifos on developmental delay for both the PDI and MDI became much stronger. Specifically, the odds ratio almost doubled for PDI and increased by 40%-60% for the MDI. These results again indicate that it is chlorpyrifos and not the other two acetylcholinesterase insecticides that is having an adverse effect on child mental and motor development and further that the effect size appears considerably greater than we had estimated before. The findings again support the suggestion, as discussed by the SAP, and as is suggested by the experimental evidence, that the effects of chlorpyrofos on child development may well be by a mode of action other than acetylcholinesterase inhibition.

Table 6. Effects of chorpyrifos on the odds of ps;ychomotor delay on the Bayley Scales of Infant Development at age 36 month. **Values shown are regression coefficient (B), 95% confidence interval, and p-value *s***

	Model 1	Model 2	Model 3	Model 4
Chlorpyrifos[2]	4.5 (1.6, 12.7)	8.4 (2.4, 30.2)	4.0 (1.4, 11.8)	7.9 (2.1, 29.1)

	p=0.004	p=0.001	p=0.01	p=0.002
(ln)Diazinon		0.45 (0.18, 1.13) p = 0.09		0.4 (0.2, 1.1) p=0.08
(ln)2-isopropoxyphenol			1.3 (0.65, 2.8) p=0.4	1.4 (0.67, 3.0) p=0.37

All models controlled for race/ethnically, gender, ETS, gestational age, maternal IQ, maternal education, HOME score[1]OR = Odds ratio
[2]>6.17 pg/g versus ≤ 6.17 pg/g in umbilical cord blood

Table 7. Effects of chorpyrifos on the odds of mental delay on the Bayley Scales of Infant Development at age 36 months. Values shown are regression coefficient (B), 95% confidence interval, and p-value

	Model 1	*Model 2*	*Model 3*	*Model 4*
Chlorpyrifos[2]	2.3 (1.1, 5.2) p=0.03	3.8 (1.5, 9.4) p=0.004	2.0 (0.9, 4.5) p=0.11	3.2 (1.3, 8.2) p=0.02
(ln)Diazinon		0.57 (0.35, 0.95) p = 0.03		0.6 (0.3, 0.9) p=0.03
(ln)2-isopropoxyphenol			1.5 (0.9, 2.5) p=0.12	1.5 (0.9, 2.6) p=0.11

All models controlled for race/ethnically, gender, ETS, gestational age, maternal IQ, maternal education, HOME score[1]OR = Odds ratio
[2]>6.17 pg/g versus ≤ 6.17 pg/g in umbilical cord blood

9. SAP comment: Is it possible to determine quantitative contribution of chlorpyrifos in exposure to mixture by accounting for differences in chemicals, potency, pharmacokinetics and possible chemical interactions.

Response: Dr. Hattis can address this question after his PK model is implemented and calibrated.

10. SAP comment: Look at intermediate markers of biologically effective dose and early effect that further link exposure and outcome and or a clearer idea of mode of action from exposure studies.

Response: As described in our letter, we have been conducting Magnetic Resonance Imaging (MRI) scans on cohort children with either high prenatal chlorpyrifos exposure (n=20) or no exposure to any of the contaminants that have been associated with adverse child cognitive development among the CCCEH cohort (n=20). The MRI results will provide a marker of biologic effect. These analyses are being undertaken under the direction of Dr. Bradley Peterson, Director of Child and Adolescent Psychiatry at the New York State Psychiatric Institute. We anticipate that a manuscript on the results will be submitted to a peer-reviewed journal by Aug 1 2010.

COLUMBIA CENTER
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ENVIRONMENTAL
HEALTH

MAILMAN SCHOOL OF PUBLIC HEALTH
Columbia University

Memo to: Deborah Smegal
From: Robin Whyatt
Date: March 8, 2010

We are pleased that our answers to the questions raised by the Science Advisory Panel (SAP) will be of value to EPA as you move forward to update the chlorpyrifos risk assessment. Below are our responses to the questions raised by Dow Agrosciences which you also asked us to address in your email of March 5,2010. Please let us know if you need any additional information or if we can be of more help.

Question one: The Columbia birth cohort includes cord blood chlorpyrifos levels for most children and urine from many mothers (Whyatt et aL. 2005,2009). However, the authors state that "we found no association between chlorpyrifos levels in maternal and cord blood and TCPy levels in maternal urine samples during pregnancy or after delivery." (Whyatt et aL. 2009, pg 563). Since the health effect papers are based solely upon chlorpyrifos levels in blood, and because blood chlorpyrifos measurements have not been reported in any other human exposure study at these trace-level concentrations, this is a critical point to evaluate with full transparency. Specific questions include:

a. Why is there no association between blood chlorpyrifos and urinary TCPy?
b. How many mother infant pairs in 1999 have both urine TCPy and blood chlorpyrifos?
c. Are the 1999 urine and blood levels correlated?
d. Are the 2000 urine and blood levels correlated?
e. Are the 2001 urine and blood levels correlated?
f. With personal monitoring, air monitoring, blood chlorpyrifos and urinaryTCPy, what is the estimated internal dose for the infant-mother pairs in each year of measurement?

Answer: *Please note that the primary biomarker we have measured in our cohort is chlorpyrifos concentrations in maternal and umbilical cord blood. We did not measure TCPy in maternal urine samples except for a small subset of subjects. Specifically, as described in our 2009 manuscript (Whyatt et aI., EHP,* 117;559-567, *2009), we measured TCPy in repeat urine samples collected from only n=97 women during pregnancy. These urine samples were collected among women who delivered between 2001-2004. Thus the answer to la through 1d above is that no mother/infant pairs have both chlorpyrifos in blood and TCPy in urine in either* 1999 *or*

2000 and we thus do not have the data to look at the correlation between TCPy in urine and chlorpyrifos in blood among subjects who delivered prior to 2001. For subjects who delivered in 2001, we can look at this relationship. It should be noted, however, that these data are not particularly helpful in evaluating the correlations between urinary TCPY and blood chlorpyrifos concentrations, due to small sample size. But more importantly, chlorpyrifos in blood samples had already dropped substantially by 2001 following the residential ban. Specifically, by 2001 among subjects that had both TCPy measured in maternal urine and chlorpyrifos measured in blood, chlorpyrifos was only detected in 24% *of maternal blood samples and* 19% *of umbilical cord blood samples (See Figure* 2, *Whyatt et at., 2009). By contrast, as shown in Figure* 1 *from in the Columbia Response to the SAP Queries (2/19/2010), chlorpyrifos was detected in* 98% *and 85% of maternal and umbilical cord blood samples, respectively, during* 1999 *and* 84% *and 80% during 2000. Given these caveats, the results for 2001 are as follows: For these subjects, the average chlorpyrifos concentrations in two-week integrated indoor air samples collected over the final 6-8 weeks of pregnancy were positively correlated with average TCPy levels in maternal prenatal spot urine samples (r=0.5, p=0. 005). However, there was no correlation (p>0.2) between TCPy levels and chlorpyrifos in either maternal blood samples (n=29) or cord blood samples (n=21) at delivery. Maternal and umbilical cord blood levels were highly correlated (r=0.85, p<0.001, n=21).*

Regarding question "If" above, please note, that as discussed in our answers to the SAP querries, Dr. Dale Hattis at Clark University is conducting PBPK modeling of the Columbia data under a Us. EPA Star Grant. The grant is entitled "Use of Biomarkers and Physiologically Based Pharmacokinetic [PBPK] Modeling in Risk Analysis for Developmental Effects of Chlorpyrifos ". We believe that the PBPK model being developed by Dr. Hattis will prove helpful in clarifying the exposure concentrations that correspond to the chlorpyrifos levels in umbilical cord blood at which we begin to see statistically significant adverse effects on fetal growth and child neurocognitive development and also in estimating the internal dose for the infant/mother pairs in each year of measurement. Dr. Hattis estimates that his analyses should be completed relatively shortly. We recommend that you contract Dr. Hattis.

Question Two: In the Rauh et al. (2006) paper the authors note a nonlinear dose trend with using 4 exposure groups. The final analyses are limited to two exposure groups. An analysis of 4 exposure groups is more information from a risk perspective and is critical for full transparency of the data. Analysis with 4 exposure groups for the results presented in Tables 2-8 should be replicated and made publically available.

Answer: *We have already addressed this comment in our answer #* 3 *from the Columbia Response to the SAP Queries (2/19/2010). To reiterate, we prepared a new table (Table 1 below) which provided the same type of information as Table* 2 *in the 2006 Pediatrics paper (Rauh et at., Pediatrics,* 118*:el 845..;el* 859, *2006) with respect to the* % *of subjects whose tests scores indicated developmental delay with respect to the PDI and the MDI, as a function of chlorpyrifos exposure. However, we provided the* % *delayed for all four exposure groups, rather than just the 'high' and 'low' exposure groups shown in the paper. For both the PDI and MDI the rate of delay is clearly highest in the high chlorpyrifos group (Group* 4). *For the* 36 *month PDI, the rate of developmental delay is relatively low in all* 3 *low-exposure groups, and is about three times higher in the highest exposure group. And for the 36 month MDI, while the <LOD group experienced a higher rate of delay than 2 and 3, the very highest rate of delay is among the most exposed children. In univariate analyses, the difference in* % *delay between highest*

exposure (>6.17pg/g) group versus the lowest exposure group (<LOD) was statistically significant for PDI (chi-square p=0.018, 1 sided; p-0.031 two-sided), but not significant for MDI However, importantly, was no significant difference in the rate of delay between groups 1, 2 and 3 *for either the PDI (chi-squared=0.97, p=0.* 61) *or MDI (chi-squared=*4.4, *p=0.11). And the rate of developmental delay in terms of both PDI and MDI in group four was significantly greater than in groups 1-*3 *combined, as reported in our paper.*

Table 1. The proportion of children at age 36 month who were at risk of delay (score < 85) on the Psychomotor Development Index (PDI) or Mental Development Index (MDI) of the Bayley Scales of Infant Development by chlorpyrifos exposure group

	Group 1 (<LOD)	**Group 2 (1st tertile > LOD)**	**Group 3 (2nd tertile > LOD)**	**Group 4 (3rd tertile > LOD)**
PDI	7/80 (8.8%)	3/65 (4.6%)	3/38 (7.95)	11/45 (24.4%)
MDI	30/80 (37.5%)	14/65 (21.5%)	11/39 (28.2%)	20/44 (45.5%)

Question Three: In the Rauh et al. (2006) paper, the authors compare results of the Bayley Mental Development Index (MDI) and Bayley Psychomotor Development (PDI) in different years. Using significance testing only, they claim there is a "preban" difference and no "postban" difference. This natural experiment is important to control for the many confounding factors associated with the home environment of these children. A more comprehensive and transparent analysis of these data over would provide information about role of chlorpyrifos and MDI and PDI this unique population.

Answer: *Please note that most cohort children in the 2006 paper by Rauh et at. were born prior to the residential ban on chlorpyrifos. Specifically, as described in our answers to SAP queries, subjects were eligible for inclusion in the Pediatric paper if they had been born by 7/12/02 and thus had reached age three by the time of the analyses and also had:* (1) *measures of chlorpyrifos in blood at birth;* (2) *neurologic assessments at least once between ages 12-36 months and* (3) *data on model covariates. Therefore, we were not able to undertake statistical analyses relating the changes in chlorpyrifos concentrations to changes in MDI and PDI following the residential ban. However, authors concurred that this is an important research question and state that "larger epidemiologic studies will to assess the full impact of the ban (see page e1854 of the Pediatric paper)." As discussed in our February 17th letter to Dr. Jack Fowle, we are currently preparing a manuscript on the relationship between prenatal chlorpyrifos exposure and outcomes on the Weschler Intelligence Scale for Children (Fourth Edition) and the Child Behavior Check List, which have been administered to cohort children at age* 7 *years. However, we also plan to undertake additional analyses of the 36th month MDI and PDI in relationship to prenatal chlorpyrifos exposures following the ban, since many more children in our cohort have now completed the* 3 *year developmental assessments and we have the sample size to undertake the analysis outlined above, while controlling for the many potential confounders.*

Question Four: The exposure estimates in the Columbia and CHAMACOS studies are reported differently, thus preventing a meta-analysis from being undertaken. Both studies reported Bayley MDI and Bayley PDI results at 24 months and neither was statistically significant (Eskenazi et al. 2007). This can be interpreted as a tendency but underpowered, or as a chance finding. Obtaining the

raw data and pooling the results should be undertaken and has several benefits.

a. The exposure estimates can be unified (either by calculating an estimated dose or estimating a TCP level from the blood data). The dose cut points can be set to at least 3 doses to determine the presence of a dose response relationship.

b. The power will doubled by combining the two cohorts, thereby increasing the statistical power.

Answer: *As discussed in our February 17th letter to Jack Fowle, a multi-site study has been funded the National Institute of Environmental Health Sciences ($568,000 total costs) that will pool the children's centers at the University of California-Berkeley, Columbia University (our children's center) and the Mount School of Medicine to address questions about relationships across the three cohorts between prenatal organophosphate exposure, fetal growth and child neurocognitive development. Dr. Brenda Eskenazi at Berkeley is the principal investigator on project.*

Appendix 3. Epidemiology Study Specific Evaluations

APPENDIX 3
Study Specific Evaluations of Children's Health Epidemiology Studies

Article 1. Whyatt et al. (2004)

Prenatal Insecticide Exposures and Birth Weight and Length among an Urban Minority Cohort. Robin M. Whyatt, Virginia Rauh, Dana B. Barr, David E. Camann, Howard F. Andrews, Robin Garfinkel, Lori A. Hoepner, Diurka Diaz, Jessica Dietrich, Andria Reyes, Deliang Tang, Patrick L. Kinney, and Frederica P. Perera. Environmental Health Perspectives. 112:1125–1132 (2004).

Study Summary

In this report, Whyatt et al. report on the relationship between pesticide exposures and developmental outcomes in newborns in the Columbia Mother's and Newborn Study to include additional insecticides (the organophosphate diazinon and the carbamate propoxur), a larger sample size (n = 314 mother–newborn pairs), and insecticide measurements in maternal personal air sampled in the home, as well as in umbilical cord plasma sampled at delivery.

Study participants were recruited during early pregnancy (≤20th week) among African-American and Dominican women age 18-35 years, and registered at New York Presbyterian Medical Center and Harlem City. Women who smoked, had a history of drug abuse, diabetes, hypertension, or HIV infection were excluded from participation in the study, as were women who resided in New York City for less than 1 year. The study sample presented in this report was recruited between 1998 and 2002, a period which straddles the December, 2000 voluntary cancellation by registrants of indoor residential chlorpyrifos use.

Insecticides, including chlorpyrifos, were measured in 48-hr personal air samples collected during the third trimester of pregnancy. Analyses of air samples for pesticide levels were carried out at the Southwest Research Institute.

Chlorpyrifos levels in umbilical cord blood samples were available for 256 newborns, sampled as close to the time of delivery as possible, and within 2 days post-partum. Cord blood plasma chlorpyrifos levels were imputed from maternal blood levels for 31 newborns for whom no cord blood sample was obtained. Quantification of chlorpyrifos levels in plasma were conducted by the Centers for Disease Control and Prevention (CDC).

Insecticide levels in both maternal personal air and blood samples were available for 259 of the 314 mother–newborn pairs (82%). Measures in either personal air or blood were available for the remaining 55 pairs (18%). Pesticide levels in personal air and blood samples below the level of detection (LOD) were assigned a value of half the LOD; levels were then $\log_2$-transformed for statistical analysis. For pesticides in blood, 31% of chlorpyrifos levels were below the LOD. Infants with below LOD cord blood chlorpyrifos levels were used as a comparison group and compared to groups with increasing levels, categorized by tertile of above LOD levels. Infants' weight, length, and head circumference were assessed at birth and obtained for this study

through medical record abstraction.

Multiple linear regression analyses were performed to evaluate associations between prenatal insecticide exposure and the indicators of infant development at birth. Covariates were selected from variables known to be associated with insecticide exposure or fetal growth. Effect modification by race/ethnicity was evaluated, but not observed.

Controlling for potential confounders, the researchers found no association between maternal personal air insecticide levels and birth weight, length. Head circumference was not significantly associated with maternal personal air or cord blood chlorpyrifos levels. However, 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). Combined measures of (ln)cord plasma chlorpyrifos and diazinon (adjusted for relative potency) were also inversely associated with birth weight and length ($p < 0.05$). 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 (combined p-value = 0.01). When stratified by date of birth prior to or after 2001 (before and after the voluntary cancellation by registrants of indoor residential chlorpyrifos use was implemented), associations between birth weight and length and cord plasma chlorpyrifos were statistically significant ($p \leq 0.007$) among newborns born before the cancellation. Among newborns born after January 2001, exposure levels were substantially lower, and no associations with fetal growth outcomes were observed ($p > 0.8$). Results suggest that prenatal chlorpyrifos exposures may have impaired fetal growth among this inner-city, low income cohort.

Study Review

This was a very well conducted study with numerous strengths, and some noted limitations. The prospective nature of the study limits the influence of systematic errors in the exposure assessment on the association observed, such that mismeasurement of chlorpyrifos exposure would likely be non-differential with respect to the indicators of fetal growth.

A notable strength of this study, relative to investigations in other cohorts, is the direct measurement of chlorpyrifos in cord blood and personal air samples, rather than non-specific markers of organophosphate pesticide exposure. Errors in estimation of cord blood chlorpyrifos levels were likely to have been non-differential with respect to the indicators of fetal development. However, authors did not formally assess the variability in the exposure estimate resulting from the imputation of missing cord blood chlorpyrifos levels.

A limitation of the specific biomarker exposure indicators in this study is the single sampling period (at delivery). It is not clear to what extent this single measurement reflects exposures over critical windows of fetal growth and development during pregnancy. If the exposure is chronic, a biomarker measured at a single time point can provide a representative dosimeter, even if the toxicant has a short half-life, as is the case for chlorpyrifos. However, if pesticide exposures are sporadic or otherwise vary over short time scales, the biomarker measurement will not be representative of "usual" exposure, or of the exposure during critical periods of fetal development. An observation effect could also influence the validity of the 48-hour personal air

sampling if, for example, the act of wearing the dosimeter inhibited participants from using pesticides or otherwise altered their behavior patterns in a manner that affected exposure levels. These limitations are assessed in subsequent validation studies conducted within this cohort (Whyatt, 2007 and Whyatt, 2009).

The health outcomes in this study, birth weight, birth length, and head circumference were obtained through direct linkage with the mothers' and infants' computerized medical records, and were likely to have been accurately measured. Any errors in outcome measurement would have occurred in the clinic, and are likely to be small and likely to be non-differential with respect to pesticide exposure.

The investigators collected data on, and adjusted for, important potential confounders. Covariate selection proceeded appropriately by assessing relationships between potential confounders and the indicators of fetal growth, change-in-estimate of the main effect of chlorpyrifos exposure, and assessments of model fit. Covariates included in final models were race/ethnicity, gestational age, parity, maternal pre-pregnancy weight, and net weight gain during pregnancy, maternal self-reported environmental tobacco smoke in the home, sex of the newborn, and season of delivery. Models for head circumference also included an indicator for delivery type (vaginal versus cesarean section).

Maternal exposure to polycyclic aromatic hydrocarbons (PAHs) during pregnancy was not included in the final models because it did not improve model fit. However, to ensure that PAH exposure did not confound the pesticide associations with fetal development, models including PAHs were evaluated. The authors' consideration of other pesticides and environmental contaminants as covariates in their multivariate models is a notable strength of this study.

Confounding by maternal smoking behaviors was controlled by restriction (*i.e.,* excluding smokers from the study sample). Confounding by unmeasured factors may have been similarly controlled by design, this being a relatively homogeneous population with respect to determinants of fetal growth, compared to the general population. For example, the authors found it unnecessary to adjust for maternal alcohol consumption (which was measured), partly because few women in the study drank alcohol during pregnancy.

Bias due to confounding by unmeasured determinants of fetal growth is possible in this observational study, for example, confounding by factors related to socioeconomic condition. Annual household income, maternal education, maternal marital status, material hardship during pregnancy, and degree of housing disrepair were evaluated as possible markers for socioeconomic status but were not included in final models because they were not associated with birth outcomes, did not improve the model fit, and did not substantially alter parameter estimates, confidence intervals (CIs), or significance levels of variables included in the final models. This observation that indicators of socioeconomic condition were not associated with the health outcomes is somewhat unexpected, although perhaps explainable by the relative homogeneity of these factors in this study population (control through restriction of study population).

In categorical analyses, infants with the lowest tertile of observable cord blood levels had, as a

group, larger heights and weights at birth, compared to infants with chlorpyrifos levels below the level of detection, although the increases were not statistically significant. Among those with higher cord blood levels, increasing tertiles of chlorpyrifos were significantly associated with decreasing birth weight and height. This is suggestive of a threshold effect (a hockey stick dose-response curve). Departures from linearity in the exposure-response relationship were not assessed in models including (ln)chlorpyrifos entered as continuous variables.

The authors observed an association between chlorpyrifos and fetal growth only when the biomarkers were used as indicators of prenatal exposure and not when environmental levels (in maternal 48-hr personal air sampling conducted during the third trimester) were used. Researchers note several possible explanations for this discrepancy, including the notion that the biomarkers are integrating dosimeters of exposure. This may be particularly important for insecticide exposure by routes other than inhalation in the home (*e.g.*, dermal, ingestion, and occupational exposures). In addition, the authors speculate that the biomarkers may provide better dosimeters of the dose to the developing fetus, relative to the airborne exposures.

The voluntary cancellation by registrants of indoor residential chlorpyrifos use introduces exogenous variation in the exposure of interest. No associations were observed between the fetal growth indicators and chlorpyrifos levels among infants born after the implementation of the voluntary cancellation of chlorpyrifos containing in home pesticide products. This finding is similarly suggestive of a possible threshold effect (decreasing dose-levels before and after voluntary cancellation), although it could also be due to a lack of statistical power to assess associations at lower levels of exposure in the relatively small subsample (n=77).

The statistical analysis appeared to be conventional and appropriate. However, 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 (*i.e.* homoscedasticity, normality of error term distribution, independence of error terms, and linearity of the exposure-response relationship), other than to state that investigators log transformed chlorpyrifos levels. As discussed previously, there is some evidence in the data to suggest a non-linear exposure response relationship (*i.e.*, threshold) between the natural log of cord blood chlorpyrifos levels and birth weight and length, but no formal statistical testing of this possibility was presented or discussed.

Bias due to differential errors in the selection of study participants is unlikely to have occurred in this prospective study with low loss to follow-up. Appropriate exclusion criteria were applied, although these design decisions may reduce external validity (generalizability) when making inference to other populations using the associations observed in this narrowly defined study population.

The results presented in this expanded analysis confirm the authors' earlier findings of an inverse association between chlorpyrifos levels in umbilical cord plasma and birth weight and length in this study population of non-smoking African-American and Dominican women, age 18-35 years, living in New York City (Whyatt et al., 2003). Systematic errors in the selection of study participants or in the measurement of key analytic variables (exposure and outcome) are unlikely to explain this observed association. The possibility of unmeasured or poorly measured potentially confounding variables cannot be completely excluded, although study authors

thoroughly evaluated this possibility in the data available. Data suggest a threshold effect may be possible, although authors did not formally evaluate this characteristic of the data.

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Article 2. Berkowitz et al. (2004)

In Utero Pesticide Exposure, Maternal Paraoxonase Activity, and Head Circumference. Gertrud S. Berkowitz, James G. Wetmur, Elena Birman-Deych, Josephine Obel, Robert H. Lapinski, James H. Godbold, Ian R. Holzman, Mary S. Wolff. Environmental Health Perspectives. 112:388–391 (2004)

Study Summary

To assess the relationship of exposure to pesticides during pregnancy and subsequent risk of impaired growth and neurodevelopment, Berkowitz and colleagues (Mount Sinai Child Development Study) investigated the relationship between prenatal pesticide exposure and infant growth and neurodevelopment in a prospective, multiethnic cohort study of mothers and infants delivered at Mount Sinai Hospital in New York City.

The study population was drawn primarily from East Harlem and consisted largely of young Hispanic women (predominantly Puerto Rican), but also included African-American and Caucasian women. Mothers were enrolled during early pregnancy from a prenatal clinic and two private practices at Mount Sinai Hospital from March 1998 to March 2002. The study included only primiparous with singleton births. Mothers were excluded if they had any of the following: an initial prenatal visit after 26 weeks of gestation; serious chronic diseases; a serious pregnancy complication that could affect fetal growth and development; alcohol consumed greater than two alcoholic beverages per day; or, illicit drug use. Mothers and infants were also excluded if the child was born with a congenital malformation or severe prematurity. A total of 479 prenatal patients were recruited; 75 were excluded because of medical complications, premature birth, an infant with birth defects, inability to collect biologic specimens before birth, change of hospital or residence outside New York City, or refusal to continue to participate.

Pesticide exposure was assessed by a prenatal questionnaire and measurement of urinary concentration of 3,5,6-trichloro-2-pyridinol (TCPy), a metabolite of chlorpyrifos exposure. The survey was administered, and maternal urine and blood specimens were collected, during the third trimester. Cord blood samples were also obtained at birth. Measurement of paraoxanase-1 (*PON1*) genotype and phenotype was made using maternal and infant cord blood samples. PON1 is an enzyme that can detoxify chlorpyrifos before it can inhibit acetylcholinesterase in the peripheral and central nervous systems. The investigators assessed birth weight, length, head circumference, and gestational age of the newborns for 404 participating mother-child pairs.

Almost half of the participants in the study, (46%) reported that they, or a household member, had used indoor pesticides during the pregnancy; 72% were classified as exposed using a composite index defined as indoor pesticide use in the household or exterminator application, fumigation, or pesticide use in common areas. Detectable levels of urinary 3,5,6-trichloro-2-pyridinol (TCPy; a metabolite of chlorpyrifos) were observed in 44% of the study participants.

The authors constructed generalized linear models to evaluate the associations among pesticide exposure and PON1 activity with average birth weight, birth length, head circumference, and gestational age, adjusting for selected covariates (race/ethnicity, infant sex, and gestational age). The authors found no statistically significant associations between pesticide exposure based

either on questionnaire responses or biomarkers of pesticide exposure (TCPy) on fetal growth or gestational age.

Researchers also evaluated the potential effect modifying role of *PON1* genotype and phenotype in the relation of interest. Stratifying participants by presence of *PON1* polymorphisms and tertiles of maternal PON1 activity, researchers reported a small but statistically significant reduction in head circumference among children of mothers with levels of chlorpyrifos above the limit of detection and also in the lowest tertile of PON1 activity, *i.e.*, slowest metabolic activity. However, the interaction between TCPy level and PON1 activity was not statistically significant. In the subgroup of infants whose mothers had TCPy levels greater than the level of detection, those with low maternal PON1 had an average (SD) head circumference of 33.3cm (1.5cm) which was significantly smaller than those with medium (34.0cm (1.5cm)) and high (34.1cm (1.6cm)) maternal PON1 activity after adjusting for race/ethnicity, infant sex, and gestational age ($p = 0.014$). A similar trend was observed with head circumference when maternal PON1 activity was considered alone; the adjusted means were 33.5 cm for the low PON1, 33.9 cm for medium PON1, and 34.1 cm for high PON1 activity ($p = 0.004$). Maternal *PON1* genetic polymorphisms were not associated with reduced head size. No trends were seen for birth weight or birth length for TCPy or the other metabolite levels when maternal paraoxonase level was taken into account. Infant paraoxonase activity had no association with any of the fetal growth measures.

Because small head size has been found to be predictive of subsequent cognitive ability, the authors conclude that chlorpyrifos may have a detrimental effect on fetal neurodevelopment among mothers who exhibit low PON1 activity.

Study Review

The largely null study had notable strengths and some limitations; notably authors did not discuss any limitations of their study in the published manuscript. The prospective nature of the study limits the influence of errors in the self-reported exposure classification on the association observed; as such errors would likely be non-differential with respect to determinants of the health outcome under investigation.

The authors assessed pesticide exposure using both subjective questionnaire-based methods and biomarkers of exposure which are objective, sensitive and specific surrogates of pesticide exposure. Biomarkers of specific pesticides (*i.e.,* TCPy) were assessed in a single maternal urine sample provided during the third trimester. The authors do not discuss the impact of policies aimed at reducing use of chlorpyrifos on the prevalence and magnitude of pesticide exposures during the time period of this study; it is likely that use of chlorpyrifos exposure was decreasing over the time period of the study, as noted in other similar studies of the same time period. The authors do not deliberate on the degree to which the biomarkers assessed represent usual exposure to pesticides versus recent, acute exposure(s), nor do they discuss the relative importance of assessment of exposure during the third trimester vis-à-vis fetal growth and development. The critical window of exposure for fetal growth and neurodevelopment health effects is not fully characterized. If sources and patterns of exposure remain static, a single measurement would be a good indicator of pesticide exposure during pregnancy, even if the pesticides have short half-lives *in vivo*. If, however, sources and exposure patterns change over

short time-spans, perhaps on the order of days to weeks, the biomarker assessment would not necessarily provide a good indicator of the relevant exposure. The authors classified biomarker levels according to the assays' levels of detection (above versus below). Falsely negative exposure characterization would occur if mothers were exposed only early in pregnancy, and the pesticides were fully metabolized prior to the third trimester. Incorrect classification of participants as exposed, when in fact they were not exposed, was unlikely to have occurred using the biomarkers. Like other sources of measurement error in this study, errors in the biomarker indicators of exposure in this study are likely to have resulted in a non-differential misclassification, with respect to fetal growth and development.

The health outcomes in this study, birth length, birth weight, head circumference, and gestational age were likely to have been accurately measured in this study, as they were obtained through direct linkage with the computerized medical record. Any errors in outcome measurement would have occurred in the clinic, and are likely to be small and certainly non-differential with respect to pesticide exposure.

To some extent, the recruitment process resulted in a population that was likely homogeneous with respect to confounders of the *in utero* pesticide exposure-fetal growth relationship (compared to the general population), and the potential for confounding was thus limited by design. The authors were also able to collect data on, and adjust for, important covariates, including 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. Covariates used in the analyses were selected, according to the authors, from variables known to be associated with either pesticide exposure or fetal growth. However, factors related to socioeconomic condition of those assessed and co-contaminants to which the study participants may have been exposed were not addressed in detail in the article. To the extent that such factors may be determinants of fetal growth and development and correlated with (but not caused by) pesticide exposure, they may confound the associations of interest in the study. If these factors are positively associated with both pesticide exposure and diminished fetal development, they would bias the true association upward (towards larger mean differences associated with exposure). However, this is unlikely to have occurred in this largely null study.

Only factors that were statistically significant ($p < 0.05$) were included in the final models. The use of a statistical test as a means to select covariates from a larger set of potential confounders is discouraged in some epidemiologic circles (Miettinen 1976, Breslow and Day 1980, Greenland 1989). The assessment of confounding is generally not considered to be a statistical endeavor, in part because the association between the confounder and the outcome is just one factor that determines the degree of confounding, and also because the statistical significance of an association is a function not only of the magnitude of the association but also of other factors (*e.g.*, sample size, and sample variability) which are irrelevant to bias due to confounding. Moreover, the use of significance testing in the selection of confounders treats false negative errors (*i.e.* the deleting a confounder) as secondary to false positive error (*i.e.*, inclusion of a non-confounder). This implicit ranking of error is backward, as deleting a confounder introduces bias and is justified only if the bias induced by its exclusion is tolerably small or deemed to be worth the precision gain, whereas including a non-confounder only (potentially) reduces precision by increasing the size of the model. This is a small consideration in this study, and different

approaches are not likely to qualitatively change the study findings or conclusions. Notably, smoking was not included as a potentially confounding variable in this analysis as the prevalence of smoking was low in this cohort. According to the authors, the inclusion of smoking in final models did not alter risk estimates and added to variance as stated by the authors.

Bias due to differential errors in the selection of study participants is unlikely to have occurred in this prospective study with no loss to follow-up at the time of this study. Appropriate exclusion criteria were applied, including mothers with no prenatal medical visit prior to 26 weeks of gestation, mothers with serious chronic diseases (*e.g.*, diabetes, hypertension, thyroid disease), mothers who developed a serious pregnancy complication that could affect fetal growth and development, and mothers who consumed more than two alcoholic beverages per day or who used illicit drugs, mothers with multiple births, and infants born with a congenital malformation or severe prematurity. The subjective, survey-based pesticide exposure assessment was fortified by objective indicators of exposure (*i.e.* urinary biomarkers).

The generalizability of the study may be limited as consequence of the particulars of the study population assessed and the numerous exclusion criteria applied. Making valid inference using these results requires an assumption that the pesticide exposure-fetal development association is the same in this population as the population to which the results are being generalized. This study population consisted of primiparous women, recruited early on in their pregnancies from the prenatal clinic and two private practices at Mount Sinai Hospital in New York from March 1998 to March 2002, consisted predominantly of young Hispanic women with low education. Sixty-eight percent were less than 25 years old. Just under half were Hispanic (49.8%), although substantial numbers of White (21.0%) and African-American (27.7%) women also participated. Just under half (46.8%) classified themselves as being either single, divorced, widowed, or separated, and just over half (50.1%) had no more than a high school education. The exclusion criteria applied to potential study participants further focused the population assessed, relative to the general population of pregnant women. In the likely situation that the prevalence of important modifiers of the exposure-fetal development association in this focused study population is different from that in other populations of concern, generalizability will be limited. Important modifiers to consider may include time spent in the home, patterns of food preparation and consumption, health consciousness, the baseline health status of the mother (and father), and a host of socioeconomic determinants. The investigators finding of effect modification by PON1 activity is illustrative of limits on the generalizability of the study; populations who have high levels of paraoxonase activity may vary by genotypic and phenotypic determinants. These factors were not discussed in the report, but it has been noted previously that there is 10- to 40-fold inter-individual variability in rates of paraoxon hydrolysis (Playfer et al 1976). The potential limitation vis-à-vis generalizability is offset by a strength; the study has increased statistical efficiency, relative to one set in a general population.

Because small head size has been found to be predictive of subsequent cognitive ability, the authors conclude that chlorpyrifos may have a detrimental effect on fetal neurodevelopment among mothers who exhibit low PON1 activity. Implicit in this conclusion is the notion that small head size is a good surrogate for fetal neurodevelopment. To support their conclusion, the authors note that head circumference has been shown to correlate with brain weight, and that brain size and head circumferences are both predictive of IQ and cognitive ability. They

additionally cite research associating brain volume with attention-deficit/hyperactivity disorder. Although head circumference was significantly associated with cognitive ability, the differences in head size across tertiles of exposure were notably small (*i.e.* less than one centimeter); the clinical relevance of such small changes in the context of even a small amount of measurement error in assessment of head size is unclear.

The authors were not surprised that infant paraoxonase levels in their study were not associated with birth outcomes because PON1 is not well expressed until after birth, and infant PON1 levels are much lower during the first year of life than maternal levels. The expression of PON1 in infants is approximately one-third that of their mothers (Chen et al. 2003). They speculate that lower PON1 activity in infants may make them more susceptible to PON1-related toxicity, including exposure to organophosphate pesticides, relative to adults.

The net effects of confounding, selection bias, and information bias, are unlikely to have qualitatively changed the results of this well conducted, prospective cohort study. Non-specific biomarkers of exposure, as were assessed in this study, are likely more objective, sensitive, and likely also more accurate and reliable indicators of pesticide exposure, relative to exposure assessment methodologies that rely on self-report. However, to the extent that prenatal chlorpyrifos is the true exposure of interest, the assessment of non-specific organophosphate pesticide metabolites in a single sample of prenatal urine is a primary potential source of exposure measurement error. Almost certainly, such biases would not have resulted in the observation of a falsely positive association between pesticide exposure and fetal growth. More likely to have occurred is an attenuation of the true (unobserved) associations, toward (observed) associations that are relatively smaller, or null. Indeed, the findings of this study are either null, or associations of small magnitude. To contextualize these findings, the authors cite literature describing the finding that even small increases in prenatal or early childhood exposures to other environmental toxicants (lead and polychlorinated biphenyls) are associated with significant decrements in IQ and academic achievement up to 11 years of age. The validity of this analogy is speculative. The external validity of the study findings may be limited if the organophosphate pesticide exposure-fetal development association is modified by factors that are more, or less, prevalent in this young, urban, minority population, relative to the population(s) to which inference is being made. That said, the decision to focus on this population is reasonable, as it is a population likely to be highly exposed, and perhaps also at high risk of cognitive and behavioral deficits enhancing the likelihood of observing an association if an association exists.

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Article 3. Eskenazi et al. (2004)

Association of in Utero Organophosphate Pesticide Exposure and Fetal Growth and Length of Gestation in an Agricultural Population. Brenda Eskenazi, Kim Harley, Asa Bradman, Erin Weltzien, Nicholas P. Jewell, Dana B. Barr, Clement E. Furlong, Nina T. Holland. Environmental Health Perspectives. 115:792–798 (2007).

Study Summary

In this article by Eskenazi et al, the authors report on the relationship between prenatal urinary organophosphate metabolite levels and fetal growth and gestational duration in the CHAMACOS cohort of Mexican-American mothers and their children living Salinas Valley, an agricultural region of California.

Enrollment of the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) cohort took place at community clinics. A total of 601 out of 1,130 eligible women agreed to participate and were enrolled between October, 1999 and October, 2000, with 538 being followed through to delivery of a live born infant, whose birth weight was measured. The study populations consisted largely of low-income, agricultural workers. Women considered eligible for the study were less than 20 weeks gestation, aged 18 years or older, Medi-Cal eligible, fluent in English and/or Spanish, and planning to deliver at Natividad Medical Center.

Excluded from analyses were women with gestational or preexisting diabetes (n = 26), hypertension (n = 15), twin births (n = 5), or stillbirths (n = 3). One additional woman for whom birth weight information was out of range (< 500 g) was also excluded. The final analytic sample consisted of 488 mother-infant pairs.

Maternal/fetal exposure to organophosphate pesticides was assessed by measurement of organophosphate dialkyl phosphate metabolites (DAPs) and seven different pesticide-specific metabolites in maternal urine during pregnancy, including 3,5,6-trichloro-2-pyridinol (TCPy), a metabolite specific to chlorpyrifos. Cholinesterase (ChE) in whole blood and butyryl cholinesterase (BChE) in plasma collected from mothers during pregnancy, at delivery, and from umbilical cord blood was also assessed. Maternal urine samples were collected between 5 and 27 weeks gestation and again between 18 and 39 weeks. The dialkyl phosphates measured were dimethylphosphate, dimethyldithiophosphate, dimethylthiophosphate (DMPs), diethylphosphate, diethyldithiophosphate, and diethylthiophosphate (DEPs). Total DAPs and DMPs were available for 485 women; DEP metabolite levels were available for 486 women. Quantification of organophosphate metabolites using gas chromatography-tandem mass spectrometry and isotope dilution calibration was conducted by the Centers for Disease Control and Prevention (CDC) labs.

Total DAP metabolite level was defined as the sum of the molar concentrations of the six DAP metabolites, dimethylphosphate (DMP) metabolite level as the sum of the molar concentrations of the three dimethylphosphate metabolites only, and diethylphosphate (DEP) metabolite level as the sum of the molar concentrations of the three diethylphosphate metabolites. Total DAP, DMP, and DEP levels were determined for each participant for each of the two pregnancy urine samples. Individual metabolite levels below the limit of detection (LOD) were assigned a value

of the LOD divided by the square root of two, and this value was included in each sum. Total DAP, DMP, and DEP metabolite levels were $\log_{10}$ transformed. DAP, DMP, and DEP values were created by averaging the two log-transformed pregnancy measures. For specific metabolites, if a woman was below the LOD for both measurements, she was classified as below LOD for the average. If she was above the LOD at either or both measurements, her measurements were averaged and classified in relation to the median. For 19 women with only one DAP measurement in pregnancy, the single measure was used.

Similar to the analytic treatment of dialkyl phosphate metabolite, authors assigned a value of the LOD divided by the square root of two for TCPy measurements below LOD. Because a large proportion of women had non-detectable levels of TCPy, the chlorpyrifos-specific metabolite was categorized into three groups: TCPy less than LOD for both pregnancy measurements, and, for those with at least one detectable level, subdivided below and above the median of the average pregnancy level.

Infant birth weight, crown–heel length, and head circumference were obtained from hospital delivery logs and medical records. Infant ponderal index, a measure of proportionality of growth, was calculated as (birth weight in grams × 100)/(length in centimeters). Gestational age was obtained from medical records and was based on ultrasound procedures for 25% of women. Low birth weight was defined as < 2,500 g. Preterm delivery was defined as birth at less than 37 completed weeks of gestation. A "small for gestational age" (SGA) birth was defined as birth weight less than the 10th percentile for gestational age according to ethnicity, parity, and infant sex.

Multiple linear regression models were developed to assess associations between the markers of pesticide exposure and length of gestation, birth weight, length, head circumference, and Ponderal index. Multivariate logistic regression was used to evaluate associations between exposure measurements and low birth weight, preterm delivery, and SGA births. Covariates in the models included including maternal age, pregnancy weight gain, week of initial prenatal care, parity, infant sex, mother's country of birth, body mass index, and family income. All models of birth weight, length, head circumference, and Ponderal index were adjusted for linear and quadratic terms for gestational age.

The women in this study averaged 25 years of age (SD = 5); two-thirds were multiparous, 80% were married, 79% had not graduated from high school, 58% were overweight or obese, 88% preferred to speak Spanish, and 84% were born in Mexico, with more than half residing in the United States for < 5 years. Almost all of the women were living within 200% of the poverty level. Very few women reported smoking (6%), drug use (2%), or alcohol consumption (1%) during pregnancy. Approximately 28% of the women had worked in the fields during the pregnancy, and another 14% had worked at other jobs in agriculture. Eighty-five percent of the women in the study had agricultural workers living in their homes during their pregnancy. The median total DAP, DMP, DEP and total DAP metabolite levels for the study population were 136 nmol/L, 101 nmol/L, and 22 nmol/L, respectively. Only one woman had no detectable DAP levels and TCPy was detected in 77% of maternal urine samples. The median level for TCPy was 3.3 μg/L.

The mean (± SD) duration of gestation was 38.9 ± 1.7 weeks; mean birth weight was 3,449 ± 516 g; mean body (crown–heel) length was 50.2 ± 2.7 cm; mean head circumference was 34.1 ± 1.5 cm; and mean Ponderal index was 2.7 ± 0.3 g/m3. A total of 3.7% (n = 18) of children were born of low birth weight; 4.8% (n = 23) were SGA births, and 6.6% (n = 32) were preterm.

The authors did not observe an adverse relationship between fetal growth and any measure of *in utero* organophosphate pesticide exposure. On the contrary, they found positive associations between body length and head circumference associated with some exposure measures, but not chlorpyrifos. They did observe decreases in gestational duration associated with two measures of *in utero* pesticide exposure: urinary DMP metabolites ($\beta = -0.41$ weeks per $\log_{10}$ unit increase; 95% CI: –0.75—0.02; p = 0.02), which reflects exposure to dimethyl organophosphate compounds such as malathion, not chlorpyrifos.

Study Review

The study has numerous strengths; most notably the prospective design the longitudinal assessment of both exposure and outcomes. Strengths in the study design also include the use of multiple exposure biomarkers including quantification of non-specific (DAPs), chlorpyrifos-specific (TCPy) metabolites, cholinesterase (acetyl and butyl), and other environmental co-exposures.

The ascertainment and analysis of urinary organophosphate pesticide markers was appropriate. Quantification was conducted at the CDC, using published methods. DAP metabolites are likely more accurate and objective indicators of organophosphate pesticide exposure, relative to other ascertainment methods such as self-report. Although they are sensitive indicators of exposure, it is difficult to infer chlorpyrifos effects specifically from urinary DAPs. Urinary metabolite levels may reflect exposure not only to a host of organophosphate parent compounds, but also to potentially less toxic environmental metabolites. Also, because exposure to pesticides varies considerably from day to day, DAPs from spot urine samples may not represent average exposure over time. However, the prenatal exposure measures were, with some exceptions, the average of two measurements, and thus may better reflect chronic exposure during the pregnancy than a one-time exposure measure.

Errors in the assignment of exposure in this prospective study will likely have resulted in attenuation of observed associations. The likelihood of these errors is high, and the magnitude of the bias induced difficult to assess. Under an assumption of no other biases, the negative findings, those for the association between children DAP levels and fetal development outcomes, for example, could have resulted from exposure measurement error.

To identify "critical windows" of fetal development when exposure may have a greater impact, the authors analyzed the associations of outcomes and metabolite levels measured during moving 6-week windows of pregnancy (*e.g.*, 5–10 weeks, 6–11 weeks, 7–12 weeks) using a series of multiple regression analyses. No period of greater impact was observed in this largely null study.

The indicators of fetal growth and development (infant birth weight, length, and head circumference, Ponderal index, and gestational age) were obtained from hospital delivery logs

and medical records, or calculated using data from these records. They are likely to have been accurately and objectively measured. Although partially self-reported, any errors in gestational age are unlikely to be associated with pesticide exposures in this prospective study.

The statistical analysis used to assess the associations between the markers of exposure and fetal growth and development were appropriate. However, 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 (*i.e.* homoscedasticity, normality of error term distribution, independence of error terms, and linearity of the exposure-response relationship), other than to state that they log transformed exposure marker levels. The authors also conduct secondary and sensitivity analyses to assess influence of modeling decisions on their findings, and generally found their results to be robust.

A reasonable set of exclusion criteria were applied which likely resulted in recruitment of a study population that was relatively homogeneous with respect to confounders of the *in utero* pesticide exposure-fetal growth relationship (compared to the general population), and the potential for confounding was thus limited by design. For example, smoking, alcohol use, and illicit drug use were not included in the models because very few women reported use and controlling for these variables did not alter the results.

Bias due to confounding was also controlled in the analysis, by assessing and adjusting for risk factors for impaired fetal growth and development that are potentially associated with pesticide exposure, including maternal age, pregnancy weight gain, week of initial prenatal care, parity, infant sex, mother's country of birth, body mass index, and family income. Variables were selected as potential confounders for the multivariate models based on associations reported in the literature, and included in final models if they changed the coefficient associated with the exposure by 10% or more. Inclusion of indicators for environmental tobacco smoke exposure, consumption of caffeinated beverages, history of low birth weight and history of preterm delivery, did not alter the relationship of pesticide metabolites and birth outcome and were not included in final models. In addition to restriction of confounding by design, the selection of the CHAMACOS population, which consists mostly of children from low-income families, also served to increase the relative statistical efficiency of the study, as this population is at high risk of developmental deficits, compared to the general population.

The results of this study by Eskenazi et al (2004) in the CHAMACOS cohort failed to demonstrate an adverse relationship between fetal growth and *in utero* organophosphate pesticide exposure as assessed by multiple measures of prenatal pesticide exposure. The prospective study design and reasonable methods used protect from certain false-positive inducing biases, although it is possible that (non-differential) exposure measurement error attenuated the associations. Misclassification of the fetal growth outcomes was unlikely to have biased the results. Residual confounding by mismeasured or unmeasured factors is possible in this observational study, although it is unlikely that such confounding would have resulted in the null finding.

Article 4. Harley et al. (2011)

Association of Organophosphate Pesticide Exposure and Paraoxonase with Birth Outcome in Mexican-American Women. Kim G. Harley, Karen Huen, Raul Aguilar Schall1, Nina T. Holland, Asa Bradman, Dana Boyd Barr, Brenda Eskenazi. PLoS ONE 6(8)1-10 (2011).

Study Summary

In this article by Harley et al, the authors present a follow-up assessment of the relationship between prenatal urinary organophosphate metabolite levels and fetal growth and gestational age in the CHAMACOS cohort of Mexican-American mothers and their children living Salinas Valley, an agricultural region of California.(Eskenazi et al, 2004) They evaluate whether paraoxonase (PON1), a key enzyme involved in detoxification of organophosphate pesticides, could be an effect modifier of the association between indicators of maternal organophosphorus pesticide exposure (DAP metabolite concentrations) and poorer fetal growth previously reported in this cohort. They additionally examine the main effects of maternal and child *PON1* genotypes and PON1 activity on birth outcomes.

Enrollment of the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) cohort took place at community clinics. A total of 601 out of 1,130 eligible women agreed to participate and were enrolled between October, 1999 and October, 2000, with 538 being followed through to delivery of a live born infant, whose birth weight was measured. The study populations consisted largely of low-income, agricultural workers. Women considered eligible for the study were less than 20 weeks gestation, aged 18 years or older, Medi-Cal eligible, fluent in English and/or Spanish, and planning to deliver at Natividad Medical Center.

Excluded from analyses were women with gestational or preexisting diabetes (n = 26), hypertension (n = 15), twin births (n = 5), or stillbirths (n = 3), and one infant with a birth < 500 grams. In addition, mother-infant pairs with missing *PON1* genotype and activity levels were excluded (n =21), leaving a final sample size of 467. Of these, *PON1* genotype information was available for 451 mothers and 436 infants, and PON1 activity data were available for 371 mothers and 324 infants.

Maternal/fetal exposure to organophosphate pesticides was assessed by measurement of organophosphate dialkyl phosphate metabolites (DAPs). Maternal urine samples were collected between 5 and 27 weeks gestation and again between 18 and 39 weeks. The dialkyl phosphates measured were dimethylphosphate, dimethyldithiophosphate, dimethylthiophosphate (DMPs), diethylphosphate, diethyldithiophosphate, and diethylthiophosphate (DEPs).

Quantification of organophosphate metabolites using gas chromatography-tandem mass spectrometry and isotope dilution calibration was conducted by the Centers for Disease Control and Prevention (CDC) labs. Total DAP metabolite level was defined as the sum of the molar concentrations of the six DAP metabolites, dimethylphosphate (DMP) metabolite level as the sum of the molar concentrations of the three dimethylphosphate metabolites only, and diethylphosphate (DEP) metabolite level as the sum of the molar concentrations of the three diethylphosphate metabolites. For eight women, missing metabolite values were imputed using

linear regression to predict the missing metabolite level as a function of the other known metabolites for a given participant at a given time point.

The variables for total DAP, DMP, and DEP concentrations were log-transformed to reduce the impact of outlier points. Metabolite measurements from the two sampling time points were averaged to create a summary measure of *in utero* organophosphate pesticide exposure.

The primary analyses were conducted using DAP concentrations unadjusted for creatinine, although sensitivity analyses included creatinine as a covariate in the models. Total DAPs and DMPs were available for 485 women; DEP metabolite levels were available for 486 women.

Maternal and umbilical cord blood samples were collected at the time of delivery. Genotyping of the $PON1_{-108}$ and $PON1_{192}$ SNPs was performed using genomic DNA extracted from blood clots and Taqman PCR methods.

Two PON1 enzyme activity assays (arylesterase and paraoxonase) were performed using spectrophotometric methods. Arylesterase activity measures the rate of hydrolysis of the substrate phenyl acetate, and reflects the quantity of PON1 enzyme. Paroaxonase activity measures the rate of hydrolysis of paraoxon, and thus reflects a combination of the catalytic efficiency and quantity of the PON1 enzyme.

Birth outcome data, including infant birth weight, length, head circumference, and gestational age, were abstracted from medical records by a registered nurse.

Linear regression models were constructed to examine the main associations of *PON1* genotype and activity with length of gestation, infant birth weight, birth length, and head circumference, independent of pesticide exposure. Maternal and child *PON1* genotypes were entered into models as categorical variables; for $PON1_{-108}$, the CC genotype was the reference, and for $PON1_{192}$, the RR genotype was the reference. Arylesterase and paraoxonase activity in maternal and umbilical cord blood were examined as continuous variables, normalized by dividing by the standard deviation.

The associations of PON1 activity with birth outcomes were assessed within each $PON1_{192}$ genotype by stratifying by genotype and by testing for interaction between activity and genotype.

The presence of effect modification of associations between maternal DAP metabolite and birth outcomes by *PON1* genotype and activity was assessed by stratification, and use of interaction terms. *PON1* genotype (as a categorical variable) or activity (as a continuous variable) and cross-product terms were included in models previously used to assess pesticide associations in the 2004 report by Eskenazi et al. (Eskenazi et al, 2004) For *PON1* genotype, two cross-product terms were included in the model (*e.g.* QR*DAP concentrations and RR*DAP concentrations). Similar cross-product terms were generated using tertiles of PON1 activity. Statistical significance for interaction was set at p=0.1. In addition, effect modification by PON1 was assessed by conducting linear regressions on data stratified by *PON1* genotype and by tertiles of enzyme activity.

Models of *PON1* genotype included maternal pre-pregnancy BMI and maternal weight gain during pregnancy as covariates. Models of PON1 enzyme activity also included maternal age and country of birth, assay temperature, and $PON1_{192}$ genotype. In models of birth weight, length and head circumference, the authors also controlled for linear and quadratic gestational age to examine associations independent of gestational duration.

As previously summarized, women in this cohort were predominantly Spanish-speaking and born in Mexico; 41% of the women worked in agriculture during pregnancy. More than 60% of families were living below the federal poverty threshold, and 89% of women had never completed high school. The mean maternal age was 25.5 years (SD = 5.0). All women had detectable levels of organophosphate pesticide metabolites in their urine during pregnancy. 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). The allele frequencies of the $PON1_{192}$ Q allele and the $PON1_{-108}$ T allele were 50% and 46%, respectively, and did not differ between mothers and newborns. The mean arylesterase and paraoxonase activity was 33.6 U/mL (SD = 16) and 256.6 U/L (SD = 165), respectively, for infants and 136.6 U/mL (SD = 44) and 989.0 U/L (SD = 616) for mothers.

In this study, infants' (but not mother's) *PON1* genotype and PON1 activity were associated with gestational age and head circumference: Infants with the susceptible $PON1_{-108}$ TT genotype had shorter gestational age (=-0.5 weeks, 95%CI: -0.9, 0.0) and smaller head circumference (=-0.4 cm, 95% CI: -0.7, 0.0) than those with the $PON1_{-108}$ CC genotype. Infants' arylesterase and paraoxonase activity were positively associated with gestational age. Maternal DAP concentrations were associated with shorter gestational age among infants of the susceptible $PON1_{-108}$ TT genotype, although only the interaction between $PON1_{-108}$ genotype and DEP metabolite concentrations was statistically significant (p-value for interaction = 0.09). However, maternal DAP concentrations were associated with larger birth weight (p-value for interaction = 0.06) and head circumference (p-value for interaction =0.01) in infants with non-susceptible genotypes. The authors conclude that infants with certain *PON1* genotypes (*e.g.*, $PON1_{-108}$ TT) may be more susceptible to effects of *in utero* organophosphate pesticide exposure.

Study Review

This study improves on the work by Eskenazi et al (2004) by including an assessment of effect modification of the association between prenatal pesticide marker levels and birth outcomes by *PON1* genotype and activity. Additional strengths of this study include the prospective design, the assessment of pesticide markers at two time-points during pregnancy, and the assessment of PON1 activity by two different methods.

The authors speculate that a possible reason that they did not observe an association between DEPs and gestational age in the earlier study by Eskenazi is that it was "masked" by the effects of *PON1*. They note that the observation of an interaction only for DEP concentrations is plausible, since the known biologically relevant action of PON1 is with two DEP pesticides (chlorpyrifos and diazinon).

The finding of an independent effect of *PON1* genotype on birth outcomes is consistent with finding of reductions in average gestational age and head circumference at birth associated with the *PON1*$_{-108}$ TT genotype in the Mount Sinai cohort, as reported by Berkowitz et al. (Berkowitz et al, 2004). The association between infant PON1 (arylesterase) activity and gestational age was null in the Mount Sinai cohort. This is in contrast to the positive association reported by Harley et al. Maternal PON1 (arylesterase) activity was not associated with birth outcomes in the Haley study, but positively associated with head circumference in the Berkowitz et al study. Finally, the interactions observed in the two studies differed. Berkowitz et al reported a decrease in head circumference associated with chlorpyrifos marker levels among newborns of mothers with low arylesterase activity in the Mount Sinai cohort. Harley et al, in contrast, observed a statistically significant interaction between DEP levels and gestational age among infants in the UC Berkeley CHAMACOS cohort with the TT *PON1* genotype, but they did not observe significant interaction between PON1 activity and birth outcomes. In addition to measuring different indicators of pesticide exposure (TCPy), the *PON1* genotypes and activity assessed for the Mount Sinai cohort differed somewhat from those assessed in the UC Berkeley cohort; Berkowitz et al did not assess *PON1*$_{192}$ (only *PON1*$_{-108}$) and paraoxonase activity (only arylesterase activity)

In the Columbia Mothers and Newborn Study (Whyatt et al, 2004), organophosphate pesticide levels in maternal blood were associated with *decreased* fetal growth, while in the UC Berkeley CHAMACOS study, modest *increases* in birth weight and head circumference were associated with *in utero* DAP concentrations. In fact, stratification by PON1 status seemed to enhance these positive associations, such that in the non-susceptible groups (*e.g.* *PON1*$_{-108}$ CC, *PON1*$_{192}$ RR, and high arylesterase activity), increasing DAP concentrations were significantly associated with increased birth weight and head circumference.

The finding of a positive association between DAPs and DMPs and both head circumference and birth weight among those with the CT or CC *PON1* genotypes, and a positive association between head circumference and DAPs among infants with RR *PON1* genotypes is perplexing. The authors speculated that it is possible that, among the less susceptible groups, high urinary DAP concentrations may be an indication of rapid detoxification and excretion of organophosphate pesticides, rather than a marker of high exposure.

The ascertainment and analysis of urinary organophosphate pesticide markers was appropriate. Quantification was conducted at the CDC, using published methods. However, the use of DAP metabolites to measure exposure has some limitations, and these were discussed by the authors. Although they are sensitive and relatively specific indicators of organophosphate exposure, it is difficult to infer chlorpyrifos effects specifically from urinary DAPs. Urinary metabolite levels may reflect exposure not only to a host of organophosphate parent compounds, but also exposure to the less toxic metabolites themselves. Because exposure to pesticides varies considerably from day to day, DAPs from spot urine samples may not represent average exposure over time. However, the prenatal exposure measures were, with some exceptions, the average of two measurements taken early and late pregnancy, and thus may better reflect chronic exposure during the pregnancy. Additionally, DAP metabolites in urine may overestimate organophosphate pesticide exposure if they reflect exposure to both pre-formed DAPs in the environment and the parent organophosphate compounds.

Errors in the assignment of exposure in this prospective study will likely have resulted in attenuation of observed associations. The likelihood of these errors is high, and the magnitude of the bias induced difficult to assess. Under an assumption of no other biases, the negative findings, those for the association between children's DAP levels and attention outcomes, for example, could have resulted from exposure measurement error.

The indicators of fetal growth and development (infant birth weight, length, and head circumference, and gestational age) were obtained from hospital medical records, and ascertained by a registered nurse. They are likely to have been accurately and objectively measured, although the potential for errors in their measurement was not assessed. Although partially self-reported, any errors in gestational age are unlikely to be associated with pesticide exposures, *PON1* genotype, or PON1 activity in this prospective study.

The statistical analysis used to assess the associations between the markers of exposure and fetal growth and development were appropriate. However, despite a reasonable sample size for assessing main effects, the Harley et al study likely lacked sufficient statistical power to assess effect modification by *PON1* genotype and PON1 activity, as the CHACAMOS Cohort study was not originally designed to assess interactions between prenatal pesticide exposures and these co-factors. As a result, the analysis is at risk of failing to detect effect modification if effect modification is truly present.

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 (*i.e.* homoscedasticity, normality of error term distribution, independence of error terms, and linearity of the exposure-response relationship), other than to state that they log-transformed DAP levels to reduce the impact of outlier observations. The authors did conduct secondary and sensitivity analyses to assess influence of modeling decisions on their findings, including adjustment for creatinine.

A reasonable set of exclusion criteria were applied which likely resulted in recruitment of a study population that was relatively homogeneous with respect to confounders of the relationship between *in utero* pesticide exposure and fetal growth (compared to the general population), and the potential for confounding was thus limited by design. For example, smoking, alcohol use, and illicit drug use were not included in the models because very few women reported use and controlling for these variables did not alter the results.

The authors reported that factors known, *a priori*, to be associated with birth outcomes in this population were considered potential confounders, although they did not enumerate these variables. Covariates were retained in final models if they were also associated with *PON1* genotype or activity (in the study population, presumably) or if their exclusion from the model changed the main effect coefficient by more than 10%. The authors' final models for birth weight and head circumference adjusted for timing of urine collection, timing of entry into prenatal care, maternal age, parity, country of birth, household income, pre-pregnancy body mass index, maternal weight gain, infant sex, and gestational age using both linear and quadratic terms. Gestational age models adjusted for timing of urine collection, timing of entry into prenatal care, maternal age, parity, country of birth, and household income.

The authors controlled for gestational age in models of birth weight, length, and head circumference to examine associations independent of gestational duration. It is possible that gestational age is an intermediate on the causal pathway. If this is the case, the coefficients from these models can be interpreted as estimates of the 'direct-effect' of prenatal urinary DAPs and fetal growth outcomes due to causal pathways other than those mediated by gestational age. These pathway-specific analyses have a potential limitation that was not discussed by the authors. Namely, there is the potential for inducing bias if gestational age and fetal growth have unmeasured common causes (*i.e.* an unknown co-exposure) (Cole and Hernan, 2002).

In addition to restriction of confounding by design, the selection of the CHAMACOS population, which consists mostly of children from low-income families, also 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 results of this study by Harley et al in the CHAMACOS cohort demonstrate modest adverse associations between some measures of PON1 and fetal growth, as well as providing some evidence of effect modification of the relationship between organophosphate pesticide exposure markers and fetal growth. The prospective study design and reasonable methods used protect from certain false-positive inducing biases, although it is possible that exposure measurement error attenuated the associations. Misclassification of the fetal growth outcomes was unlikely to have biased the results. Residual confounding by mismeasured or unmeasured factors, including bias induced by adjusting for an intermediate variable (gestational age), is possible in this study, and may account for the observed associations.

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Article 5. Engel et al. (2007)

Prenatal Organophosphate Metabolite and Organochlorine Levels and Performance on the Brazelton Neonatal Behavioral Assessment Scale in a Multiethnic Pregnancy Cohort. Stephanie M. Engel, Gertrud S. Berkowitz, Dana B. Barr, Susan L. Teitelbaum, Jodi Siskind, Stefanie J. Meisel, James G. Wetmur and Mary S. Wolff. American Journal of Epidemiology. 165:1397-1404, 2007.

Study Summary

Engel et al conducted a study leveraging the Children's Environmental Health Study cohort data to investigate the impact of pesticide exposure on pregnancy outcomes and child neurodevelopment in an inner-city multiethnic cohort of women recruited during pregnancy. The study enrolled primiparous women presenting for prenatal care with singleton pregnancies at the Mount Sinai prenatal clinic and two private practices and who delivered their infants at Mount Sinai Hospital between May 1998 and July 2001. Participants in this study were predominantly young, unmarried, Hispanic and African American women with low educational attainment.

This is the second of three published analyses of prenatal pesticide exposure and child growth and development in the Mount Sinai Hospital Children's Environmental Health Study. The first (Berkowitz et al 2004) reported on assessments of birth weight, length and head circumference. The third (Engel et al 2011) reports on assessments of infant development at age 12 and 24 months and child development at ages 6-9 years.

The study population was drawn primarily from East Harlem and consisted largely of young Hispanic women (predominantly Puerto Rican), but also included African-American and Caucasian women. Mothers were enrolled during early pregnancy from the Prenatal Clinic and two private practices at Mount Sinai Hospital from May 1998 to July 2001. The study included only primiparous with singleton births. Mothers were excluded if they had any of the following: an initial prenatal visit after 26 weeks of gestation, serious chronic diseases, a serious pregnancy complication that could affect fetal growth and development; alcohol consumed greater than two alcoholic beverages per day, illicit drug use. Mothers and infants were also excluded if the child was born with a congenital malformation or severe prematurity. A total of 479 prenatal patients were recruited; 75 were excluded because of medical complications, premature birth, an infant with birth defects, inability to collect biologic specimens before birth, change of hospital or residence outside New York City, or refusal to continue to participate.

The authors assessed the relationship between total diethylphosphates (DEPs), total dimethylphosphates (DMPs), total dialkylphosphate metabolites (DAPs), as well as malathion dicarboxylic acid (MDA) and indices of abnormal neonatal behavior and/or neurological integrity using outcome measures derived from the Brazelton Neonatal Behavioral Assessment Scale (BNBAS), which was administered to the infants of participating mothers within days after birth and before they left the hospital. The authors also assessed modification of the associations between pesticide metabolite levels and metrics of neurodevelopment by maternal paraoxonase 1 activity.

Environmental exposures, sociodemographic characteristics, medical history, and lifestyle

factors were assessed by a prenatal questionnaire administered to the mothers during the third trimester. Participating mothers provided urine samples during the 3rd trimester prenatal visit which were provided to the CDC in order to assay six dialkylphosphate metabolites and malathion dicarboxylic acid (MDA). The investigators imputed values for samples with one or more missing values using methods described by Eskenazi et al. (2004), extending the method to also include imputation of metabolite values for women who were missing two metabolites in a class. Diethyl- and dimethylphosphate metabolites were summed on a molar basis (as nm/liter) to obtain total diethylphosphates (DEPs) and total dimethylphosphates (DMPs), respectively, and together to obtain total dialkylphosphates (DAP) levels. All biomarkers except MDA were included in analyses as $\log_{10}$ linear terms in the initial model selection stage. Results were reported for DEPs dichotomized at the median level. MDA was dichotomized at the limit of detection because only 21.6% of the population had detectable values. Maternal plasma paraoxonase 1 activity was classified in tertiles.

A high prevalence of pesticide use was reported in this cohort of pregnant women residing in New York City. The study cohort was enrolled between 1998 and 2002, which, the authors point out, overlaps with a period when chlorpyrifos and diazinon were being phased out of residential use. However, the authors note that in 1997, chlorpyrifos was the most heavily used insecticide by pest control operators at the New York City Housing Authority. The authors noted that all of the women who participated in personal air monitoring of their home had detectable levels of diazinon, chlorpyrifos, carbamate propoxur, and o-phenylphenol. 46% of women in the cohort reported that pesticides had been applied in their home during their pregnancy. Total diethylphosphates were calculated as the sum of diethyldithiophosphate, diethylphosphate, and diethylthiophosphate. In this cohort, the DEPs were detectable in 88.8% of the mothers, with a median (IQR) of 24.7 nm/liter (8.9–52.8 nm/liter). Total dimethylphosphates were calculated as the sum of dimethyldithiophosphate, dimethylphosphate, dimethylthiophosphate. DMPs were detectable in 90.2% of the mothers, with a median (IQR) of 47.8 nm/liter (15.6–149.2 nm/liter). Total dialkylphosphates were detectable in 96.5% of the mothers, with a median (IQR) of 82.0 nm/liter (35.2–194.7 nm/liter).

To assess infants' neurodevelopment, the investigators conducted a behavioral assessment using the Brazelton Neonatal Behavioral Assessment Scale (BNBAS). All BNBAS examinations were completed within 5 days of delivery, and 86.1% were conducted within two days of delivery. The BNBAS includes 28 behavioral items and 18 primitive reflexes. The exam does not yield a single score but instead assesses the infant across different developmental areas; infants' behaviors were scored according to seven domains:

1) Habituation - the ability to respond to and inhibit discrete stimuli while asleep;
2) Orientation -attention to visual and auditory stimuli and quality of overall alertness;
3) Motor - motor performance and quality of movement and tone;
4) Range of state - a measure of infant arousal and state lability;
5) Regulation of state - the ability to regulate state in the face of increasing levels of stimulation;
6) Autonomic stability - signs of stress related to homeostatic adjustments of the central nervous system;
7) The number and type of abnormal primitive reflexes

Of the 308 infants evaluated, all 18 primitive reflexes assessed in the BNBAS, 64% had all reflexes within the normal range, 18% had one abnormal reflex, 8% had two abnormal reflexes, and 10% had three or more abnormal reflexes. The majority of abnormal reflexes were due to hypotonicity (which includes a reflex not elicited after stimulation). Authors do not discuss the prevalence of other common causes of hypotonicity such as hypothyroidism, hypoglycemia, hypoxic encephalopathy.
The authors observed an association between both generic and pesticide-specific biomarkers of prenatal organophosphate pesticides and an increased number of abnormal primitive reflexes which are considered a critical marker of neurologic integrity. Notably, no statistically significant adverse associations were found for the dialkylphosphate metabolites and any measured behavior in the author's primary analyses.

They did observe, however, an interaction between paraoxonase 1expression levels and total dimethylphosphates on risk of abnormal reflexes, such that infants born to women in the first (interaction p = 0.002) and second (interaction p = 0.01) tertiles (slower metabolizers) had a greater risk of abnormal reflexes than infants of those in the highest tertile (fast metabolizers). In the first tertile of paraoxonase 1 expression, the relative risks (RR) of having abnormal reflexes were 1.78 (1.01, 3.14) associated with total diethylphosphates, 1.96 (1.27, 3.03) for total dialkylphosphates, and total dialkylphosphates 2.38 (1.37, 4.15). In the second tertile of paraoxonase 1 expression, the corresponding relative risks (RR) were 1.42 (0.85, 2.35) for total diethylphosphates, 1.66 (1.03, 2.65), for total dialkylphosphates, and total dialkylphosphates 1.75 (0.96, 3.17). No increased risk of abnormal reflexes with increasing exposure was found for women in the highest tertile of paraoxonase 1 expression, nor was a similar interaction observed between total diethylphosphates and paraoxonase 1.

Higher levels of total diethylphosphates and total dialkylphosphates, too, were associated with an increase in the risk of having abnormal reflexes. Subjects with prenatal total diethylphosphates levels above the median delivered infants who were 2.3 times more likely to have at least two abnormal reflexes (95% CI: 1.1, 5.0). Among women with lower tertiles of paraoxonase 1 expression (*i.e.*, slow organophosphate pesticide metabolizers) the authors also observed a significantly increased risk of abnormal reflexes with increasing exposure.

Relative to the first quartile, quartiles 2–4 of total diethylphosphates, total dimethylphosphates, and total dialkylphosphates were associated with an increased proportion of abnormal reflexes, although the associations did not increase monotonically and varied in their strength and precision. The authors speculated that this may due to a threshold effect.

With the exception of MDA, there appeared to be stronger associations between metabolite levels and abnormal reflexes for examinations performed after the first day of life, although only the total dimethylphosphates interaction $\text{p-value}_{\text{interaction}} = 0.10$. In contrast, an interaction ($p_{\text{interaction}} = 0.10$) with MDA showed that, for infants examined before day 2, the relation between MDA and abnormal reflexes was stronger (RR= 2.51, 95% CI: 1.61,3.90) compared with that for infants examined later (RR= 1.34, 95%CI: 0.72, 2.49).
The authors noted that their findings were remarkably consistent with those of Young et al. (Neurotoxicology, 2005) who reported that for each $\log_{10}$ unit increase in total

dialkylphosphates, there was a 26% increased risk of abnormal reflexes, (compared to 32% increased risk in this study). The magnitudes of association were also similar for total diethylphosphates (RR_{Young} = 1.25, $RR_{current\ study}$ = 1.49) and total dimethylphosphates (RR_{Young} = 1.20, $RR_{current\ study}$) = 1.13). Both studies reported stronger associations between dialkylphosphate metabolites and abnormal reflexes for older children, although, in the present study, the interaction with day of administration was not statistically significant.

The association between MDA, a malathion-specific metabolite, and abnormal reflexes appeared to be age dependent in this study, with the most pronounced effect observed among infants evaluated within the first day of life. The authors noted that this finding conflicts with the suggestion of later effects for dialkylphosphate metabolites, including dimethylphosphates, which encompasses nonspecific metabolites of malathion. The authors concluded that it is unclear what pesticides may be driving this disparity.

Study Review

This was a well conducted prospective study conducted in a young, predominantly minority population. The study design, analytic approach, and statistical analyses were appropriate. The authors used a sensitive albeit non-specific marker of chlorpyrifos pesticide exposure (DEP/DAP). While the analytic methods appear sound, authors assessed these markers using only a single maternal urine specimen taken during the third trimester. However, in their discussion, the authors note that organophosphate pesticide metabolites have short half-lives. If sources and patterns of exposure remain static, a single measurement would be a good indicator of pesticide exposure during pregnancy. If, however, sources and exposure patterns change over short time-spans, perhaps on the order of days to weeks, such an exposure assessment would not necessarily provide a good indicator of the relevant exposure. Like the many other sources of error possible in this study, this, too is likely to result in a non-differential misclassification of the exposure, with respect to the disease outcome. Serial measurements of urinary biomarkers of pesticide exposure in the third trimester would shed light on these patterns, although the benefits of such information are offset by the added cost, logistical complexity expense, and burden on the study participants.

The Neonatal Behavioral Assessment Scale (NBAS), used to ascertain the health endpoints in this study, is designed to examine individual differences in newborn behavior (http://www.brazelton-institute.com/intro.html). Examinations took place in a quiet, semi-darkened, warm room adjacent to the neonatal nursery, or in the mother's private room. Examiners in this study were either trained and certified by the Brazelton Institute or trained by a certified examiner. It was not specified how many examinations were conducted by the certified examiners, nor how recently the certified examiners had received their certification. Procedures for tracking the quality of the assessment process were also not discussed in the report. The health outcome assessment methodology used in this study is less than objective; assessments rely on substantial interaction between the infant and the examiner. Procedures for ensuring that the examiners were blinded to the participants' exposures were not discussed in the article. However, authors did include examiner as an indicator variable in statistical analyses to control for slight differences in examiner methodology.

Moreover, the behavioral assessment was administered before hospital discharge only on a subset of children in the cohort (n =311/404). The BNBAS was not administered if the infant was admitted to the NICU (n = 21); if the infant was delivered and discharged over a weekend (n = 43); if the parent refused (n = 5); if the infant was not testable (n = 2); or if study personnel were unavailable (n = 22). Factors related to weekend delivery (*e.g.*, fewer inductions) would be underrepresented among the tested subjects. This missing data has the potential to result in a selection bias, reduce the degree of precision with which associations were estimated, and limit the generalizability of the study findings. However, the authors' explanation for the incomplete ascertainment is presented and seems appropriate and largely logistical in nature.

The authors employed a reasonable set of exclusion criteria that likely resulted in recruitment of a study population that was relatively homogeneous with respect to confounders of the *in utero* pesticide exposure-neurodevelopment relationship (compared to the general population). The potential for confounding was thus limited by design (restriction). Bias due to confounding was also controlled in the analysis, by assessing and adjusting for risk factors for neuromuscular functioning that are potentially associated with pesticide exposure, including maternal age, race, marital status, education, cesarean delivery, delivery anesthesia, infant age at examination, infant gender, infant jaundice, and smoking, alcohol consumption, caffeine consumption, or illicit drug use during pregnancy, and the examiner conducting the BNBAS.

The use of an automated statistical procedure (backward elimination and change of effect measure) as a means to eliminate potential covariates is discouraged in some epidemiologic circles on the basis that assessment of confounding is generally not considered to be a statistical endeavor, although they continue to be used by others, largely on the grounds that it is 'objective'. The authors used a change-in-estimate approach, rather than a reliance on the statistical significance (p-value) associated with a covariate; their variable selection rule has been shown to produce more reliable models than variable selection methods based on statistical significance (Greenland, 1989). Because they used a backward elimination procedure, they considered confounding by a given risk factor in the context of adjustment for other covariates. The approach to covariate selection is a minor consideration in this study, and different approaches are not likely to qualitatively change the study findings or conclusions. A robust discussion of covariate selection concepts can be found in Chapter 15 of Modern Epidemiology (Kenneth J. Rothman, Sander Greenland, Timothy L. Lash).

The authors noted that post-entry exclusions/loss-to follow-up in the study may have biased their findings if they were associated with both organophosphate pesticide levels and neonatal behavior. Missing data has the potential to induce a selection bias if the reasons for missingness are determined by exposure (or a predictor of exposure) and are also not independent of neurodevelopment. Most of the excluded women were those who moved out of the study area, were lost-to-follow up, or lacked prenatal biologic specimens, which largely reflect socioeconomic condition. Participants included and excluded differed slighted with regard to maternal age and race; however, these factors were not associated with number of abnormal reflexes in this study. These considerations may, however, limit the generalizability of the study findings.

The statistical analysis used in the study was appropriate, although several subtleties in the

analytic procedures are worthy of mention. The main finding of the study was a statistical interaction between biomarker levels of organophosphate pesticides and paraoxon activity. It was not clear whether this effect modification was consideration *a priori*. Also, the authors imputed missing data, and this may 1) affect the precision of association estimates and 2) result in attenuated effect estimates as a result of exposure measurement error. The description of the imputation procedure used was insufficient for drawing strong conclusions regarding its validity with respect to the magnitude of measurement error/bias, and its effect on estimates of precision.

The generalizability of the study may be limited as consequence of the particulars of the study population assessed and the numerous exclusion criteria applied. This study population consisted of primiparous women, recruited early on in their pregnancies from the prenatal clinic and two private practices at Mount Sinai Hospital in New York from March 1998 to March 2002, consisted predominantly of young Hispanic women with low education. Sixty-eight percent were less than 25 years old. Just over half were Hispanic (51.1%), although substantial numbers of White (20.3%) and African-American (27.3%) women also participated. Just under half (47.6%) classified themselves as being single. The exclusion criteria applied to potential study participants further focused the population assessed, relative to the general population of pregnant women. In the likely situation that the prevalence of important modifiers of the exposure-fetal development association in this focused study population is different from that in other populations of concern, generalizability will be limited. Important modifiers to consider may include time spent in the home, patterns of food preparation and consumption, health consciousness, the baseline health status of the mother (and father), and a host of socioeconomic determinants including access to health care. The investigators finding of effect modification of the biomarker-fetal growth relationship by PON1 activity is illustrative of limits on the generalizability of the study; populations who have high levels of paraoxonase activity may vary by genetotypic and phenotypic determinants. These factors were not discussed in the report, but it has been noted previously that there is 10- to 40-fold inter-individual variability in rates of paraoxon hydrolysis (Playfer et al 1976).

The primary finding was an interaction between high paraoxonase 1expression levels and organophosphate pesticide exposure, whereby fast metabolizers had lower risk of abnormalities associated with prenatal pesticide metabolite levels in maternal urine samples. As with their previous report, bias due to confounding, and selection of study participants is unlikely to have large and qualitative impact on the association observed. To the extent that prenatal chlorpyrifos is the true exposure of interest, the assessment of non-specific organophosphate pesticide metabolites in a single sample of prenatal urine is a primary potential source of exposure measurement error. Because of the prospective design, information bias is expected to result in attenuation of the true (unobserved) associations, toward (observed) associations that are relatively smaller, or null.

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Article 6. Young et al. (2005)

Association Between In Utero Organophosphate Pesticide Exposure and Abnormal Reflexes in Neonates. Jessica G. Young, Brenda Eskenazi, Eleanor A. Gladstone, Asa Bradman, Lesley Pedersen, Caroline Johnson, Dana B. Barr, Clement E. Furlong, Nina T. Holland. NeuroToxicology. 26:199–209 (2005).

Study Summary

Young et al. assessed the relationship between *in utero* and early postnatal organophosphate pesticide exposure and neonatal neurobehavior in the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) cohort. Urinary levels of dialkylphosphate (DAP) metabolites, including dimethyl and diethylphosphate metabolites, measured at approximately fourteen and twenty-six weeks gestation, and once post-delivery, measured at approximately seven days postpartum were assessed as markers of exposure to organophosphate pesticides. Cognitive development was assessed using the Brazelton Neonatal Behavioral Assessment Scale (BNBAS).

The CHAMACOS study is a longitudinal birth cohort study of environmental exposures and the health of pregnant women and their children living in the Salinas Valley, California. The Salinas Valley is a major center of agricultural production in the United States with approximately 500,000 pounds of organophosphate pesticides applied annually, according to the CDC. (California EPA. Pesticide Use Reporting 2001 Summary Data, 2002. (www.cdpr.ca.gov/docs/pur/pur01rep/01_pur.htm) Pregnant women initiating prenatal care at the Natividad Medical Center, a county hospital located in the city of Salinas, were recruited to participate in the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) project. Both the source and study populations consisted largely of low-income, Latina agricultural workers. Women considered eligible for the study were less than 20 weeks gestation, aged 18 years or older, Medi-Cal eligible, fluent in English and/or Spanish, and planning to deliver at Natividad Medical Center. A total of 601 women were enrolled between October, 1999 and October, 2000, with 528 followed through delivery of a live born infant. The BNBAS was performed on 421 infants. Eight twins and 27 preterm infants were excluded from analysis.

Exposure was assessed by measurement of organophosphate dialkylphosphate (DAP) metabolites in maternal urine samples collected between 5 and 27 weeks gestation and again between 18 and 39 weeks. Maternal urine specimens were generally collected at the time of the prenatal and post-delivery interviews. The post-delivery urines were collected within 1 week of delivery for 73% of the sample, with the remainder obtained up to 176 days afterwards. Quantification of metabolites was conducted by Centers for Disease Control and Prevention (CDC) labs. Total DAP metabolite level was defined as the sum of the molar concentrations of the six DAP metabolites, dimethylphosphate (DMP) metabolite level as the sum of the molar concentrations of the three dimethylphosphate metabolites only, and diethylphosphate (DEP) metabolite level as the sum of the molar concentrations of the three diethylphosphate metabolites. Total DAP, DMPT, and DEP levels were determined for each participant for each of the two pregnancy urine samples and for the post-delivery urine sample. Individual metabolite levels below the limit of detection (LOD) were assigned a value of the LOD divided by the

square root of two, and this value was included in each sum. A small number of missing metabolite values were imputed using regression to predict the missing metabolite level as a function of the other known metabolites for given participant at a given time point. The total DAP, DMP, and DEP metabolite levels were then $\log_{10}$ transformed.

For 30% of participating infants, developmental outcomes were assessed prior to discharge from the hospital; the remainder was assessed at the CHAMACOS research office or in the participant's home. Assessments were performed away from the mother in a private room with low-level light and noise control. The BNBAS, consists of 28 behavioral measurements scored on a nine-point scale and 18 reflex items scored on a four-point scale. These scores were classified into seven clusters: habituation, orientation, motor performance, range of state, regulation of state, autonomic stability, and reflex. Higher scores on the behavioral clusters indicate more optimal functioning while higher scores on the reflex cluster indicate less optimal functioning. Missing individual BNBAS items for the six behavioral clusters were imputed as the median value of known scores within the cluster associated with the missing item. If all items in a particular cluster were missing, then the cluster score was considered missing. Missing individual reflex items comprising the reflex cluster were not imputed.

Separate linear regression models were fit for six of the seven clusters of the BNBAS. Poisson regression was used to model the association between urinary metabolite levels and number of abnormal reflexes. Independent variables included the average of prenatal metabolite levels, the postnatal level, and relevant covariates. Models relating metabolite levels to each cluster score were initially fit including the postnatal metabolite level and the average of the two prenatal metabolite levels. For eighteen mothers missing one of the two pregnancy urine samples, the one remaining was used to represent the average. All models adjusted for covariates, and were rerun with creatinine-adjusted metabolite levels. Analyses were performed for the entire study sample and stratified by the median age at BNBAS assessment (3 days).

Of the 378 infants included in this analysis, 47.5% were male. The mean maternal age was 26±5 years. Most mothers lived at or below the poverty line (64%), were born in Mexico (85%), had previous live births (68%), were married (81%), and had not completed high school (81%). Few women smoked (5%) or drank alcohol (16%) during pregnancy, although most reported caffeine use (75%). Only 1% of the sample reported any illicit drug use during pregnancy.

The authors observed a statistically significant association between organophosphate pesticide exposure and the reflex cluster of the BNBAS, both for the entire sample and for infants assessed at greater than 3 days of age. 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: $\beta = 0.53$, 95% CI = 0.23, 0.82; DMP: $\beta = 0.41$, 95% CI = 0.12, 0.69; DEP: $\beta = 0.37$, 95% CI = 0.09, 0.64), and the proportion of infants with more than three abnormal reflexes (total DAP: OR = 4.9, 95% CI = 1.5, 16.1; DMP: OR = 3.2, 95% CI = 1.1, 9.8; DEP: OR = 3.4, 95% CI = 1.2, 9.9). No other associations between either prenatal or postnatal DAPs with any other developmental outcomes were observed.

Study Review

Overall, authors performed a well-conducted study of pre- and post-natal pesticide exposure and adverse neurodevelopmental outcomes. The prospective nature of the study, use of multiple biomarkers of exposure, and extensive data collection allowing for adjustment of potentially confounding variables are key strengths of this work. For the purpose of informing the nature of the relation between chlorpyrifos *per se* and these outcomes, use of non-specific OP metabolites is a disadvantage. Other limitations of this work are also noted.

The ascertainment and analysis of urinary organophosphate pesticide markers was appropriate. Quantification was conducted at the CDC, using published methods. DAP metabolites are sensitive albeit non-specific markers of organophosphate pesticide exposure. They are likely more accurate and objective indicators of organophosphate pesticide exposure, relative to other ascertainment methods such as self-report. In their discussion, the authors note that, "presently, there are no better methods of exposure assessment to capture exposure to multiple organophosphate compounds." It is difficult to infer chlorpyrifos effects specifically using metabolite levels, as they also indicate exposure to other organophosphates. The study population was likely sustained many other potentially relevant exposures to multiple chemicals and other pesticides which may affect development. It is not possible to disentangle the various effects from these potential co-exposures and chlorpyrifos in this study. Moreover, the degree to which metabolites in prenatal maternal urine samples reflect 'usual' levels, and thus usual exposure, is uncertain. The authors' observation of large within-mother variability in pesticide marker levels between the two prenatal sampling periods is evidence that a single sample may not be representative of longer-term levels in this population, particularly when exposures are transient and highly variable. Averaging of the two prenatal exposure measures may ameliorate this issue somewhat.

Metabolite levels in postnatal urines likely reflect early postnatal, rather than *in utero* exposure, given the short half-life of organophosphate pesticides. This is particularly likely for the 27% of postnatal samples collected greater than one week after delivery. The effect of errors in the assessment of organophosphate pesticide exposure, whether due to mismeasurement of the metabolites, imperfect correlation between metabolite levels and pesticide exposure, or imputation of missing data, is likely to be an attenuation of the association (a bias toward no association) in this prospective study.

The BNBAS was appropriately administered by one of four examiners trained by a BNBAS certified trainer. BNBAS is considered to provide an appropriate evaluation of newborn development when administered up to the second month of life, and is considered a valid tool, when properly conducted. The cluster approach to classification of BNBAS scores has precedence in the literature. Missing BNBAS data points were imputed, if possible. Indeed, there was a substantial amount of missing outcome data, due largely to the particular requirements of the assessment (*e.g.* that the infant be asleep immediately prior to the assessment of habituation and that the infant by crying prior to assessment of consolability and self-quieting). For example, fifty-four percent of the sample was missing all habituation items and an additional 22% required at least one imputation. Forty percent of the sample required at least one imputation of the four items comprising the regulation of state cluster, with 13, 25, and 0.5% of the sample requiring one, two, and three items imputed, respectively. Individual items with the most missing values for the regulation of state cluster were consolability and self-quieting. Two infants were missing

all items comprising the orientation cluster score. The number of abnormal reflexes was calculated based on non-missing values only. Reflexes with the most missing values included incurvation (9%), nystagmus (7%), and tonic neck reflex (7%). Missing values did not exceed 4% for the remaining reflex items. Again, the effect of errors in the ascertainment of developmental outcomes is likely to result in attenuation of observed associations as it is unlikely that missingness in outcome measure is related to pesticide exposure, as examiners were blind to the infant's exposure status and the imputation procedures were unlikely to induce bias.

The analyses presented were appropriate to the data collected and the research question posed. The authors averaged participants' pesticide marker levels from the two prenatal urine samples (if available), and, as a separate variable, the postnatal metabolite level. Sixteen mothers were missing the post-delivery urine. In order not to exclude those with missing post-delivery urines from the statistical models, results were re-examined with the variable representing post-delivery metabolite level excluded from all models. The effect of the missing exposure data is a decrease in the precision, and also likely attenuation of observed associations due to measurement error.

Potential confounders considered in this investigation included primarily predictors of BNBAS performance, including: maternal age, pre-pregnancy body mass index, self-reported smoking, alcohol, caffeine, and illicit drug use during pregnancy, vitamin use during pregnancy, gestational age at which prenatal care was initiated, total number of prenatal care visits, average blood pressure during pregnancy, parity, method of delivery, general anesthesia use during delivery, breastfeeding initiated after delivery, and poverty level. Other covariates included infant sex, age in days at BNBAS assessment, minutes since last fed at BNBAS, and BNBAS examiner. Missing covariate values were imputed by random selection of an individual among all participants with known values for the covariate and assigning the missing value the selected participant's value.

Covariates were selected for inclusion in final models if they were statistically significantly associated with each of the seven cluster scores in univariate models, (alpha = 0.15). The authors used a less conservative alpha level as a cut-off for selection of covariates, a somewhat more conservative approach to covariate selection, relative to use of the conventional alpha level of 0.05. However, the use of a statistical test as a means to select covariates from a larger set of potential confounders is discouraged in some epidemiologic circles (Miettinen 1976, Breslow and Day 1987, Greenland 1989). The assessment of confounding is generally not considered to be a statistical endeavor, in part because the association between the confounder and the outcome is just one factor that determines the degree of confounding, and also because the statistical significance of an association is a function not only of the magnitude of the association but also of other factors (*e.g.*, sample size, and sample variability) which are irrelevant to bias due to confounding. Moreover, the use of significance testing in the selection of confounders treats false negative errors (*i.e.* the deleting a confounder) as secondary to false positive error (inclusion of a non-confounder). This implicit ranking of error is backward, as deleting a confounder introduces bias and is justified only if the bias induced by its exclusion is tolerably small or deemed to be worth the precision gain, whereas including a non-confounder only (potentially) reduces precision by increasing the size of the model. This is a small consideration in this study, and different approaches are not likely to qualitatively change the study findings or conclusions. Birth weight and gestational age were considered potential causal intermediates in

this relation, and not confounders.

Regardless of the confounder evaluation conducted by the investigators, residual confounding due to poorly measured or unmeasured confounders is likely to have influenced the observed associations. Missing covariate data were imputed. The resulting analysis is relatively more precise, owing to the larger sample size analyzed, but this statistical efficiency comes at the trade off of bias due to residual confounding by imperfectly measured covariates. Socioeconomic conditions and other difficult to measure determinants of early life development may further confound the reported associations in the direction of more deleterious effects associated with the organophosphate metabolites and with unknown magnitude. The potential for this bias may be mitigated somewhat due to restriction in the variability of these confounding factors in this relatively homogeneous study population.

The generalizability of the findings to other populations may be limited, due to the particular nature of this study population with respect to factors which may modify the association between organophosphate exposures and infant development.

The results of this investigation are suggestive of a detrimental association between prenatal organophosphate exposure, as measured by maternal urinary metabolite levels, and abnormal reflexes, as measured by the BNBAS, particularly in infants assessed after the first 3 days of life. This study may be limited in its direct contribution to the literature base regarding the specific association between chlorpyrifos and developmental outcomes; although approximately 500,000 pounds of organophosphate pesticides are applied annually in Salinas Valley, chlorpyrifos constitutes only a small percentage of the pesticides applied. Although the prospective study design and reasonable methods protect from certain false-positive inducing biases, there remains the potential of residual confounding in the direction of an adverse effect on development by unmeasured factors.

Article 7. Rauh et al. (2006)

Impact of Prenatal Chlorpyrifos Exposure on Neurodevelopment in the First 3 Years of Life Among Inner-City Children. Virginia A. Rauh, Robin Garfinkel, Frederica P. Perera, Howard F. Andrews, Lori Hoepner, Dana B. Barr, Ralph Whitehead, Deliang Tang, Robin W. Whyatt. Pediatrics. 18(6): 1835-1859.

Study Summary

To evaluate the affect/impact of prenatal exposure to ambient and indoor pollutants including pesticides, the investigators conducted a prospective cohort study among inner-city (NYC) mothers and their babies born between February 1998 and May 2002. A major aspect of the protocol was to assess pesticide exposure, specifically chlorpyrifos, and its impact on pregnancy outcome, neurodevelopment, and behavior. Previous publications presented outcomes of this cohort such as birth weight and length findings (Whyatt, et al, 2004 and Perera et al, 2003.) This publication presents findings focused on the neurodevelopmental assessments made at the 12, 24, and 36 month time points.

The women enrolled into the study were identified during their pregnancy, through the New York Presbyterian Medical Center and Harlem Hospital. To be eligible for the study they were required to be between the ages of 18 and 35 years, non-smokers (later verified by blood cotinine levels,) and without a history of diabetes, hypertension, HIV infection, or drug abuse. They must have sought prenatal care by the 20th week of pregnancy and self-identified as African American or Dominican. At the time of this report, the authors explain that out of the original 648 pregnant women consented, 536 (83%) of them and 254 children were still actively enrolled at the 3 year milestone. Of these children, 228 completed evaluations at 12 months of age, 227 at 24 months, and 228 at 36 months, with 189 children having full data at all 3 time points.

The primary source of chlorpyrifos measurements were samples of cord blood collected at delivery as well as samples of maternal blood collected shortly after (within 2 days of) delivery. These serum levels were supplemented by maternal self-report of residential pesticide use during the third trimester of pregnancy. Using findings from previous analyses of chlorpyrifos effects on birth weight (Whyatt, et al, 2004 and Perera et al, 2003,) the investigators chose a cord blood chlorpyrifos limit of 6.17pg/g to define the exposure groups. Children were categorized as having either high (>6.17pg/g) or low ($\leq$6.17pg/g) prenatal exposure.

Two instruments were used to evaluate neurodevelopment and child behavior. The Bayley Scales of Infant Development II (BSID-II) was used to generate a Mental Development Index (MDI) and a Psychomotor Development Index (PDI) at 12, 24, and 36 months of age. Second, the behavior of the children was described by the mother using the 99-item Child Behavior Checklist (CBCL) and the resulting syndrome scale scores. Finally, the Home Observation for Measurement of the Environment (HOME) instrument was used to assess the quality of the child-care environment. This instrument collects data regarding the physical and interactive qualities of the child's home as a measure of mental stimulation and interactions.

The maternal and cord blood samples also provided cotinine levels to confirm maternal report of environmental tobacco smoke (ETS) exposures and lead levels to understand the role lead

exposure may play interacting with the outcomes of measure. During their third trimester of pregnancy, the women completed a 45-minute questionnaire to collect demographics, education and occupational history, income, active and passive smoking, alcohol and drug use during pregnancy, and residential pesticide use. From this self-report questionnaire and additional analyte measurement in biological sample, authors obtained information regarding potentially confounding variables.

Multivariate linear regression and logistic regression were used to estimate associations between prenatal chlorpyrifos exposure and developmental outcomes. General linear modeling of the repeated measures (12, 24, 36 month evaluations) was also used to estimate the effect over time that chlorpyrifos had on neurodevelopment. All models included prenatal ETS exposure, gender, ethnicity, gestational age at birth, quality of home care-taking environment, maternal education, and maternal IQ. Other measured co-variables including blood lead levels were evaluated as to the potential confounding influence on the main effect, and were not included in final models due to lack of statistical or biological evidence of confounding bias.

The descriptive statistics present the cohort as relatively homogenous with respect to socioeconomic variables but showing a wide range of chlorpyrifos exposure. Chlorpyrifos levels ranged from undetectable to 63 pg/g with 20.6% of the cord blood results falling into the high exposure category. Significant differences in chlorpyrifos exposure were seen across ethnic categories with 24.2 % of African American participants and 14.9% of Dominican participants classified as highly exposed (p=.02). Birth weight and prenatal exposure to ETS were also significantly associated with the exposure categories with infants in the high exposure group weighing approximately 211g less than babies in the low exposure group ($p < .05$) and 56% of those in the high exposure group reporting prenatal ETS versus 32% in the low exposure group ($p = .002$).

When the BSID-II results were analyzed in terms of exposure group, no significant differences were seen at the 12 and 24 month time points. At the 36-month milestone, although MDI scores did not show a significant difference across the exposure groups, PDI scores were significantly lower among children in the highly exposed group (95.69 vs. 101.63, $p = .006$). Statistically significant differences were also seen at the 36 month milestone, in mental (cognitive) and psychomotor delays ($p = .048$ and .002 respectively.)

When analyzing the 36-month child behavior checklist (CBCL) (behavioral) scores, results from the 12 and 24 month milestones were not significantly associated with exposure status. At 36 months, significant differences were observed in the general category of attention-problems (p=.010) and in the more specific DSM-IV scale for ADHD problems ($p = .018$).

The multivariate regression models included gender, ethnicity, gestational age at birth, home environment, prenatal ETS exposure, maternal education, and maternal intelligence. The authors reported that the difference in MDI scores was "marginally significant" ($p = .06$) with the exposed group scoring an average of 3.3 points lower. Among the significant covariants were race/ethnicity (at 24 and 36 months), gender of infant (at 12, 24, and 36 months), gestational age (at 24 and 36 months), low maternal education (at 36 months), and HOME score (at 36 months) with Dominicans, male children, low gestational age, low maternal education (< high school,)

and low HOME score all linked to lower MDI scores. It was also reported that environmental exposures (chlorpyrifos and ETS) and sociodemographic covariables accounted for 25% of the variance seen in the 36-month MDI scores. When the same multivariate regression models were calculated regarding the PDI scores, none of the 12 or 24 month PDI scores showed significant effects. The 36 month scores did show significant effects for chlorpyrifos exposure, race, and gestational age.

Next, investigators calculated estimates of adjusted risk for developmental delays (MDI and PDI) related to chlorpyrifos exposure. Adjustments were made for gestational age, gender, ethnicity, maternal education, maternal intelligence, and home environment. The findings showed that before 36 months of age, delays were no more likely in the highly exposed group. But, 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 = .02) and motor delays were 4.9 times greater (95% CI: 1.78-13.72; p = .002) than those with lower prenatal exposure.

General linear models (GLM) were fit to describe longitudinal developmental trajectories for high and low chlorpyrifos exposure groups, adjusting for race/ethnicity, gender, gestational age, maternal educational level, HOME score, and ETS exposure. The GLM results were consistent with the 12, 24, and 36 month linear regression analyses indicating that associations between developmental scores and chlorpyrifos were most pronounced at 3 years of age. The authors describe an initial decrease in scores in both groups between the ages of 12 and 24 months, but remark that such decreases are commonly observed among low-income groups followed by a rebound in scores after 24 months of age. The rate of increase in the MDI scores after 24 months was greater among the low exposure group than the high exposure group but was not significantly greater (p = .23). 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 = .01).

The authors summarize three main findings from the study: 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.

Study Review

The investigators conducted a well-designed prospective cohort study and retained a high percentage (83%) of their participating mothers over the three year follow-up period. With very narrow inclusion criteria, the investigators selected a homogenous cohort of minority, inner-city women, most of whom fall into low income and low education categories. This homogeneity serves to equalize the study comparison groups with regard to potential measured and unmeasured confounders, but may also limit the generalizability of the findings.

While the high retention rate of the mothers provides as stable study population, the authors do not address that only 53% of the children from these mothers reached the three year milestone with study data collected. It is unclear what, if any, percentage of these children did not survive,

were lost to follow-up, or were too sick/affected by neurodevelopmental delays to participate. Authors did not provide information concerning the degree of missingness by chlorpyrifos exposure category in the published analyses, however in supplemental analyses suggested by the 2008 FIFRA SAP on chlorpyrifos authors did provide this information (See Appendix 4-1). In these additional analyses, authors concluded the degree of missingness was relatively equal by exposure category and therefore not likely to have imposed a selection bias.

As discussed by the authors, one weakness of the study is the reliance on a single exposure level (prenatal/cord blood.) It is acknowledged that this snapshot of exposure may not accurately reflect both pre- and post-natal exposure levels, although in general, for chronic exposures with a high prevalence and consistent frequency of use as indicated in this study population, a single measure may be sufficient to accurately rank participant exposure levels. The authors further explain that previous analyses of this cohort showed that exposures to chlorpyrifos did not show significant variation throughout pregnancy (Whyatt et al., 2007) and therefore concluded that this single measure can accurately represent prenatal exposure. While this may accurately represent prenatal exposure, there is no control for exposure over the subsequent 3 years which may be critical, especially as the authors note that their GLM analyses show a critical developmental re-bound period between 24 and 36 months. From this study, it is unclear if an increase or decrease in exposure during the period of early childhood could also influence the neurodevelopment of these children. Finally, the creation of a dichotomous exposure variable also brings limitations to the interpretation of the study results due to the amount of within-group variation likely present using this method of exposure classification. By setting the cut-off at 6.17pg/g, there are members of their "low exposure" group who may approximate high exposure levels and vice versa, resulting in non-differential measurement error.

The authors also address limitations to the sensitivity and predictive validity of the developmental tests, especially among children less than 3 years of age. Although, these tests are considered to be most valid among school-aged children they are also used as indicators for early intervention to prevent continued developmental delay. The authors describe a statistically significant reduction in scores associated with high exposure levels but they do not discuss whether this 7-point deficit is clinically relevant in terms of diagnosing or treating a child at this stage of development.

Also complicating this analysis is the pervasive, non-specific nature of neurological effects and the difficulty in attributing causal pathways related to just one environmental exposure. Although the authors site previous research linking chlorpyrifos exposure to inhibition of neural growth (Dam, 1998) it is still difficult to attribute developmental and behavioral effects to a single exposure variable measured at one point in time. In future studies it would be advantageous to include other potential covariates such as family history of developmental delay or attention disorders (especially among parents and siblings) as well as other co-morbidities not adjusted for by design (*i.e*, restriction using eligibility criteria to exclude co-morbidities). Not only is the etiology of attention and related behavioral disorders not well understood but also the diagnosis of these disorders is often influenced by complex outside forces.

This well-designed study provides suggestive findings linking prenatal chlorpyrifos exposure with neurodevelopmental deficits at the three years of age. The lack of outcome information for

a high percentage of cohort children is a concern which is not directly addressed by the authors. The use of a single exposure estimate (which likely led to non-differential exposure misclassification bias in this study) and an imprecise outcome characterization (although a clinical tool, neurodevelopmental milestones are 'soft' outcomes to measure) may have an accumulative effect to underestimate the true effect (bias results toward to null value), if a true effect exists. As mentioned previously, at the time of this publication, these analyses covered only a snapshot of the findings of the ongoing cohort study. The authors allude to the fact that they will use the subsequent evaluations, especially the 7-year scores to further describe the effects of chlorpyrifos exposure on neurodevelopment and behavior.

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Article 8. Lovasi et al. (2010)

Chlorpyrifos Exposure and Urban Residential Environment Characteristics as Determinants of Early Childhood Neurodevelopment. Gina S. Lovasi, James W. Quinn, Virginia A. Rauh, Frederica P. Perera, Howard F. Andrews, Robin Garfinkel, Lori Hoepner, Robin Whyatt, Andrew Rundle. American Journal of Public Health. 101(1):63-70 (2005).

Study Summary

Lovasi et al evaluated associations between neighborhood characteristics and early-life neurodevelopment in the Columbia Mother's and Newborn Study Cohort, and assessed whether such characteristics confound the association between exposure to chlorpyrifos and neurodevelopment previously reported. US Census data (2000) were used to estimate neighborhood-level measures of physical infrastructure, socioeconomic status, crowding, demographic composition, and linguistic isolation, augmenting previously reported prenatal chlorpyrifos exposure, psychomotor development, and mental development data on a New York City birth cohort.

Study participants were recruited during early pregnancy ($\leq 20^{th}$ week) among African-American and Dominican women age 18-35 years, and registered at New York Presbyterian Medical Center and Harlem Hospital, in New York City. Women who smoked, had a history of drug abuse, diabetes, hypertension, or HIV infection were excluded from participation in the study, as were women who resided in New York City for less than 1 year.

Cord blood plasma chlorpyrifos levels were assessed, and dichotomized as high (greater than the third tertile level of 6.17 pg/g) or low (<6.17pg/g). Maternal blood levels of chlorpyrifos were used to estimate cord blood levels for the 12% of infants for whom cord blood was not obtained.

The Bayley Scales of Infant Intelligence–Revised (BSID-II) were used to assess cognitive and psychomotor development at age 36 months. Development was reported using a Psychomotor Development Index (PDI) and a Mental Development Index (MDI), which served as the continuous outcome measurements in the study.

Participants' neighborhoods were defined using a 1-kilometer network buffer. Using 2000 US Census data, network buffer characteristics were determined, including: 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 (all household members aged 14 years and older have at least some difficulty with English).

Indicators of building disrepair were self-reported by mothers during prenatal interviews, and summed to create an index of disrepair that included one point for each of the following problems: holes in ceilings or walls, peeling or flaking paint, water damage, leaking pipes, and

lack of gas or electricity in the prior 6 months.

Generalized estimating equation models were created for the continuous outcomes (PDI and MDI) and Bayley Scores of Intelligence at 36 months. Robust standard errors were used to correct for possible autocorrelation within community district areas. All models included the following potential confounders: gender, gestational age at birth, Dominican versus African American ethnicity, maternal education, maternal intelligence quotient, the presence of secondhand smoke in the home during pregnancy, and an index of the quality of the home environment with respect to caretaking. Using hierarchal regression techniques which allow for control of individual level as well as group-level variables, authors were able to further clarify the nature of the association between mental and psychomotor development (Bayley scales of infant development) and prenatal chlorpyrifos exposure. Other variables including blood lead, other environmental exposures among other factors were not considered confounders in the prenatal chlorpyrifos and Bayley score association.

Of 266 children included as participants, 47% were male, 59% were Dominican, and 41% were African American. After adjusting for potentially confounding variables, high chlorpyrifos exposure (greater than 6.17 pg/g) was associated with a 6.5-point decrease in PDI and a 3.3-point decrease in MDI at age 36 months, as previously reported. These associations remained statistically significant and similar in magnitude in models additionally adjusting for dilapidated housing and neighborhood characteristics.

Study Review

This study helps clarify the nature of the relation between prenatal chlorpyrifos exposure and childhood neurodevelopment by controlling for the potential influence of socioeconomic status (SES) using advanced statistical techniques. Unique to this research, authors were able to measure variability due to SES using several different variables measured at both the individual-level and the group-level. This work enhances the precision and accuracy of the epidemiologic measures previously reported.

The authors note that participants lost to follow-up were not significantly different from continuing participants with respect to maternal age, ethnicity, marital status, education, income, or gestational age and birth weight of the newborn; authors cited a retention rate at the 3-year follow-up of 83%. How they arrived at that number is unclear. Originally, 648 pregnant women consented to participate and 327 children completed a developmental assessment at approximately 36 months of age. In this report, analyses were conducted among 266 children with available chlorpyrifos exposure data, yielding an effective retained proportion of 41%. This leaves open the possibility of selection bias resulting from loss to follow-up; one may only speculate as to the magnitude and direction of such bias.

The direct measurement of cord blood chlorpyrifos in this study is a substantial strength, compared to the assessment of non-specific markers as assessed in similar studies of organophosphate pesticide and child neurodevelopment. Umbilical cord blood was obtained at delivery for 88% of the children in the study. For the remaining 12% for whom no cord blood was obtained, cord blood plasma chlorpyrifos was estimated from maternal plasma levels using

blood sampled two days prior to delivery. Any chlorpyrifos measurement error resulting from this estimation is almost assuredly non-differential with respect to cognitive development outcomes. Children excluded for missing chlorpyrifos exposure data were similar to the analytic sample except that excluded children had more indicators of building dilapidation, so the likelihood of selection bias of large magnitude is unlikely. The decision to dichotomize cord blood chlorpyrifos levels using the highest tertile as a cut-point reduces the ability to assess exposure-response relationships, and may somewhat decrease statistical efficiency relative to using a continuous measure. If a large number of participants had levels of chlorpyrifos below the level of detection, however, using a categorical approach dispenses with the need to estimate or otherwise define exposure at low levels. The prenatal exposures assessed in this study are likely correlated with other prenatal exposures and factors in the postnatal environment which themselves predict cognitive development. This presents a challenge for a causal interpretation of prenatal chlorpyrifos exposure *per se*, as the agent and time period responsible for observed cognitive deficits.

The Bayley Scales of Infant Development–Revised (BSID-II) is a widely used norm-referenced developmental test for young children, it can be used to diagnose developmental delay, and it is known to be sensitive to the effects of toxic exposures such as low-level intrauterine lead. The assessment was appropriately administered, with each child being evaluated under controlled conditions by a trained bilingual research assistant. Inter-observer reliability was assessed but data were not presented in this report. The authors note that, when administered at age 3 years, the BSID-II demonstrates only moderate predictive power for subsequent intelligence and school performance, but cite previous literature as evidence that the test is nevertheless clinically useful for children performing in the subnormal range.

The statistical analyses were generally appropriate. Missing data on covariates were estimated using multiple imputations, and the variance estimates presented appropriately reflect the degree of uncertainty caused by missing covariate data. Robust standard errors were estimated to account for potential autocorrelation. The authors presented multiple model results, each with a different indicator of neighborhood character. Only neighborhood covariates representing socioeconomic conditions (*i.e.* percentage of individuals living in poverty, and percentage with high school education) were statistically significant predictors of PDI and MDI. Interestingly, and without explanation, the authors did not present the results of a model which simultaneously included all of the neighborhood characteristics as covariates. Presumably, this was done for the sake of parsimony, as one could argue against the inclusion of covariates considered not to be predictors of cognitive development. Alternatively, these factors were, *a priori*, considered potential confounders, and the use of statistical significance tests in the assessment of confounding is discouraged in many epidemiologic circles.

The observation that neighborhood characteristics did not confound the observed association between chlorpyrifos levels and cognitive development in this study has several possible explanations. This was a relatively homogenous study population living in the New York City Area, and it is possible that confounding is limited by the small degree of variability in neighborhood characteristics between those with and without high exposure levels of chlorpyrifos. It is also possible that the neighborhood characteristics are measured with error, such that they fail to capture aspects of socioeconomic condition, neighborhood composition,

and neighborhood condition that effect cognitive development, even within this context of measurement of several individual-level as well as group-level SES measures. This residual bias, if it exists, could (at least partially) explain the decrement in cognitive development associated with chlorpyrifos exposure, assuming that poorer neighborhood conditions are both predictors of reduced cognitive function and comparatively more prevalent among those with high chlorpyrifos exposure. The first of these two explanations has no effect on internal validity; under the assumption of no other bias, the observed association is unbiased, but external validity may be limited due to the restricted study population. The investigators' methods to assess neighborhood characteristics were well conceived and well implemented, and reflective of commonly utilized variables to measure important aspects of the multi-dimensional characteristic of socio-economic status. However, such exposures are notoriously difficult to measure well even using these advanced statistical methods and approaches.

The authors did not assess effect modification of the association between prenatal chlorpyrifos exposure and cognitive development by neighborhood conditions, which is an equally, if not more, interesting area of inquiry than assessment of confounding. The present study simply does not have the sample size/statistical power to address these effects, but, in the discussion, the authors speculate that large studies that collect both neighborhood context and exposure data could reveal patterns of effect modification. The relative homogeneity of the study population may have precluded statistically robust examination of effect modification by factors related to SES in this study.

The setting of the investigation in a sample drawn from low-income African American and Dominican communities is both a strength and a limitation of the study. The population is likely at high risk of developmental deficits, which increases the power of the study relative to a similar study set in a population at low risk. The assessment of confounding by demographic and neighborhood characteristics is limited by the restriction of the study sample to low-income communities (a weakness), but so too is the bias induced by these potential confounders (a strength). The generalizability of the study results may also be limited because participants were recruited from a restricted range of neighborhoods in New York City (a weakness), but the study population is likely among the most highly exposed and also at risk for cognitive impairment (Landrigan, 1990a, 1990b; Landrigan et al., 1999).

This study represents an extension of previously published work with additional adjustment for multiple neighborhood characteristics. The work was well conceived and conducted, representing perhaps the best assessment of the association between chlorpyrifos and cognitive development in the epidemiologic literature. Chief among its strengths is the direct assessment of cord blood plasma chlorpyrifos, the prospective design, and the assessment and control of potential confounders. Using the methods of data collection, statistical analyses, and enhanced methods to evaluate the role of SES as a potentially confounding variables, authors report no substantial difference in the reported association between prenatal chlorpyrifos exposure and child neurodevelopment as previously reported (Rauh et al. 2006).

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Article 9. Engel et al. (2011)

Prenatal Exposure to Organophosphates, Paraoxonase 1, and Cognitive Development in Childhood. Stephanie M. Engel, James Wetmur, Jia Chen, Chenbo Zhu, Dana Boyd Barr, Richard L. Canfield, and Mary S. Wolff. Environmental Health Perspectives 2011; 119:1182-1188

Study Summary

This is the third of three published analyses of prenatal pesticide exposure and child growth and development in the Mount Sinai Hospital Children's Environmental Health Study. The first (Berkowitz et al 2004) reported on assessments of birth weight, length and head circumference. The second (Engel et al 2007) reported on assessments of infant neurocognitive development and reflexes shortly after birth. In this study, Engel and colleagues extended their 2007 to include analysis of PON1 activity, PON1 polymorphisms, and cognitive development at ages 12 and 24 months and 6–9 years.

Of the 404 women originally enrolled between May, 1998 and July, 2001 (n = 404), a subset (n = 360) had organophosphate metabolite levels quantified in urine samples collected during the third trimester. Prenatal maternal blood and cord blood samples were analyzed for PON1 activity and genotype. Children of these mothers returned for neurodevelopment assessments at ages 12 months (n = 200), 24 months (n = 276), and 6–9 (n = 169) years of age. Participants in this study were predominantly young, unmarried, Hispanic women with low educational attainment. Most women delivered term infants of normal birth weight, as prematurity and very low birth weight were exclusion criteria for study enrollment.

The study population was drawn primarily from East Harlem and consisted largely of young Hispanic women (predominantly Puerto Rican), but also included African-American and Caucasian women. Mothers were enrolled during early pregnancy from the Prenatal Clinic and two private practices at Mount Sinai Hospital from May 1998 to July 2001. The study included only primiparous with singleton births. Mothers were excluded if they had any of the following: an initial prenatal visit after 26 weeks of gestation, serious chronic diseases, a serious pregnancy complication that could affect fetal growth and development; alcohol consumed greater than two alcoholic beverages per day, illicit drug use. Mothers and infants were also excluded if the child was born with a congenital malformation or severe prematurity. A total of 479 prenatal patients were recruited; 75 were excluded because of medical complications, premature birth, an infant with birth defects, inability to collect biologic specimens before birth, change of hospital or residence outside New York City, or refusal to continue to participate.

A questionnaire was administered to participants during their third trimester of pregnancy to obtain information on environmental exposures, sociodemographic characteristics, obstetrical and medical history, and lifestyle factors.

Generalized linear models were used to analyze the relationship between total diethylphosphates (DEPs), total dimethylphosphates (DMPs), total dialkylphosphate metabolites (DAPs), and subsequent cognitive development evaluated at age 12 and 24 months using the Bayley Scales of Infant Development, 2nd edition (BSID-II) and using the Wechsler Preschool and Primary Scale

of Intelligence, 3rd edition (WPPSI-III) at approximately age 7 years. The authors also assessed modification of pesticide metabolite - cognitive development associations by maternal and child PON 1 activity and PON 1 polymorphisms.

The authors presented their results stratified by level of race/ethnicity and *PON1* genotype, having observed apparent effect modification by these variables. The authors found that prenatal total dialkylphosphate metabolite level was associated with a decrement in mental development at 12 months among blacks and Hispanics. The associations appeared to be strongest among children of mothers who carried the *PON1* Q192R QR/RR genotype. In later childhood, increasing prenatal total DAP and DMP metabolites were associated with decrements in perceptual reasoning in the maternal *PON1* Q192R QQ genotype (imparting slow catalytic activity for chlorpyrifos oxon), with a monotonic trend consistent with greater decrements with increasing prenatal exposure. The authors concluded that their findings are suggestive of an association between prenatal exposure to organophosphates and decrements in cognitive development, particularly perceptual reasoning, with evidence of effects beginning at 12 months and continuing through early childhood, with *PON1* being a potentially important potential susceptibility factor for these deleterious effects. The authors did not observe any effect modification by PON1 enzyme activity.

The authors noted that the associations they observed between the organophosphate pesticide metabolites and cognitive development are similar to those reported in a similar prospective cohort study set in an agricultural area of California (the Center for the Health Assessment of Mothers and Children of Salinas Study, Eskenazi et al. 2010).

Urine samples obtained during the 3rd trimester prenatal visit were provided to the CDC to quantify levels of six dialkylphosphate metabolites. In cases where individual dialkylphosphate metabolite levels were missing because of analytic interference, metabolite levels were imputed using regression analysis to predict the missing metabolite on the basis of the other non-missing metabolites measured for that woman within the group of correlated metabolites (Eskenazi et al. Environ Health Perspect, 2004). Samples below the limit of detection (LOD) were defined as LOD/√–2. Diethyl- and dimethylphosphate metabolites were then summed on a molar basis (as nm/liter) to obtain total diethylphosphates (DEPs) and total dimethylphosphates (DMPs), respectively, and together to obtain total dialkylphosphates (DAP) levels. Twenty-six very dilute urine samples with < 20 μg/dL creatinine were excluded from organophosphate metabolite analyses. Approximately 97%, 89%, and 90% of the cohort had detectable levels of DAP, DEP, and DMP metabolites, respectively. Prenatal organophosphate marker levels were dichotomized using median exposure as the cut-off.

Plasma was separated from samples of prenatal maternal peripheral blood and cord blood and used to measure PON1 activity. *PON1* polymorphisms in both maternal and child DNA were also assessed using the blood samples provided by participants. Prenatal and cord blood PON1 activity levels were classified in tertiles for analysis of effect modification of the relationship between pesticide marker levels and subsequent cognitive development.

The Bayley Scales of Infant Development, 2nd edition (BSID-II), was administered to participating children at 12 and 24 months of age, approximately, to evaluate mental and

psychomotor development. The Mental Development Index (MDI) portion of the assessment rates a child's cognitive ability (*i.e.* memory, habituation, problem solving, early number concepts, generalization, classification, vocalizations, language, and social skills). The Psychomotor Development Index (PDI) rates fine and gross motor coordination. Between the ages of 6 and 9 years of age, a subset of participating children was again assessed using psychometric intelligence tests. Before age 7, children were administered Block Design, Information, Matrix Reasoning, Vocabulary, Picture Concepts, Symbol Search, Word Reasoning, and Coding subtests of the Wechsler Preschool and Primary Scale of Intelligence, 3rd edition (WPPSI-III). Composite Verbal, Performance, Processing speed, and Full-Scale IQ (FSIQ) scores were derived using age-standardized WPPSI-III norms. Children between the ages of 7 and 9 years were administered the Block Design, Similarities, Digit Span, Picture Concepts, Coding, Vocabulary, Letter-Number Sequence, Matrix Reasoning, Comprehension, and Symbol Search subtests of the Wechsler Intelligence Scale for Children, 4th edition (WISC-IV). Working Memory, Processing Speed, and FSIQ scores were derived using age-standardized WISC-IV norms.

The BSID-II was administered at Mount Sinai Hospital. Interviews and examinations were conducted in English or Spanish. The WPPSI-III was administered in English or Spanish by one of four examiners, as was the WISC-IV. by one of four examiners Both the WPPSI-III and the WISC-IV were administered in a private room, without the parent being present.

Of the 200 children who were administered the BSID-II at approximately 12 months of age (mean ± SD, 13.1 ± 1.6 months), one was excluded from analyses because they refused to compete a substantial portion of the examination; two children were excluded from the 12-month analysis because their parent reported a diagnosis of pervasive developmental disorder and 20 children were excluded because their corresponding maternal urine samples were deemed to dilute to analyze. Of the 177 eligible children who completed 12-month exams, 174 had organophosphate metabolites measured in prenatal urine. At the 24-month BSID-II (mean ± SD, 27.4 ± 4.5 months), 276 children completed the exam. Two were excluded from the 24-month analysis because the parent reported a diagnosis of pervasive developmental disorder. Children of mothers who submitted very dilute samples of urine (less than 20 μg/dL creatinine) were also excluded from analysis (n = 23). Of the remaining 251 eligible observations, 247 had organophosphate metabolites measured in prenatal urine. MDI scores for 10 children were not usable due to extensive refusals; they were included in the PDI analyses because their scores were valid. The authors reported an association between prenatal total dialkylphosphate metabolite level and a decrement in mental development at 12 months among blacks and Hispanics. These associations appeared to be enhanced among children of mothers who carried the *PON1* Q192R QR/RR genotype. In later childhood, increasing prenatal total dialkyl- and dimethylphosphate metabolites were associated with decrements in perceptual reasoning among those with maternal *PON1* Q192R QQ genotype, which imparts slow catalytic activity for chlorpyrifos oxon, with a monotonic trend consistent with greater decrements with increasing prenatal exposure.

Study Review

This was a well conducted prospective study conducted in a young, predominantly minority

population. The study design, analytic approach, and statistical analyses were appropriate. Differential measurement error of exposure and outcomes is likely to be minimal, although other sources of bias may have influenced the study findings.

The dramatic loss-to-follow-up over time is a possible cause of selection bias in this study. The authors noted that mothers returning for follow-up assessments tended to be older, with a disproportionately low fraction of women in the youngest age category; they also had disproportionately higher level of education, on average, relative to the original cohort. However, the subset of participants who returned for follow-up visits did not differ substantially from the originally enrolled cohort with respect to racial/ethnic composition, breast-feeding behaviors, and self-reported alcohol use during pregnancy.

The authors employed a reasonable set of exclusion criteria that likely resulted in recruitment of a study population that was relatively homogeneous with respect to confounders of the *in utero* pesticide exposure - cognitive development relationship (compared to the general population), and the potential for confounding was thus limited by design (restriction). Bias due to confounding was also controlled in the analysis, by assessing and adjusting for known risk factors for cognitive development that are potentially associated with pesticide exposure, including 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. The authors considered gestational age at delivery and birth weight, not as confounders, but as potential causal intermediates. They used a change-in-estimate approach to covariate selection, beginning with a full model, which allowed them to consider confounding by a given risk factor in the context of adjustment for other covariates. Given the limited sample size in this study, a full model with many covariates may be unstable, although it is unclear from the article whether such instability was observed.

Residual confounding by unmeasured or poorly measured confounders is likely in this study. Socioeconomic determinants, which are notoriously difficult to measure well, are likely predictive of pesticide exposure, and risk factors for poor cognitive development. The authors adjusted for some factors which may be surrogates for socioeconomic status (*e.g.*, race/ethnicity, marital status, maternal education), but adjustment for these factors is unlikely to have fully accounted for confounding by socioeconomic status. If such residual confounding exists, it is likely to result in effect estimates which overestimate the effect of organophosphate pesticide exposure. However, the extent of this confounding was likely limited by the recruitment of a relatively homogenous study population, vis-à-vis socioeconomic conditions.

Presence of environmental contaminants in the home, either during pregnancy or after birth, may confound the relationship under study. The authors do not discuss co-contaminants present in the home during the pregnancy; such factors would likely result in the observation of a greater than effect of pesticide exposure on cognitive development if they are positively associated with pesticide exposure and predictive of deficits in cognitive development. The authors stated that they considered a "Home Observation for Measurement of the Environment" (HOME) score as a potential confounder, and, in fact, they included this score in their multivariate models. The degree to which this "HOME" score is a good proxy for relevant co-exposures is unclear, and

leaves open the possibility of residual confounding by environmental contaminants in the home.

The authors observed apparent effect modification by maternal race/ethnicity. For example, among non-whites, increasing DAP and DMP tertiles of exposure were associated with a decrease in the MDI. However, among whites, the opposite was observed; higher metabolite levels were associated with an increase in MDI scores. Such an observation may be indicative of a true interaction between race/ethnicity and prenatal pesticide exposure, but it may also be explained by residual and differential confounding by co-contaminants that are correlated with pesticide exposure and race. Indeed, in this study population, race/ethnicity was strongly associated with housing type (*i.e.* public versus private housing), and the authors did, indeed, observe similar trends in the effect estimates when stratifying by housing type rather than race/ethnicity. The authors speculated that this observed heterogeneity may indicate that whites, or those living in private or owner-occupied housing, may have experienced a different source of exposure to pesticides or their metabolites that may have contributed substantially to their urinary concentrations, and identify pesticide residues from fresh fruit and vegetable consumption as one such source. The authors adjusted for seasonal variation in exposure, although they note that other time-related variability may influence exposure assessment.

Although they observed heterogeneity by *PON1* genotype, the authors did not observe any effect modification by PON1 enzyme activity in blood sampled during the third trimester, as they had in their earlier assessments of pesticide metabolites and cognitive development assessed shortly after birth. They speculated that this was because genotype is a more stable, long-term predictor of metabolism potential, relative to prenatal phenotype as measured by PON1 activity.

The metabolites assessed in this study are sensitive, but non-specific, markers for chlorpyrifos. The analytic methods appear sound. However, they assessed these markers using only a single maternal urine specimen taken during the third trimester – a critical period for neurodevelopment. If sources and patterns of exposure remain static, a single measurement would be a good indicator of pesticide exposure during pregnancy. If, however, sources and exposure patterns change over short time-spans, perhaps on the order of days to weeks, such an exposure assessment would not necessarily provide a good indicator of the relevant exposure. Like the many other sources of error possible in this study, this, too is likely to result in a non-differential misclassification of the exposure, with respect to the cognitive outcome. Serial measurements of urinary biomarkers of pesticide exposure in the third trimester would shed light on these patterns, although the benefits of such information are offset by the added cost, logistical complexity expense, and burden on the study participants.

The instruments used to assess neurodevelopment in this study (*i.e.,* the Bayley Scales of Infant Development, 2nd edition (BSID-II), the Wechsler Preschool and Primary Scale of Intelligence, 3rd edition (WPPSI-III), and the Wechsler Intelligence Scale for Children, 4th edition (WISC-IV)) are commonly used in both a clinical and research setting and are widely accepted tools.

In this study, prenatal maternal DEP urinary metabolite concentrations were associated with slight decrements in FSIQ, Perceptual Reasoning, and Working Memory between the ages of 6 and 9 years. Among children of QQ mothers, DAP and DMP urinary metabolite concentrations were associated with poorer scores on Perceptual Reasoning and FSIQ. The authors reported

stronger Q192R interactions for DAP and DMP urinary metabolites compared to that for the DEP metabolites, although the explanation is unclear and may have been influenced by small sample size. The authors note that *PON1* Q192R exhibits substrate specificity, but the strongest interactions were observed for DMP, not DEP (into which chlorpyrifos and diazinon both metabolize). Interestingly, researchers state that malathion spraying for mosquitoes carrying West Nile virus was a well-publicized source of exposure during the period of enrollment which may have increased the levels of DMP in this population.

The statistical analyses used in the study appear to have been appropriate, although they are not explained in detail. The authors stratified their findings by both race/ethnicity and *PON 1* genotype, although it is unlikely that the study was sufficiently powered to provide optimal statistical power to assess pesticide effects in the presence of such effect modification. To maximize the number of participants included in the analysis, the investigators defined a combined population of children who came for at least one of the Wechsler psychometric intelligence exams. The WISC-IV composite scores were included preferentially, and WPPSI-III composite scores substituted if the child did not return for the later exam. Regarding the use of a composite population for the outcome evaluations at 6-9 years, the authors report 'convergent validity' between the WISC-IV and WPPSI-III. In a previous report, the correlation between WPPSI-III and WISC-IV FSIQ scores was 0.89; WPPSI-III Verbal IQ and WISC-IV Verbal Comprehension, 0.83; and WPPSI-III Performance IQ and WISC-IV Perceptual Reasoning, 0.79. In the present study, correlations between the composite scores on the WPPSI-III and WISC-IV were likewise strong: FSIQ = 0.83, Verbal IQ/Verbal Comprehension = 0.84, and Performance IQ/Perceptual Reasoning = 0.78. In their statistical modeling, the authors included a variable to indicate whether the WISC-IV and WPPSI-III was used.

Regarding the measurement of developmental outcomes using the Bayley Score, in general, associations observed during the 12-month follow-up were either attenuated or non-existent at the 24-month visit, including the observation of race-based heterogeneity. The authors speculated that, although exposures likely decreased over time, the 0- to 12-month exposure window may have included less hand-to-mouth childhood exposure (from crawling exposure to house dust, and/or fresh fruit and vegetable consumption) than in the 12- to 24-month window; thus, more childhood exposure in the 12- to 24-month window may modify the effect of prenatal exposure.

The prospective nature of the study is a primary strength. Pesticide exposure indicators and outcomes metrics are likely to have been measured with some error, but these errors are likely to be non-differential. On average, such non-differential measurement error results in attenuation of the measures of association, and are thus unlikely to result in false-positive findings. A further strength attributable in part to the prospective design was the investigators' ability to consider potential confounders and effect-modifiers *a priori*, measure them by design, and adjust for/assess their influence in the analysis.

Importantly, this study was conducted at a time during which important regulatory changes in residential use of chlorpyrifos were being implemented (occurring approximately midway through the study). As a result, the participating children's exposures to chlorpyrifos may have decreased over time. The investigators reported that they did not observe any time-period

interactions, but they did not present data on the presence or absence of temporal changes in participants' pesticide metabolite levels (*i.e.* comparing those that were enrolled earlier versus later in time). In the discussion, they did mention that it was likely that childhood exposure to chlorpyrifos and diazinon was lower post-2000 (following the voluntary cancellation of indoor residential chlorpyrifos use). To the extent that post-natal exposures were largely reduced, the associations observed can be more definitively linked to prenatal exposures. If however, pesticide exposures persisted into childhood, it would be less clear whether deficits in cognitive functioning observed in this study were caused specifically by pre-natal exposures, rather than subsequent exposure early in life, assuming that pesticide exposure is causally associated with such decrements.

In this third study of organophosphate pesticide markers in maternal urine samples collected during the third trimester and subsequent cognitive development in children participating in the "Mt. Sinai Child Growth and Development Study", the authors report on findings out to age nine. The specter of selection bias induced by loss-to-follow-up is a marked weakness in an otherwise well conducted study. Unfortunately, it is difficult to assess the magnitude and direction of such bias. Confounding by unmeasured or poorly measured factors is yet another limitation, specifically with reference to the measurement of socio-economic factors and other environmental co-exposures. Non-specific biomarkers of exposure, as were assessed in this study, are likely more objective, sensitive, and likely also more accurate and reliable indicators of pesticide exposure, relative to exposure assessment methodologies that rely on self-report. However, to the extent that prenatal chlorpyrifos is the true exposure of interest, the assessment of non-specific organophosphate pesticide metabolites in a single sample of prenatal urine is a primary potential source of exposure measurement error. Because of the prospective design, information bias is expected to result in attenuation of the true (unobserved) associations, toward (observed) associations that are relatively smaller, or null. The finding of apparent effect modification by maternal race/ethnicity in this study could result from differential loss to follow-up, measurement error in the assessment of actual pesticide exposure, and/or differential residual confounding.

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Article 10. Eskenazi et al. (2007)

Organophosphate Pesticide Exposure and Neurodevelopment in Young Mexican-American Children. Brenda Eskenazi, Amy R. Marks, Asa Bradman, Kim Harley, Dana B. Barr, Caroline Johnson, Norma Morga, Nicholas P. Jewell. Environmental Health Perspectives. 115:792–798 (2007).

Study Summary

In this article by Eskenazi et al, the authors report on the relationship between prenatal and child urinary organophosphate metabolite levels and child neurodevelopment up to two years of age in the CHAMACOS cohort.

Enrollment for the CHAMACOS took place from 1999–2000 at community clinics, and included women who were ≥ 18 years old, pregnant at < 20 weeks gestation, Spanish- or English-speaking, eligible for low-income health insurance, and planning to deliver at the local public hospital. Researcher followed 531 women to delivery of a live-born, surviving, neonate. Excluded from the analysis were children that did not have a neurodevelopmental assessment, (n=71), who did not have prenatal and relevant concurrent DAP metabolite measurements (n = 3), were not singletons (n=8), had a medical condition that could affect performance on the neurobehavioral assessment (n = 3; deafness, Down syndrome, hydrocephalus). Also excluded from analysis were Bayley results with raw scores which were too low for standardization (n=3, 1, and 1, for the 6-month, 12-month, and 24-month assessments, respectively).

Maternal/fetal exposure was assessed by measurement of six non-specific organophosphate dialkylphosphate (DAP) metabolites in maternal urine samples collected between 5 and 27 weeks gestation and again between 18 and 39 weeks. Maternal urine was also analyzed for 3,5,6-trichloro-2-pyridinol (TCPy), a metabolite specific to chlorpyrifos. Post-natal early childhood urine samples were also collected, and DAP metabolites quantified, for the participating children at the time of the neurodevelopmental assessments. Quantification of organophosphate metabolites using gas chromatography-tandem mass spectrometry and isotope dilution calibration was conducted by the Centers for Disease Control and Prevention (CDC) labs.

To asses exposure, investigators measured six nonspecific organophosphate DAP metabolites in maternal and child urine: three dimethyl (DMP) phosphate metabolites (dimethylphosphate, dimethylthiophosphate, dimethyldithiophosphate); and three diethyl (DEP) phosphate metabolites (diethylphosphate, diethylthiophosphate, and diethyldithiophosphate) (Bradman et al. 2005). These six metabolites represent the by-products of approximately 80% of OPs used in the Salinas Valley. Values below the limit of detection (LOD) were assigned a value of LOD/(√2). The urine analysis was completed by the CDC. Maternal prenatal urine was also analyzed for specific metabolites including TCPy, a metabolite specific chlorpyrifos. Because a large proportion of women had non-detectable levels of TCPy, the chlorpyrifos-specific metabolite was categorized into three groups: TCPy less than LOD for both pregnancy measurements, and, for those with at least one detectable level, subdivided below and above the median of the average pregnancy level.

The outcome, child neurodevelopment, was assessed using the Bayley Scales of Infant

Development (Mental Development (MDI) and Psychomotor Development (PDI) Indices) (Bayley, 1993) and the Child Behavior Checklist (CBCL). The Bayley Scales of Infant Development were completed at the 6 months (n = 396), 12 months (n = 395), and 24 months (n = 372) of age. Both scales were administered in Spanish and/or English by psychometricians blinded to exposure. Psychometricians were trained using these standardized protocols and were supervised for quality assurance by a clinical neuropsychologist. Assessments were performed in a private room at the CHAMACOS research office or in a recreation vehicle (RV) modified to be a mobile testing facility. Scores > 1 SD below the mean (*i.e.*, < 85) indicate possible developmental delay.

Mother's completed the Child Behavior Checklist (CBCL) at the 24 month visit (n = 356). From the CBCL report, the authors assessed three scales as indicators of child neurodevelopment: The Attention Problems syndrome scale, which includes such items as "can't concentrate" and "can't sit still"; the DSM-oriented Attention-Deficit/Hyperactivity Disorder (ADHD) scale, which additionally includes such items as "gets into everything"; and the DSM-oriented Pervasive Developmental Disorder (PDD) scale, which includes such items as "avoids eye contact," "rocks head, body," and "unresponsive to affection". Experts consider a score greater than the 98th percentile of a national normative sample of clinical significance; they consider a score greater than the 93rd percentile of borderline clinical significance.

Many confounders were ascertained including, intelligence of the mother, maternal depression, a measure of how stimulating the child's environment is, and prenatal exposure to some known or suspected neurotoxins. Maternal intelligence was assessed via the Peabody Picture Vocabulary Test (PPVT). Maternal depression was assessed using the Center for Epidemiologic Studies Depression Scale (CES-D). 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, 12-month, and partially at the 24-month visit. The following known or suspected neurotoxicants were measured: polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), p,p'-dichlorodiphenyltrichlorethane (DDT), p,p'- dichlorodiphenyltrichlorethylene (DDE), and lead. Lead was measured in cord blood. PBDEs, PCBs, DDT, and DDE were measured in maternal serum samples collected at 26 weeks gestation.

Statistical analyses of the relationship between metabolite levels and Bayley performance were conducted using multiple linear regression models and generalized estimating equations (*i.e.*, longitudinal models) for MDI and PDI were developed for each of the three assessment time points (*i.e.*, 6, 12, and 24 months). Neurodevelopmental scores were modeled as continuous variables. Covariates in the models included indicators for the psychometrician conducting the assessment and the location of assessment, age at assessment, sex, duration of breast-feeding, HOME score, and household income, parity and indicator of maternal intelligence (PPVT score).

Other covariates that were considered but *not* included in the analysis were: 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 hexachlorobenzene.

Furthermore, the authors examined interactions between child DAPs and child sex and, because they previously observed an association with maternal DDT and 24-month MDI, between maternal DAPs and DDT. Finally, authors also reran the models using log-transformed creatinine-adjusted metabolites for comparative purposes.

Child behavior checklist score at the 24-month assessment was modeled using separate multivariate logistic regression models for the five child behavior checklist indices, dichotomized at the clinical significance level and at the borderline clinical significance level. The same covariates that were included in the final Bayley models were used for the CBCL models with three exceptions: maternal depression was added, and psychometrician and assessment location were dropped, because scores were based on maternal report.

Of the 348 infants included in this analysis, 49.4% were male. Most mothers were less than 30 years old at baseline (75.6), born in Mexico and Spanish-speaking (89%), had household income below the poverty line (62%). Many of the participating mothers worked in agriculture during pregnancy (43%) and had symptoms of depression (51%). Most of the women were married, non-smokers, and did not drink alcohol during pregnancy.

In this study population, prenatal DAP levels were adversely associated with MDI, while early life DAP levels were positively associated with MDI. At 24 months of age, these associations reached statistical significance (per 10-fold increase in prenatal DAPs: $\beta = -3.5$ points; 95% CI: –6.6 to –0.5; child DAPs: $\beta = 2.4$ points; 95% CI: 0.5 to 4.2). Neither prenatal nor child DAPs were associated with PDI or child behavior checklist attention problems. Both prenatal and postnatal DAPs were associated with risk of pervasive developmental disorder (per 10-fold increase in prenatal DAPs: OR = 2.3, $p = 0.05$; child DAPs OR = 1.7, $p = 0.04$). TCPy was not associated with any neurodevelopment outcome in this study.

Study Review

This study tested whether *in utero* or postnatal exposures to organophosphate pesticides have a detrimental impact on neurodevelopment, as assessed by the Bayley Scales of Infant Development and the child behavior checklist (CBCL). The study population was appropriate to the hypothesis, given that there were a range of exposure levels to OPs and there was some homogeneity in the population, *e.g.*, mostly low SES, Hispanic, agricultural workers. The homogeneity of the population restricted confounding by design. For example, another paper reported that smoking, alcohol use, and illicit drug use were rare the CHAMACOS cohort. The homogeneous population also increased the relative statistical efficiency of the study because it is likely at higher risk of deficits in neurodevelopment than the general population (Landrigan, 1990; Landrigan et al., 1999). However, the exclusion of children who lacked a neurodevelopmental assessment (n = 71, approximately 13% of the study population), has the potential to induce a selection bias if the reasons for the missing data are determined by exposure (or a predictor of exposure) and are also not independent of neurodevelopment. For example, participants with high pesticide exposures could have missed neurodevelopmental assessments for reasons related to uncontrolled determinants of exposure which are also related to neurodevelopment. Comparisons of maternal DAP metabolite levels between those included in this analysis and those not included in the analysis were not presented.

The exposure assessment methods in this study were good. Quantification of the metabolite levels was conducted at the CDC, using published methods. The study measured the organophosphate biomarker, DAP metabolites, in maternal and child spot urine samples, and the chlorpyrifos specific biomarker, TCPy, in maternal urine only. Measurement of biomarkers in urine is a more objective and likely more accurate indicator of organophosphate pesticide exposure than other ascertainment methods such as self-report. However, these biomarkers do present a few limitations, because study participants could be exposed to less toxic preformed DAP or TCPy metabolites (Lu et al. 2005; Wilson et al. 2003; Morgan et al. 2004), and exposure to pesticides varies over time and the half-life of chlorpyrifos in the body is short. However, the prenatal exposure measures were, with some exceptions, the average of two measurements, and thus may better reflect chronic exposure during pregnancy than in studies where only a single spot urine sample was collected. Under an assumption of no other biases, the negative findings could have resulted from exposure measurement error.

The outcome measurements, the Bayley Scales of Infant Development and the CBCL, were also appropriate. The Bayley Scales of Infant Development is considered a gold standard assessment. The assessments were reportedly appropriately administered. Both the MDI and PDI scales were administered in Spanish and/or English by psychometricians masked to exposure status of the children. Psychometricians were trained using standardized protocols and were supervised for quality assurance by a clinical neuropsychologist. Assessments were performed in a private room. The influence of inter-observer reliability was controlled by inclusion for an indicator for the assessed but data were not presented in this report.

The Child Behavior Checklist (CBCL) has been widely used in cross-cultural research and collects data on a range of behavior problems, yielding scores for several syndrome scales and five scales designed to be consistent with Diagnostic and Statistical Manual of Mental Disorders (DSM) diagnoses. The three scales used in this assessment were selected by the investigators *a priori* because the effects they measured were similar to effects seen in relation to DAP exposure in animal models. The child behavior checklist is limited in that it is reliant on maternal report and the authors note the findings from the child behavior checklist are not directly equivalent to a DSM diagnosis.

The statistical analysis used to assess the associations between the markers of exposure and neurodevelopment were appropriate. However, 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 (*i.e.* homoscedasticity, normality of error term distribution, independence of error terms, and linearity of the exposure-response relationship), other than to state that they log transformed DAP levels. The authors also conducted a host of sensitivity analyses to assess influence of modeling decisions on their findings, and generally found their results to be robust. For example, they controlled for birth weight, gestational age, and abnormal reflexes which were considered causal intermediates, re-ran models excluding low birth weight and preterm infants, and ran models with and without adjustment of biomarkers for creatinine levels. The authors also assessed effect modification by gender and DDT exposure because population of primarily immigrant women who may have been exposed to DDT and other U.S. banned substances in country of origin; associations between pesticide markers and the indicators of

neurodevelopment did not differ by either of these co-variables.

To increase the statistical efficiency of the study by preserving the size of the analytic population, missing covariate values were imputed by randomly selecting a value from participants with non-missing values. Maternal depression had the largest percentage of values requiring imputation (5%). Of remaining covariates, between 0% and 1.8% of values were imputed. Although a reasonable approach, poorly measured confounders (*i.e.* residual confounding) may also be influenced by imputed covariate data, although perhaps to a lesser degree because imputed data may still be preferable to no data at all.

Bias due to confounding was also controlled in the analysis, by assessing and adjusting for risk factors for neurodevelopment that are potentially associated with pesticide exposure, including age at assessment, sex, duration of breast-feeding, HOME score, and household income, parity and indicator of maternal intelligence. In addition to the variables included in the final models, the authors examined potential confounding by several other variables suggested by the literature (*i.e.*, 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, childcare), but they did not markedly alter the observed associations. Covariates were selected for inclusion in the final analyses if they were related to conditions of testing, related to neurodevelopment in the literature and associated ($p < 0.10$) with most outcomes or consistently related to neurodevelopment in the literature, even if not in the data. This is a reasonable approach to assessment of confounding.

The authors' quantification of environmental co-exposures (DDT, DDE, β-hexachlorocyclohexane, hexachlorobenzene, PCBs, and lead) is a significant strength of this study. Children and mothers, especially those from economically impoverished environments and émigrés from home countries in which these compounds were frequently use in the recent past, may be exposed to other environmental agents that may affect neurodevelopment. Potential confounding by these exposures, as well as by indicators of fetal development (shortened gestation and poorer reflexes) was assessed, and observed associations in these models were consistent with the primary findings.

This study has apparent inconsistencies in the findings which are worth noting. First, while DAPs were associated with an increased risk of PDD in this population, post-natal DAPs were positively associated with MDI. No explanation was presented by the authors. One possible explanation is that children with higher cognitive functioning may be more interactive with their environment, leading to higher exposure to pesticide residues, an example of "reverse causation", whereby the temporal order of the exposure and outcome are reversed. The measurements of prenatal pesticide markers do not suffer from the same issues of unknown temporal order; they clearly preceded the measures of post-natal child development. Another possible explanation suggested by the authors is confounding by diet. Children who eat more fruits and vegetables may have higher DAP levels, but because of better diets have higher functioning. Second, although the investigators observed associations between neurodevelopment and the non-specific metabolite markers of organophosphate pesticides (DAPs), no such associations were observed for metabolites specific to chlorpyrifos (TCPy), nor for a malathion-specific metabolite (MDA). The organophosphate metabolites (DAPs) cannot be

traced back to individual pesticides, and the observed associations with DAPs may be attributed to other pesticides, oxydemeton-methyl for example, for which no specific metabolite is measureable, or may be due to the short-half life and lower storage stability of the pesticide specific metabolites as compared to the non-specific metabolites.

The study has numerous strengths, most notably the prospective design the longitudinal assessment of both exposure and outcomes. Strengths in the study design also include the quantification of non-specific (DAPs), chlorpyrifos-specific (TCPy) metabolites, and other environmental co-exposures. The results of this investigation by Eskenazi et al in the CHAMACOS cohort are suggestive of a detrimental association between prenatal organophosphate exposure, as measured by maternal urinary metabolite levels and neurodevelopment up to 24 months of age. Although the prospective study design and reasonable methods protect from certain false-positive inducing biases, there remains the potential of residual confounding in the direction of an adverse effect on development by unmeasured factors leading to the observation of a false-positive association. The authors reasonably suggest that their results should be interpreted with caution because, although they observed adverse associations between prenatal DAPs and mental development and pervasive developmental problems at 24 months of age, they also observed a positive relationship between neurodevelopment and postnatal DAPs.

Article 11. Eskenazi et al. (2010)

PON1 and Neurodevelopment in Children from the CHAMACOS Study Exposed to Organophosphate Pesticides *in utero*. Brenda Eskenazi, Karen Huen, Amy Marks, Kim G. Harley, Asa Bradman, Dana Boyd Barr, and Nina Holland. Environmental Health Perspectives. 118:1775-1781 (2010).

In this article by Eskenazi et al. (2010), the authors report on the relationship between *PON1* genotype and PON1 activity level and child neurodevelopment, and the potential effect modifying role of PON1 status in the relation between prenatal and child urinary organophosphate metabolite levels and child neurodevelopment up to two years of age in the CHAMACOS cohort.

Methods and analyses employed in this investigation were similar to Eskenazi et al. 2007, with the exception of the measurement of PON1 status in association with this outcome, and assessment of interaction between PON1 and DAPs with child neurodevelopment. Enrollment for the CHAMACOS took place from 1999–2000 at community clinics, and included women who were ≥ 18 years old, pregnant at < 20 weeks gestation, Spanish- or English-speaking, eligible for low-income health insurance, and planning to deliver at the local public hospital. Researcher followed 528 women to delivery of a live-born, surviving, neonate. Excluded from the analysis were children that did not have a neurodevelopmental assessment, (n=71), who did not have prenatal and relevant concurrent DAP metabolite measurements (n = 3), were not singletons (n=8), had a medical condition that could affect performance on the neurobehavioral assessment (n = 3; deafness, Down syndrome, hydrocephalus). Also excluded from analysis were Bayley results with raw scores which were too low for standardization (n=3, 1, and 1, for the 6-month, 12-month, and 24-month assessments, respectively), and participants for whom PON1 information was not available. The study included 371 mother-infant pairs.

Maternal/fetal exposure was assessed by measurement of six non-specific organophosphate dialkylphosphate (DAP) metabolites in maternal urine samples collected between 5 and 27 weeks gestation and again between 18 and 39 weeks. Specifically, investigators measured six nonspecific organophosphate DAP metabolites in maternal and child urine: three dimethyl (DMP) phosphate metabolites (dimethylphosphate, dimethylthiophosphate, dimethyldithiophosphate); and three diethyl (DEP) phosphate metabolites (diethylphosphate, diethylthiophosphate, and diethyldithiophosphate) (Bradman et al. 2005). These six metabolites represent the by-products of approximately 80% of OPs used in the Salinas Valley. Chlorpyrifos and diazinon were the most commonly used organophosphates in the region that metabolize to the diethyl phosphate metabolite. Values below the limit of detection (LOD) were assigned a value of LOD/(√2). Quantification of organophosphate metabolites using gas chromatography-tandem mass spectrometry and isotope dilution calibration was conducted by the Centers for Disease Control and Prevention (CDC) labs using published, validated, and accepted methods.

Maternal and umbilical cord blood samples were collected at the time of delivery. Genotyping of the *PON1*$_{-108}$ and *PON1*$_{192}$ SNPs was performed using genomic DNA extracted from blood clots and Taqman PCR methods. Two PON1 enzyme activity assays (arylesterase and paraoxonase) were performed using spectrophotometric methods. Arylesterase activity measures the rate of hydrolysis of the substrate phenyl acetate, and reflects the quantity of PON1 enzyme.

Paroaxonase activity measures the rate of hydrolysis of paraoxon, and thus reflects a combination of the catalytic efficiency and quantity of the PON1 enzyme. Using triplicate samples, quality assurances methods illustrated the coefficient of variation was within the acceptable range (<10%).

The outcome, child neurodevelopment, was assessed using the Bayley Scales of Infant Development (Mental Development (MDI) and Psychomotor Development (PDI) Indices) (Bayley, 1993) and the Child Behavior Checklist (CBCL). The Bayley Scales of Infant Development were completed at the 6 months (n = 396), 12 months (n = 395), and 24 months (n = 372) of age. Both scales were administered in Spanish and/or English by psychometricians blinded to exposure. Psychometricians were trained using these standardized protocols and were supervised for quality assurance by a clinical neuropsychologist. Assessments were performed in a private room at the CHAMACOS research office or in a recreation vehicle (RV) modified to be a mobile testing facility. Scores > 1 SD below the mean (*i.e.*, < 85) indicate possible developmental delay.

Mother's completed the Child Behavior Checklist (CBCL) at the 24 month visit (n = 356). From the CBCL report, the authors assessed three scales as indicators of child neurodevelopment: The Attention Problems syndrome scale, which includes such items as "can't concentrate" and "can't sit still"; the DSM-oriented Attention-Deficit/Hyperactivity Disorder (ADHD) scale, which additionally includes such items as "gets into everything"; and the DSM-oriented Pervasive Developmental Disorder (PDD) scale, which includes such items as "avoids eye contact," "rocks head, body," and "unresponsive to affection". Experts consider a score greater than the 98th percentile of a national normative sample of clinical significance; they consider a score greater than the 93rd percentile of borderline clinical significance.

Many confounders were ascertained including, intelligence of the mother, maternal depression, a measure of how stimulating the child's environment is, and prenatal exposure to some known or suspected neurotoxins. Maternal intelligence was assessed via the Peabody Picture Vocabulary Test (PPVT). Maternal depression was assessed using the Center for Epidemiologic Studies Depression Scale (CES-D). 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, 12-month, and partially at the 24-month visit. The following known or suspected neurotoxicants were measured: polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), p,p'-dichlorodiphenyltrichlorethane (DDT), p,p'- dichlorodiphenyltrichlorethylene (DDE), and lead. Lead was measured in cord blood. PBDEs, PCBs, DDT, and DDE were measured in maternal serum samples collected at 26 weeks gestation. However, these variables were not included in final models. The following variables were adjusted in the interaction analysis: age at assessment, sex, parity, breast-feeding duration, HOME score, maternal PPVT, and household poverty status. For the MDI and PDI models an indicator variable for the psychometrician was included, and maternal depression included in the model for PDD.

Statistical analyses of the relationship between PON1 status and Bayley performance were conducted using multiple logistic regression models; interaction terms and stratified analyses were presented to evaluate the potential effect modifying role of PON1 status in the DAPs and

child neurodevelopment association. Neurodevelopmental scores were modeled as continuous variables. *PON1* genotype was modeled as a three-level variable, using the wild type as the referent. PON1 enzyme levels were categorized as tertiles. Authors log_{10} transformed DAP levels, and averaged the two measures of urinary DAPs taken during pregnancy in the analyses, when available (n=22 for which only one sample available). Covariates in the models included indicators for the psychometrician conducting the assessment and the location of assessment, age at assessment, sex, duration of breast-feeding, HOME score, and household income, parity and indicator of maternal intelligence (PPVT score). Authors imputed missing values to preserve sample size, particularly in the stratified analyses. Statistical significance of the main effect was considered a p-value of 0.05, and significance of an interaction term was considered a p-value of 0.15.

Among the selected participants, mothers were generally young age (average 26.4 years, sd 5.2), born in Mexico, were predominantly married or living as married, and were poor, 60% living at or below the poverty line. Forty-four percent of the women reported working in agriculture during pregnancy, 82% resided with a household member who works in agriculture, and 23% live near an agricultural operation. Most mothers reported breast feeding, and the duration of breast feeding was on average 6 months. Authors detected dimethyl phosphates more frequently than diethyl phospahtes. The average score for the MDI was 85 and PDI 98; 14% of children evaluated exceeded the concern level of the CBCL.

Overall, infants with the variant $PON1_{108TT}$ allele who produced lower amounts of PON1 enzyme had reduced scores on the MDI and PDI, and increased risk of PDD using the CBCL. Authors observed no association with $PON1_{192QQ}$ genotypes in the relation with MDI, PDI or PDD. Similarly, authors observed no association between enzyme levels and any measure of child neurodevelopment in this study. The interaction between maternal DAP and *PON1* genotype with the Bayley assessed outcomes was non-significant (p=0.98), however researchers observed a trend of lower scores by the number of $PON1_{108}$ variants alleles. For each 10-fold increase in total DAPs, the MDI score reduced by 3.2 $PON1_{108CC}$, 3.7 $PON1_{108CT}$, and 5.5 $PON1_{108TT}$ (p-interaction 0.98). A similar trend was observed for the diethyl phosphates, but neither strata-specific estimates nor trend tests were significant. No similar trend was apparent across the number of variant alleles at the $PON1_{192}$ genomic locus. No associations were observed between DAPs and PDI by level of the $PON1_{108}$ genotype. However, there was limited evidence of heterogeneity by $PON1_{192}$ genotype in the relation between DAPs and PDI, but results were not significant.

Study Review

Overall, researchers offer some evidence of a link between infant *PON1* status and child neurodevelopment state, and authors' state they cannot conclude the study provides evidence of effect modification by *PON1* genotype or phenotype status in the relation between DAPs and MDI, PDI or PDD in this study. Similarly to the accompanying investigation (Eskenazi et al. 2007), the study population was appropriate to the hypothesis, given that there were a range of exposure levels to OPs and there was some homogeneity in the population, *e.g.*, mostly low SES, Hispanic, agricultural workers. The homogeneity of the population restricted confounding by design. For example, another paper reported that smoking, alcohol use, and illicit drug use were

rare the CHAMACOS cohort. The homogeneous population also increased the relative statistical efficiency of the study because it is likely at higher risk of deficits in neurodevelopment than the general population (Landrigan, 1990; Landrigan et al., 1999). Authors did not share whether DAP levels were similar in mothers of infants for whom the MDI and PDI scores were not available; the potential for selection bias therefore cannot be assessed. The measurement of both urinary DAPs and PON1 status were well conducted, however limitations previously noted regarding the non-specific nature of a DAP measurement to the question of chlorpyrifos toxicity and the use of one- or two measures during the latter part of pregnancy as an estimate for gestational exposure apply equally to this investigation.

The outcome measurements, the Bayley Scales of Infant Development and the CBCL, were also appropriate. The Bayley Scales of Infant Development is considered a gold standard assessment. The assessments were reportedly appropriately administered. Both the MDI and PDI scales were administered in Spanish and/or English by psychometricians masked to exposure status of the children. Psychometricians were trained using standardized protocols and were supervised for quality assurance by a clinical neuropsychologist. Assessments were performed in a private room. The influence of inter-observer variability was controlled by inclusion of an indicator variable for the psychomatrician.

The Child Behavior Checklist (CBCL) has been widely used in cross-cultural research and collects data on a range of behavior problems, yielding scores for several syndrome scales and five scales designed to be consistent with Diagnostic and Statistical Manual of Mental Disorders (DSM) diagnoses. The three scales used in this assessment were selected by the investigators *a priori* because the effects they measured were similar to effects seen in relation to DAP exposure in animal models. The child behavior checklist is limited in that it is reliant on maternal report, and the authors note the findings from the child behavior checklist are not directly equivalent to a DSM diagnosis.

Again, the statistical analysis used to assess the associations between the markers of exposure and neurodevelopment were appropriate. However, 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 (*i.e.* homoscedasticity, normality of error term distribution, independence of error terms, and linearity of the exposure-response relationship), other than to state that they log transformed DAP levels. The authors also conducted a host of sensitivity analyses to assess influence of modeling decisions on their findings, and generally found their results to be robust. For example, investigators controlled for maternal depression in the relation between pesticide exposure and PDD, and ran models with and without adjustment of biomarkers for creatinine levels.

To increase the statistical efficiency of the study by preserving the size of the analytic population, missing covariate values were imputed by randomly selecting a value from participants with non-missing values. Maternal depression had the largest percentage of values requiring imputation (5%). Of remaining covariates, between 0% and 1.8% of values were imputed. Although a reasonable approach, poorly measured confounders (*i.e.* residual confounding) may also be influenced by imputed covariate data, although perhaps to a lesser degree because imputed data may still be preferable to no data at all.

Bias due to confounding was also controlled in the analysis, by assessing and adjusting for risk factors for neurodevelopment that are potentially associated with pesticide exposure, including age at assessment, sex, duration of breast-feeding, HOME score, and household income, parity and indicator of maternal intelligence. In addition to the variables included in the final models, the authors examined potential confounding by several other variables suggested by the literature (*i.e.*, 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, childcare), but they did not markedly alter the observed associations. Covariates were selected for inclusion in the final analyses if they were related to conditions of testing, related to neurodevelopment in the literature and associated ($p < 0.10$) with most outcomes or consistently related to neurodevelopment in the literature, even if not in the data. This is a reasonable approach to assessment of confounding.

The authors' quantification of environmental co-exposures (DDT, DDE, β-hexachlorocyclohexane, hexachlorobenzene, PCBs, and lead) is a significant strength of this study. Children and mothers, especially those from economically impoverished environments and émigrés from home countries in which these compounds were frequently use in the recent past, may be exposed to other environmental agents that may affect neurodevelopment. Potential confounding by these exposures, as well as by indicators of fetal development (shortened gestation and poorer reflexes) was assessed, and observed associations in these models were consistent with the primary findings.

Similarly to the previous investigation by Eskenazi et al. (2007), the study has numerous strengths, most notably the prospective design the longitudinal assessment of both exposure and outcomes. Strengths in the study design also include the quantification of non-specific (DAPs), PON1 status, and other environmental co-exposures. The results of this investigation by Eskenazi et al. (2007) in the CHAMACOS cohort were suggestive of a detrimental association between prenatal organophosphate exposure, as measured by maternal urinary metabolite levels and neurodevelopment up to 24 months of age, and this investigation presents some additional, albeit limited evidence of potential effect modification by PON1 status. While results were not significant, sample size within strata of *PON1* genotype and question as to the relevance of measurement of just PON1 among the suite of enzymes involved in organophosphate metabolism remain. Although the prospective study design and reasonable methods protect from certain false-positive inducing biases, there remains the potential of residual confounding in the direction of an adverse effect on development by unmeasured factors leading to the observation of a false-positive association, however less so with respect to PON1 status as these factors would not likely be correlated. The authors reasonably suggest that their results should be interpreted with caution as the evidence for true effect modification in this relation is weak.

Article 12. Marks et al. (2010)

Organophosphate Pesticide Exposure and Attention in Young Mexican-American Children: The CHAMACOS Study. Amy R. Marks, Kim Harley, Asa Bradman, Katherine Kogut, Dana Boyd Barr, Caroline Johnson, Norma Calderon, and Brenda Eskenazi. Environmental Health Perspectives. 118:1768–1774 (2010).

Study Summary

Using data collected as part of the CHAMACOS Study, Marks et al conducted a study to investigate the association between urinary dialkyl phosphate (DAP) metabolites in pregnant women and their children, as a marker of organophosphate exposure, and attention-related outcomes among Mexican-American children living Salinas Valley, an agricultural region of California.

Enrollment for the CHAMACOS took place from 1999–2000 at community clinics, and included women who were ≥ 18 years old, pregnant at < 20 weeks gestation, Spanish- or English-speaking, eligible for low-income health insurance, and planning to deliver at the local public hospital. The investigators followed 526 women to delivery of a live born, surviving singleton. Excluded from analyses were children who did not have a prenatal DAP metabolite measurement (n = 2), had a medical condition that could affect performance on the neurobehavioral assessment (n = 3; deafness, Down syndrome, hydrocephalus), were lost to follow-up, or did not participate at the 3.5- or 5-year study visit (n = 173) were excluded from the study. The study population consisted of the remaining 348 children who had available data at 3.5 and/or 5 years.

Exposures were assessed by measurement of organophosphate dialkylphosphate (DAP) metabolites in maternal and child urine samples. Maternal urine was collected between 5 and 27 weeks gestation and again between 18 and 39 weeks. The post-delivery urines were collected within 1 week of delivery for 73% of the sample, with the remainder obtained up to 176 days afterwards. Urine samples were also collected from participating children at the 3.5 year and 5 year visits. Quantification of organophosphate metabolites was conducted by Centers for Disease Control and Prevention (CDC) labs. Total DAP metabolite level was defined as the sum of the molar concentrations of the six DAP metabolites, dimethylphosphate (DMP) metabolite level as the sum of the molar concentrations of the three dimethylphosphate metabolites only, and diethylphosphate (DEP) metabolite level as the sum of the molar concentrations of the three diethylphosphate metabolites. Total DAP, DMP, and DEP levels were determined for each participant for each of the two pregnancy urine samples and for the children's urine samples. Individual metabolite levels below the limit of detection (LOD) were assigned a value of the LOD divided by the square root of two, and this value was included in each sum. Total DAP, DMP, and DEP metabolite levels were $\log_{10}$ transformed. "Pregnancy" DAP, DMP, and DEP values were created by averaging the two log-transformed pregnancy measures. For 19 women with only one DAP measurement in pregnancy, the single measure was used. Several children were missing DAP measures at 3.5 years (n = 58) and 5 years (n = 14).

Attention-related outcomes were assessed in three ways: 1) maternal report of child behavior at 3.5 and 5 years of age; 2) direct assessment of the child at 3.5 and 5 years; 3) and by a psychometrician's report of the behavior of the child during testing at 5 years.

Mothers completed the Child Behavior Checklist (CBCL) for 1.5–5 years of age as part of the maternal interview at the 3.5- and 5-year visits to assess emotional/behavioral problems and competencies of the children. Attention was evaluated as continuous raw scores on these scales, and by the proportion above a cutoff score greater than the 93rd percentile (a borderline clinical range).

Children completed a battery of neurodevelopmental assessments at each visit: The visual attention subtest of the NEPSY-II at the 3.5-year visit, and the Conners' Kiddie Continuous Performance Test (K-CPT), during the 5-year neurodevelopmental assessment. The NEPSY-II was administered to children by trained psychometricians. Children were asked to circle specific images on a page of pictures that included distracters. For data analysis, the authors used continuous scores scaled to a normative sample of U.S. children; the age-standardized mean ± SD for this subtest is 10 ± 3. The K-CPT is a 7-min computerized test that assesses reaction time, accuracy, and impulse control. Briefly, children were instructed to press the space bar when they saw any image on the computer screen except a ball. The computer program yields T-scores age-standardized to a general U.S. population (mean ± SD = 50 ± 10) for errors of commission (*i.e.*, the child responds when he or she should not), errors of omission (*i.e.*, the child fails to respond when he or she should), and hit reaction time. The authors examined T-scores continuously and categorically using the cutoff of T-score > 65, which is reportedly considered markedly atypical. The program used also combined measures to generate a clinical ADHD Confidence Index score (range, 0–100). For statistical analyses, the authors used continuous Confidence Index percentiles and selected a cut-point of > 70th percentile (meaning that 70% of children performing similarly on the test could be correctly classified as having clinical ADHD).

Following the 5-year neurodevelopmental assessment, psychometricians blinded to exposure status answered several subjective questions evaluating the behavior of the child during the 2-hr visit, including four questions derived from the seven-item Hillside Behavior Rating Scale. The authors summed responses to two questions assessing motor activity and distractibility to create an adapted ADHD symptoms scale. The Hillside Scale is reportedly associated with parent and teacher ratings and has been found to add significantly to the clinical prediction of ADHD. For analysis the authors created a dichotomized Hillside outcome variable with scores ≥ 7 of 12 possible points (representing < 10% of children) to flag children displaying a higher degree of attention problems based on psychometricians' observation.

Statistical analyses were conducted using multivariate linear and logistic regression models to evaluate the associations between the markers of organophosphate pesticide exposure and the indicators of attention deficits summarized above. Separate models were run with maternal DAPs only, child DAPs only, and the two together in the same model. Odds ratios greater than one and β-estimates greater than zero indicate positive associations between exposure and increased risk of adverse outcome, except for the NEPSY visual attention subtest, where a β-estimate greater than zero indicates better performance. All models adjusted for the following covariates: an indicator of the evaluating psychometrician (not included in CBCL models), age at assessment, sex, maternal education, depressive symptoms, maternal intelligence, out-of-home child care, and breast feeding duration. The covariates 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 were also considered but not included in the final models because they did not markedly alter the observed associations and were therefore not used in final models. To preserve the size of the analytic population, remaining missing covariate values (six instances for maternal depression and three for maternal PPVT) were imputed by simple random selection of a value from participants with non-missing values.

The authors also conducted a number of sensitivity analyses. They reran all models using creatinine-adjusted DAP metabolite concentrations. In addition, they added to final models some factors potentially on the causal pathway (*i.e.*, birth weight, gestational duration). The authors also considered whether controlling for lead (log_{10}-transformed), a known neurotoxicant, altered results for DAP concentrations in the subsamples with cord (n = 229) or 2-year (n = 296) lead values. Finally, because rates of ADHD vary considerably by sex in the general population (Pastor and Reuben 2008) and effects of other toxicants have been found to vary by sex (Delaney-Black et al. 2004), the authors tested for interactions between DAP concentrations and child sex, using $p < 0.15$ for the interaction term to determine whether associations of DAP concentrations with measures of attention differed for boys and girls.

Of the 378 infants included in this analysis, 47.5% were male. The mean maternal age was 26.5±5.2 years. Most mothers were born in Mexico (86%), spoke only Spanish in the home (90%), had low income (97% within 200% of the federal threshold for poverty; 63% within 100%), had not completed high school (79%), were nonsmokers (96%), had previous live births (66%), and were married (82%). Many of the participating mothers had symptoms of depression (44%).

In this study population, higher concentrations of organophosphate 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. Prenatal DAPs were non-significantly associated with maternal report of attention problems and ADHD at age 3.5 years but were significantly related at age 5 years (CBCL attention problems: $\beta = 0.7$ points; 95% CI: 0.2–1.2; ADHD: $\beta = 1.3$; 95% CI: 0.4–2.1). 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).

Study Review

This study tested whether *in utero* or postnatal exposures to organophosphate pesticides have a detrimental impact on attention. The study population was appropriate to the hypothesis, given that there were a range of exposure levels to OPs and there was some homogeneity in the population, *e.g.*, mostly low SES, Hispanic, agricultural workers. The homogeneity of the population restricted confounding by design. For example, another paper reported that maternal smoking, alcohol use, and illicit drug use were rare this cohort. The homogeneous population also increased the relative statistical efficiency of the study because it is likely at higher risk of

deficits in neurodevelopment than the general population. The exclusion of children who did not complete the 3.5- or 5-year assessment (n = 173), has the potential to induce a selection bias if the reasons for the missing data are determined by exposure (or a predictor of exposure) and are also not independent of the development of attention disorders. However, the authors stated that the levels of DAPs were similar in the mothers of the children which were not included relative to those included in the analysis. The authors also report that distributions of other covariates measured before 3.5 years were also similar, except that more boys than girls dropped from the study. This provides some indication that selection bias may not be a large factor in this analysis.

The ascertainment and analysis of urinary organophosphate pesticide markers was appropriate. Quantification was conducted at the CDC, using published methods. DAP metabolites are likely more accurate and objective indicators of organophosphate pesticide exposure, relative to other ascertainment methods such as self-report. Although they are sensitive indicators of exposure, it is difficult to infer chlorpyrifos effects specifically from the metabolite levels, as they also indicate exposure to other organophosphates. The study population likely sustained many other potentially relevant exposures to multiple chemicals and other pesticides which may affect development. The six organophosphate metabolites cannot be traced back to individual pesticides but, according to the authors, they represent the breakdown products of approximately 80% of the total organophosphate pesticides used in the Salinas Valley. It is not possible to disentangle the various effects from these potential co-exposures and chlorpyrifos in this study.

Because organophosphate pesticides have short half-lives, the degree to which metabolites in urine samples reflect 'usual' levels, and thus usual exposure, is uncertain. The prenatal exposure measures were, with some exceptions, the average of two measurements, and thus may better reflect chronic exposure during the pregnancy. The absence of findings associated with child levels may be due, in part, to the fact that the child's levels are poor indicators of exposure during critical windows of exposure with respect to development of attentional difficulties.

Errors in the assignment of exposure in this prospective study will likely have resulted in attenuation of observed associations. The likelihood of these errors is high, and the magnitude of the bias induced difficult to assess. Under an assumption of no other biases, the negative findings, those for the association between children's concurrent DAP levels and attention outcomes, for example, could have resulted from exposure measurement error.

The outcome in this study is very difficult to measure. Instead of opting for a single best instrument/indicator of attention deficits, the investigators chose to use multiple instruments, and also to combine the results of these various instruments to form a composite indicator of attentional difficulties. There is no evidence presented in the report that would suggest that the outcome assessments were improperly conducted. Nevertheless, outcome measurement error is likely. As with the exposure assessment, errors in the assignment of attention deficits in this prospective study will likely have resulted in attenuation of observed associations. Again, the likelihood of these errors is high, and the magnitude of the bias induced difficult to assess. The authors did not report on an assessment of changes in attentional difficulties between the 3.5 and 5 year visits. If such changes did occur, it would have been interesting to the reader to present results of a longitudinal analysis between this change in the outcome, and the child DAP levels over time.

The statistical analysis used to assess the associations between the markers of exposure and attention deficits were appropriate. Model diagnostics were performed and, according to the authors, found acceptable. The results of the model diagnostics were not presented. The authors also conducted a host of sensitivity analyses to assess influence of modeling decisions on their findings, and generally found their results to be robust.

In addition to those included in final models, the following covariates were considered, but not included in multivariate models: maternal age, parity, marital status, active/passive smoking exposure, regular alcohol use during pregnancy, presence of father in the home, maternal work status, and household income at the time of assessment. Confounding by blood lead levels was also assessed in a subset of children with measurements of cord blood (n = 229) and 2-year (n = 296) lead levels, and found not to influence the DAP and attentional outcomes.

Covariates were selected for these analyses if they were related to conditions of testing or related to attention deficits in the literature and/or associated with more than one outcome ($p < 0.10$ or changes to the main effect coefficient by $\geq 10\%$). The authors used a less conservative alpha level as a cut-off for selection of covariates, a somewhat more conservative approach to covariate selection, relative to use of the conventional alpha level of 0.05. However, the use of a statistical test as a means to select covariates from a larger set of potential confounders is discouraged in some epidemiologic circles (Miettinen 1976, Breslow and Day 1980, Greenland 1989). The assessment of confounding is generally not considered to be a statistical endeavor, in part because the association between the confounder and the outcome is just one factor that determines the degree of confounding, and also because the statistical significance of an association is a function not only of the magnitude of the association but also of other factors (*e.g.*, sample size, and sample variability) which are irrelevant to bias due to confounding. Moreover, the use of significance testing in the selection of confounders treats false negative errors (*i.e.* the deleting a confounder) as secondary to false positive error (inclusion of a non-confounder). This implicit ranking of error is backward, as deleting a confounder introduces bias and is justified only if the bias induced by its exclusion is tolerably small or deemed to be worth the precision gain, whereas including a non-confounder only (potentially) reduces precision by increasing the size of the model. This is a small consideration in this study, and different approaches are not likely to qualitatively change the study findings or conclusions. Despite the confounder evaluation conducted by the investigators, residual confounding due to mismeasured or unmeasured confounders is likely to have somewhat biased the observed associations.

To preserve the sample size the authors appropriately imputed or replace data for missing covariates. Data on maternal depression were missing for 5% of observations and maternal intelligence scores for less than 1%. Values of maternal depression measured when the child was 1 year old were available and substituted for 13 instances where maternal depression when the child was 3.5 years was missing. Other missing covariate values (six instances for maternal depression and three for maternal PPVT) were imputed by simple random selection of a value from participants with non-missing values. The resulting analysis is relatively more precise, owing to the larger sample size analyzed, but this statistical efficiency comes at the tradeoff of bias due to residual confounding by imperfectly measured covariates. Socioeconomic conditions and other difficult to measure determinants of early life development may further confound the

reported associations in the direction of more deleterious effects associated with the organophosphate metabolites and with unknown magnitude. The potential for this bias may be mitigated somewhat due to restriction in the variability of these confounding factors in this relatively homogeneous study population.

The generalizability of the findings to other populations may be limited, due to the particular nature of this study population with respect to factors which may modify the association between organophosphate exposures and infant development. The organophosphate pesticide exposure markers and the prevalence of attention deficits in this low-income Mexican-American population sampled from an agricultural area are almost certainly not generalizable to the U.S. population, although exposure levels are within a comparable range to the U.S. general population (see Section 5.0).

The study has numerous strengths, most notably the prospective design and the longitudinal assessment of both exposure and outcomes. The positive association between organophosphate metabolites in the urine of pregnant women and increased odds of attention problems and poorer attention scores in their offspring was relatively consistent using different assessments of attentional difficulties (*i.e.* by maternal report, psychometrician observation, and neuropsychological testing). Associations were stronger at age 5 years compared to age 3.5 years, and there was evidence of a stronger association among boys, compared to girls. The results of this investigation by Marks et al in the CHAMACOS cohort are suggestive of a detrimental association between prenatal organophosphate exposure, as measured by maternal urinary metabolite levels, and attentional difficulties at age 5 years. This study may be limited in its direct contribution to the literature base regarding the specific association between chlorpyrifos and developmental outcomes because the assessment of non-specific organophosphate pesticide metabolites (DAPs) is a primary potential source of exposure measurement error. Because of the prospective design, information bias is expected to result in attenuation of the true (unobserved) associations, toward (observed) associations that are relatively smaller, or null.

Article 13. Rauh et al. (2011)

Seven-Year Neurodevelopmental Scores and Prenatal Exposure to Chlorpyrifos, a Common Agricultural Pesticide. Virginia Rauh, Srikesh Arunajadai, Megan Horton, Frederica Perera, Lori Hoepner, Dana B. Barr, and Robin Whyatt. Environmental Health Perspectives. 119:1196–1201 (2011).

Study Summary

Having previously reported an association between prenatal exposure to chlorpyrifos and neurodevelopmental problems at three years of age in the Columbia Center for Children's Environmental Health, Rauh et al now evaluate the relationship between prenatal chlorpyrifos exposure and neurodevelopment among cohort children at age seven.

Study participants were recruited during early pregnancy (≤20th week) among African-American and Dominican women age 18-35 years, and registered Hospital, in New York at New York Presbyterian Medical Center and Harlem City. Women who smoked, had a history of drug abuse, diabetes, hypertension, or HIV infection were excluded from participation in the study, as were women who resided in New York City for less than 1 year. The study sample presented in this report was recruited between 1998 and 2002, a period which straddles the January, 2001 implementation of a voluntary cancellation by registrants of indoor residential chlorpyrifos use. Of originally 725 consenting women, 535 were active participants in the ongoing cohort study at the time of this report. This report uses data from 265 of their children 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.

Chlorpyrifos levels in umbilical cord blood samples were available for 256 newborns, sampled as close to the time of delivery as possible, and within 2 days post-partum. In cases where the umbilical cord blood sample was not collected (12% of subjects), cord blood chlorpyrifos levels were imputed from mothers' values. Quantification of chlorpyrifos levels in plasma were conducted by the Centers for Disease Control and Prevention (CDC).

Neurodevelopment at age 7 years was assessed using 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. These indices are associated with, but distinct from, overall intelligence quotient (IQ) and are sensitive to cognitive deficits related to learning and working memory, which have been linked to chlorpyrifos exposure in rodent studies. A Full-Scale IQ score combines the four composite indices. A General Ability Index score is a summary score of general intelligence, similar to Full-Scale IQ, but excludes contributions from both Working Memory Index and Processing Speed Index.

Multivariate linear regression models were used to estimate associations. Chlorpyrifos exposure level (picograms per gram) was entered as a continuous variable. The WISC-IV Composite Index scores were natural log (ln) transformed to stabilize their variances and to improve the linear model fit, based on regression diagnostics. Linear and non-linear functional relationships

between chlorpyrifos exposure and each of the log-transformed WISC-IV indices were assessed and compared.

Participating mothers were predominantly from low income households, with 31% failing to complete high school by child's age 7 years, and 66% were never married. The children in the study were largely full term and included very few low-birth-weight infants through study design. Chlorpyrifos exposure levels as measured in cord blood ranged from non-detectable to 63 pg/g.

Unadjusted correlations showed significant inverse associations between chlorpyrifos levels and Working Memory (r=-0.21, p=<0.0001); and Full-scale IQ (r=-0.13, p=0.02). Because cord lead levels were not significantly correlated with chlorpyrifos levels, and in an effort to avoid excluding those with missing lead levels, lead was not included in the regression models. ETS was significantly correlated with chlorpyrifos levels (Spearman coefficient 0.113, p=0.01) but not with any of the WISC-IV indices. In addition, birth weight was not significantly associated with any of the WISC scales and not included in the final models.

The investigators used both fully-adjusted linear regression modeling and the Least Absolute Shrinkage and Selection Operator (LASSO) technique, described as a more parsimonious selection of covariates that addresses unstable estimates while avoiding overfitting (Houwelingen 2001; Tibshirani, 1996.) Both methods yielded very similar estimates, finding associations between chlorpyrifos exposure and Full-Scale IQ as well as the Working Memory Index. For Full-Scale IQ both techniques generated a coefficient (B) of -0.003 with matching CIs of -0.006, 0.001 but the P-values were calculated as 0.064 for the LASSO and 0.048 for the fully-adjusted model. The fact that both CI's contain zero while the fully-adjusted model's P-value is lower, suggests that the LASSO technique does in fact shrink unstable estimates towards zero. For the Working Memory Index, both techniques generated a coefficient (B) of -0.006 with the LASSO calculating a CI of -0.009 to -0.002, p<0.001 and the fully-adjusted model calculating a CI of -0.010 to -0.002, p=0.003. 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. In order to understand the magnitude of these effects, the investigators calculated the neurodevelopmental deficit associated with the increase in exposure and 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% reduction in Working Memory. Authors explain that their full model results did not show any significant interactions between ETS or polycyclic aromatic hydrocarbons (PAH) with measures of prenatal chlorpyrifos exposure during the prenatal period and intelligence measures in this study.

The investigators conducted a sensitivity analysis to determine if the association between chlorpyrifos levels and Working Memory Index was partially due to the effect on general intelligence. To do this, they looked at the General Ability Index of the WISC-IV, a general intelligence scale calculated without the inclusion of the items included in the Working Memory Index or Processing Speed Index. When the General Ability Index was added to the linear regression model, the estimated chlorpyrifos effect did not change and they did not observe an interaction between chlorpyrifos and General Ability Index. From this they concluded that the

Working Memory Index is a targeted measure, independent of general intelligence.

The investigators further explored their findings by conducting a supplemental analysis to evaluate whether the observed associations between chlorpyrifos and the Working Memory Index were related to underlying behavioral problems. They used the results of the Child Behavior Checklist and its clinically-oriented behavioral scales and concluded that there was no evidence of mediation caused by behavioral issues.

Lastly the investigators addressed the possible influence of their method and frequency of use of LOD imputations. To do this they recalculated all of the association estimates using only those records that had detectable chlorpyrifos levels and explained that this method would provide an unbiased estimate of the association (Little, 1992.) They did not find any consistent differences in estimates between the full sample (including imputed exposure levels) versus those calculated without those imputed levels.

Study Review

This was a very well conducted study with numerous strengths including selection of a relevant and appropriate cohort, the prospective study design, direct measurement of chlorpyrifos, comprehensive consideration of confounding, and analytic design choices which maximize statistical efficiency. However, some limitations are also noted.

The direct measurement of chlorpyrifos in cord blood and personal air samples, rather than self-reported indirect exposure assessment or use of biomarkers of organophosphate pesticide exposure that are not specific to chlorpyrifos exposure is a notable strength of the Columbia Mother's and Newborn Study cohort. Unlike previous analyses among this cohort that used a dichotomous variable (low vs. high exposure) to classify exposure status, these findings employ a continuous chlorpyrifos level, which provides a more meaningful look at the data including the ability to assess potential threshold effects and linear and non-linear dose-response trends. The investigators rigorously evaluated their methods for imputing values for undetectable chlorpyrifos levels; analyses illustrated imputation methods were valid. However, regarding the LOD estimations, the authors did not describe the methodology used (*e.g.* bootstrap estimation), if any, to incorporate the added uncertainty (*i.e.* added imprecision to estimates) inherent in the use of imputed data into the estimation of the corresponding variance estimates. However, errors in estimation of cord blood chlorpyrifos levels were likely to have been non-differential with respect to the indicators of childhood neurodevelopment and intelligence.

A limitation of the specific biomarker exposure indicators in this study is the single sampling period (at delivery). It is not clear to what extent this single measurement reflects exposures over critical windows during pregnancy. If the exposure is chronic, a biomarker measured at a single time point can provide a representative dosimeter, even if the toxicant has a short half-life, as is the case for chlorpyrifos. However, if pesticide exposures are sporadic or otherwise vary over short time scales, the biomarker measurement will not be representative of "usual" exposure, or of the exposure during critical periods of fetal neurodevelopment. In previous publications from this same cohort, the investigators explain that analyses were performed showing that, among these women, exposures to chlorpyrifos did not show significant variation throughout pregnancy

(Whyatt et al, 2007) and therefore concluded that this single measure can accurately represent prenatal exposure. This assumption is further assessed in subsequent validation studies conducted by Whyatt et al (2007 and 2009) within this cohort.

Whether or not these cord blood measurements accurately represent prenatal exposure, 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. From this study, it is unclear if an increase or decrease in exposure during this influential postnatal period, caused by age-related or seasonal dietary changes or changes in pesticide application, could also impact the neurodevelopment of these children. Because this imprecise measure of exposure is unlikely to vary differentially, this error would bias towards the null.

Another influence unaccounted in the analysis is the effect of increased awareness of the risks of pesticide exposures over the time period of cohort follow-up in this study (1998-2004) and the potential for differential exposure or outcome (CBCL) measures. It is feasible to assume that those parents of children with suspected or diagnosed neurodevelopmental issues in early childhood would be more aware of issues concerning organophosphate exposure, and thus more likely to subsequently reduce their child's exposures, especially through a reduction in pesticide or dietary sources in childhood. If this is true, and post-natal exposures play a role in childhood neurodevelopment measured in this study, those children with neurodevelopmental deficits may be less likely to experience post-natal exposures than those not diagnosed, thus making the pre-natal effects appear weaker. Similarly, mothers of infants in the high exposure category may differentially report behavioral issues on the maternal-reported child behavior checklist . Either type of differential measurement error may lead to information bias, with unknown consequences on the estimate of risk in these studies. It would be meaningful to assess the mother's awareness of the effects of organophosphate exposures as well as details regarding dietary exposures and how they may have changed over the 7-year period.

The prospective nature of the study limits the influence of errors in the exposure assessment on the association observed, such that mismeasurement of chlorpyrifos exposure would likely be non-differential with respect to the indicators of neurodevelopment and intelligence at age 7 years. The voluntary cancellation of the residential use of chlorpyrifos introduces exogenous variation in the exposure of interest. After the cancellation, levels of chlorpyrifos in personal and indoor air samples in this cohort decreased by more than 65%, and plasma blood levels dropped by more than 80%.

The Wechsler Intelligence Scale for Children (WISC-IV) instrument measures four areas of mental functioning: the Verbal Comprehension Index is a measure of verbal concept formation, a good predictor of school readiness; the Perceptual Reasoning Index measures nonverbal and fluid reasoning; the Working Memory Index assesses children's ability to memorize new information, hold it in short-term memory, concentrate, and manipulate information; and the Processing Speed Index assesses ability to focus attention and quickly scan, discriminate, and sequentially order visual information. The authors explain that the sensitivity of the WISC-IV among 6 to 7.5 year old children has been validated in previous neurodevelopmental studies of environmental lead exposure, even at low-dose levels (Chiodo et al. 2004). However, WISC-IV scores are also known to be influenced by socioeconomic background and/or child behavior

problems, particularly those related to anxiety (Wechsler, 2003). The narrowly restricted cohort and evaluation of behavioral issues, which may in part reflect anxiety issues, within this study somewhat limits this potential bias of the outcome assessment tool in this study.

One potential weakness is that the investigators do not give the details of the administration of the WISC-IV; specifically the credentials of the interviewers, whether or not they were blinded to the child's exposure status, any trainings or certifications, and whether or not intra- or inter-interviewer reliability or quality assurance was conducted. These types of administration issues can result in less-sensitive measures and misclassification, but there is no reason to believe that there would be a differential effect based on exposure level.

Also complicating this analysis is the pervasive, non-specific nature of neurological effects and the difficulty in attributing causal pathways to one agent. In future studies it would be meaningful to include other potential co-variants such as family history (especially among parents and siblings) of developmental delay or attention disorders as well as other co-morbidities. Not only is the etiology of attention and related behavioral disorders not well understood but also the diagnosis of these disorders is often influenced by complicated outside forces. Because the study population is relatively homogenous with respect to socio-economic variables, it is unlikely that differences in co-morbidities or family history would be associated with exposure status, thus biasing the association towards the null.

The authors describe an elegant and methodologically sound statistical analysis, addressing many of the potential shortcomings of their exposure data and co-variables. Out of the five WISC-IV scores, two were described as being significantly influenced by chlorpyrifos exposure. After they conducted both full-adjusted linear regression and LASSO techniques, they concluded that both Full Scale IQ and Working Memory index were affected by prenatal chlorpyrifos exposure. For each standard deviation increase in exposure (4.61pg/g), there was a 1.4% reduction in Full-Scale IQ and a 2.8% reduction in Working Memory. The Working Memory Index conclusions seem reasonable, but the associations for Full Scale IQ were not statistically significant (LASSO: -0.006, 0.001, p=0.064; fully-adjusted: -0.006, 0.001, p=0.048.) In addition, the authors do not address the clinical relevance of the 1.4% and 2.8% reductions and how this may or may not influence a child or his/her psychological or educational success.

In their confirmatory analyses they used the General Ability Index (the intelligence score calculated after removing Working Memory and Processing Speed items) to verify that the association seen between chlorpyrifos exposure and Working Memory was not an artifact of the association with Full Scale IQ. With the General Ability Index included in the linear regression model, the estimated chlorpyrifos effect did not change and they did not observe an interaction between chlorpyrifos and General Ability Index. Subsequently they concluded that the deficits in Working Memory Index were associated with chlorpyrifos exposure, independent of general intelligence but analyses could have also taken an additional step. These results could also be used to conclude that since the General Ability Index was not found to be affected by chlorpyrifos exposure, the Full Scale IQ findings were caused by the influence of the Working Memory module of the instrument. The influence of chlorpyrifos exposure on the working memory aspect of the Full Scale IQ score was strong enough to overcome the non-significant results from the other modules of the instrument.

Confounding by maternal smoking behaviors was controlled by restriction (*i.e.*, excluding smokers from the study sample). Confounding by unmeasured factors may have been similarly controlled by design, this being a relatively homogeneous population with respect to determinants of neurodevelopment, compared to the general population. Authors evaluated several potentially confounding variables including exposure to other environmental chemicals including blood lead, and only included those variables which displayed evidence of confounding.

While controlling confounders by restriction, the choice to study low income, urban dwellers likely resulted in a relative increase in statistical efficiency (*i.e.* increased power) because this population likely experiences a greater variability of pesticide exposure and is at higher risk of neurodevelopmental deficits, relative to the general population.

Although blood lead levels were not included as covariates in the multivariate models, the authors reported that umbilical cord lead was not significantly correlated with chlorpyrifos level or WISC-IV scores among the subgroup of children in this population for whom blood lead was assessed (n=89). Polyaromatic hydrocarbons were weakly correlated with chlorpyrifos in this cohort but were not significantly associated with WISC-IV scores. Birth weight was not significantly associated with any of the WISC-IV indices and was not included in the final models.

Bias due to confounding by unmeasured determinants of neurodevelopment is possible in this observational study, for example, confounding by factors related to socioeconomic condition. The urban dwelling, minority children assessed in this study may experience other unmeasured exposures or underlying health problems that could potentially confound (or modify) associations with pesticide exposure. However, both adjustment through restriction of study population, as well as control for several measures of SES in multi-variable models, as well as additional methodological analyses of the role of SES in this association (Lovasi et al. 2011), does not suggest a significant additional role of unmeasured confounding due to variation in SES within this study population. Effect modification by all of the covariates was assessed, including for environmental tobacco smoke and polycyclic aromatic hydrocarbons, but no interactions were observed.

The authors state that the retention rate for the full cohort was 82% at the 7-year follow-up, and reported that there were no significant sociodemographic differences between subjects retained in the study and those lost to follow-up, although these data were not provided. However, the 265 children included in this analysis were born to a 36.6% subsample of the 725 women who originally consented to participate in the study. The resulting large proportion of missing data has the potential to induce a selection bias if the reasons for missingness of outcome data are determined by exposure (or a predictor of exposure) and are also not independent of neurodevelopment. For example, participants with high pesticide exposures could have missed neurodevelopmental assessments for reasons related to uncontrolled determinants of exposure which are also related to neurodevelopment. This was not discussed.

A comprehensive set of sensitivity analyses and regression diagnostics were conducted,

including examination of residuals for normality and homoscedasticity, influential observations, and the linearity of the exposure-response relationship, and assessment of the influence of design choices (*e.g.* imputation) on observed results. Models were determined to be consistent with modeling assumptions.

Non-linearity of the exposure response curves were assessed by superimposing smoothed cubic spline regression curves over scatterplots of the data. The log-transformed Working Memory Index and Full-Scale IQ were approximately linear, whereas the other functions demonstrated some curvature across exposure levels, with sparse observations at the highest exposures. However, formal goodness of fit testing did not demonstrate that non-linear models provided a better fit to the data, compared to models that assume a linear exposure-response relationship (*i.e.* no observed threshold effect).

Results of this study showed that higher prenatal chlorpyrifos exposure, as measured in umbilical cord blood plasma, was associated with decreases in cognitive functioning on two different WISC-IV indices, in this cohort of urban minority children at 7 years of age. For each SD increase in exposure (4.61 pg/g), Full-Scale IQ declined, on average, by 1.4% (0.94–1.8 points) and Working Memory Index scores declined by 2.8% (1.6–3.7 points). These results are consistent with earlier findings of an inverse association between chlorpyrifos levels in umbilical cord plasma and neurodevelopment in the same cohort. The study represents perhaps the most rigorous study design and analysis of the studies reviewed.

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Article 14. Bouchard et al. (2011)

Prenatal Exposure to Organophosphate Pesticides and IQ in 7-Year-Old Children. Maryse F. Bouchard, Jonathan Chevrier, Kim G. Harley, Katherine Kogut, Michelle Vedar, Norma Calderon, Celina Trujillo, Caroline Johnson, Asa Bradman, Dana Boyd Barr, Brenda Eskenazi. Environmental Health Perspectives. 119:1189–1195 (2011)

Study Summary

Researchers with the Center for the Health Assessment of Mothers and Children of Salinas study (CHAMACOS study) published an analysis which investigated the relationship between prenatal and early life exposures to organophosphate pesticides (OPs) and cognitive development. The CHAMACOS study is a prospective birth cohort study of environmental exposures and the health of pregnant women and their children living in the Salinas Valley, an agricultural area of California.

A 2007 report from this cohort suggested that prenatal, but not postnatal, exposure to OPs was associated with increased odds of pervasive developmental disorder and lower scores of mental development at two years (Eskenazi B 2007). Additionally, a 2010 paper reported an association between organophosphate pesticide exposure and poorer attention skills as well as hyperactive behaviors at 5 years (Marks AR 2010). This 2011 study by Bouchard et al was conducted to determine whether cognitive deficits associated with prenatal exposure to organophosphate pesticides are persistent, since previous cohort studies have not followed children to school-age, when cognitive deficits may have greater implications for school performance. This paper reports the association between prenatal and postnatal exposure to organophosphate pesticides, indicated by urinary dialkyl phosphate (DAP) metabolite concentrations, and cognitive abilities of 7-year olds.

Enrollment took place from 1999–2000 at community clinics, and included women who were ≥ 18 years old, pregnant at < 20 weeks gestation, Spanish- or English-speaking, eligible for low-income health insurance, and planning to deliver at the local public hospital. The initial 601 women who were enrolled into the study delivered 526 infants. From this analysis, 2 participants with missing DAP concentration measurements were excluded, 4 children with a medical condition that would affect the assessment, children who were lost to follow-up or who did not participant in the 7-year visit were excluded (72 moved, 59 refused, 24 unable to trace, 21 unable to schedule, 2 deceased), and children missing the cognitive assessment at the 7-year visit (13) were also excluded.

Prenatal urine was collected between 5 and 27 weeks gestation and again between 18 and 39 weeks. Urine was collected from the children at 6 months and 1, 2, 3 ½ and 5 years of age. Six nonspecific organophosphate DAP metabolites (3 DEP metabolites and 3 DMP metabolites) were measured in maternal and child urine which represent the breakdown products of about 80% of the total organophosphate pesticides used in the Salinas Valley (CDC 2009). The most commonly used organophosphate pesticides in the Salinas Valley include chlorpyrifos and diazinon (which are metabolized to DEP) as well as malathion and oxydemeton-methyl (which devolve to DMP). Concentrations below the limit of detection (LOD) were randomly imputed based on a log-normal probability distribution whose parameters were estimated using maximum

likelihood estimation. The DAP metabolite concentrations were expressed on a molar basis and summed to yield total DEP, DMP, and DAP concentrations.

Cognitive abilities were measured at the 7-year study visit using the Wechsler Intelligence Scale for Children - Fourth Edition (WISC-IV) (Wechsler 2003). Scores for four domains were calculated based on the following subtests: Verbal Comprehension (comprised of the Vocabulary and Similarities subtests), Perceptual Reasoning (Block Design and Matrix Reasoning subtests), Working Memory (Digit Span and Letter-Number Sequencing subtests), and Processing Speed (Coding and Symbol Search subtests). All subtests were administered in the dominant language of the child, which was determined through administration of the Oral Vocabulary subtest of the Woodcock-Johnson/Woodcock-Muñoz Tests of Cognitive Ability in both English and Spanish (Woodcock and Johnson 1990). Ultimately, 67% of children were tested in Spanish, and 33% in English. WISC-IV scores are standardized against U.S. population-based norms for English and Spanish-speaking children.

Many confounders were measured, including intelligence of the mother, 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. The following known or suspected neurotoxicants were measured: polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), p,p'-dichlorodiphenyltrichlorethane (DDT), p,p'-dichlorodiphenyltrichlorethylene (DDE), and lead. Lead was measured in maternal blood at 26 weeks gestation, in cord blood for a subset of participants, and children's blood a 2 years of age. PBDEs, PCBs, DDT, and DDE were measured in maternal serum samples collected at 26 weeks gestation.

Nonspecific total DAP, DEP, and DMP metabolites (nmol/L) were log10-transformed. The authors examined the association between non-creatinine adjusted urinary DAP concentrations and cognitive scores using multiple linear regression, with point estimates representing the change in cognitive scores for each 10-fold increase in DAP concentrations. For prenatal exposure, they examined associations with the DAP concentrations measured separately for urine collected during the 1st and 2nd half of pregnancy (≤20 vs. >20 weeks gestation). However, since they found similar relations between cognitive scores and DAPs measured earlier and later than 20 weeks gestation, they averaged the two DAP measures for further analyses. For 20 children (6%) only one prenatal measure was available for the analyses. Because DMP and DEP metabolites might have different relationships to the outcomes, the authors examined them separately. For postnatal exposure, they examined the cross-sectional association of cognitive scores with DAP concentrations measured in children's urine collected at different ages in separate models.

They also calculated the cumulative DAP level between 6 and 60 months using the area under the curve (AUC), calculated using the trapezoidal method. For 46 children with 1 missing DAP measurement at 1, 2, or 3½ years, they imputed the mean of the 2 measures closest in time for the AUC calculation. Forty-nine children who were missing DAP measures at either the 6-month

or 5-year visit, or missing more than 1 DAP measure from the 3 other time points were excluded from the AUC analysis. For comparison with prenatal exposure, they calculated the mean urinary DAP concentrations measured during childhood for children with at least 3 out of 5 measures (taken at 6 months, 1, 2, 3 ½, and 5 years); this excluded 20 children.

The authors compared effect estimates for urinary DAPs measured in early vs. late pregnancy and in the prenatal vs. postnatal periods using seemingly unrelated estimation and they used the mean postnatal DAP concentrations (as opposed to AUC) for these analyses in order to compare metrics with similar units. They used generalized additive models with 3-degree-of-freedom cubic splines to evaluate the shape of dose-response curves, test the linearity assumption, and investigate potential thresholds while controlling for covariates. They did not observe evidence of departure from linearity, or threshold for effect, thus, they retained the simpler models based on linear regression. For illustration purposes, they grouped DAP concentrations into quintiles, entered this categorical variable in the multiple regression model with the same covariates described above, and obtained the mean IQ score for each quintile.

The authors explored potential confounding effects for the following variables: maternal intellectual abilities, maternal education, HOME score at 6 months, 1, 2, 3 ½, and 5 years, breastfeeding duration, maternal age, birth order, poverty category, marital status, children's age at WISC testing, and maternal levels of PBDEs, PCBs, DDE, DDT, and lead during pregnancy. They added each of these variables added individually to the final model. However only maternal intellectual abilities (PPVT score, continuous), maternal education (3 categories), and continuous HOME score at 6 months were retained in the final models. Other variables were dropped from the final models because they did not change the magnitude of the coefficient for urinary DAP concentrations by >10%.

In separate analyses, the authors also investigated potential confounding and effect modification by variables possibly on the causal pathway (*i.e.* birth weight and gestational age, assessed continuously). Because the majority of children (67%) were tested in Spanish, the authors reran the analyses restricted to this subset. The authors also examined the interaction between sex and DAP concentrations, based on previous findings in this cohort (Marks AR, 2010).

To explore possible synergistic effects between pre- and postnatal DAP concentrations, the authors included an interaction term for mean prenatal DAP concentrations×AUC. However, since this term was not statistically significant ($p > .15$) they did not include it in the final models.

The authors observed evidence of an association between prenatal exposures to organophosphate pesticides as measured by urinary DAP metabolites in women during pregnancy, and decreased cognitive functioning in children at age 7. This finding was seen for total DAP, DMP, and DEP metabolites. Children in the highest quintile had of maternal DAP concentrations had an average deficit of 7.0 IQ points compared with those in the lowest quintile. The associations were linear with no threshold. Urinary DAP concentrations in childhood were not associated with cognitive scores in this cohort of children.

Study Review

This study tested whether *in utero* or postnatal exposures to organophosphate pesticides have a detrimental impact on cognitive abilities at age seven years. The study population was appropriate to the hypothesis, given that there were a range of exposure levels to OPs and there was some homogeneity in the population, *e.g.*, mostly low SES, Hispanic, agricultural workers. The homogeneity of the population restricted confounding by design. For example, another paper reported that maternal smoking, alcohol use, and illicit drug use were rare this cohort. The recruitment of an inner-city cohort may also have increased the relative statistical efficiency of the study if the study population is at higher risk of neurodevelopmental deficits compared to the general population. Selection bias may have been a factor in this analysis as only 329 families out of 601 who were originally enrolled into the study were included. The authors state that the families included in this analysis did not differ on most attributes, including urinary DAP concentrations during pregnancy, maternal measures of cognitive ability, maternal education, marital status, poverty category, and child's birth weight. However, mothers of children included in the present study were slightly older and breastfed longer than the initial cohort. Differences between maternal blood lead and other environmental exposures which are potential confounders were not discussed.

The ascertainment and analysis of urinary organophosphate pesticide markers was appropriate. Quantification was conducted at the CDC, using published methods. DAP metabolites are likely more accurate and objective indicators of organophosphate pesticide exposure, relative to other ascertainment methods such as self-report. Although they are sensitive indicators of exposure, it is difficult to infer chlorpyrifos effects specifically from the metabolite levels, as they also indicate exposure to other organophosphates. The study population likely sustained many other potentially relevant exposures to multiple chemicals and other pesticides which may affect development. The six organophosphate metabolites cannot be traced back to individual pesticides but, according to the authors, they represent the breakdown products of approximately 80% of the total organophosphate pesticides used in the Salinas Valley. It is not possible to disentangle the various effects from these potential co-exposures and chlorpyrifos in this study.

The IQ test used the WISC-IV is very widely utilized in both clinical and research setting, and has a long history of use, and was appropriately administered by psychometrician. Additionally, the test was administered at age 7 years, which is above the floor for administration (age 6) (Wechsler 2003). However, IQ tests are notoriously susceptible to possible biases due to differences in cultural and/or ethnic composition of the population, although the homogeneity of the population may ameliorate the effect of this potential bias. The authors did perform the analysis both by looking at only the tests that were administered in Spanish and with the Spanish and English included. However, language may not have controlled for all potential biases which are inherent in IQ testing, *e.g.*, degree of acculturation.

The statistical models used are appropriate and well powered. For exposure measures that were less than the laboratory analytic limit of detection, the measures were imputed. Imputation is a better method than substitution of the LOD/$\sqrt{2}$ or LOD/2, which is the method used in many studies. It appears that the investigators selected parsimonious models by only including terms which changed the outcome measure and were statistically significant. Additionally, the authors tested the linearity assumption which is inherent in linear regression using cubic splines.

Residual confounding by unmeasured or poorly measured confounders is possible in this study. The authors did measure a large number of potential confounders, but not all were evaluated notably, childhood exposure to neurotoxins, and maternal drug used during pregnancy. A number of environmental chemicals were considered, although only measurements of exposure during pregnancy were included in the analysis. Additionally, exposure to carbamates, which are also potentially neurotoxic, was not included in the analysis. The authors also did not consider maternal alcohol, tobacco, or drug use in this analysis. However, another study with this cohort reported that these confounders are very rare in this study population.

The authors did not discuss paternal intelligence or intelligence of other caregivers. This is probably because only the mothers and children were enrolled into the study, and it is difficult to enroll fathers and other caregivers into birth cohort studies. Although it is understandable that these measures were not addressed, it seems highly possible that intelligence of the fathers and other caregivers could be associated with the exposure, organophosphate pesticides, and the disease, cognitive impairment of the child. Thus these factors could have confounded the study results, and it is a limitation that they were not included in the measurement or analysis.

A potential limitation with respect to generalizability is the high level of exposures observed in the study cohort, compared to the US population. The mean level of urinary DAP metabolites in this cohort is higher than the mean which has been seen among women of child bearing years in NHANES. However, the investigators report that 25% of the US population have urinary DAP levels exceeding the median in this study. So, the concentrations in this cohort are not out of the range of normal for the US as a whole. Generalizing beyond the range of exposure observed in this study requires an assumption that the linear exposure-outcome relationship observed in the study is applicable to populations with different levels of exposure.

This study has many strengths, the longitudinal design, the measurement of urinary DAP at many 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. Additionally, the authors noted their findings were consistent with findings from previous reports from this cohort (Eskenazi, 2007) and other investigations of adverse associations between prenatal exposure to organophosphate pesticides and cognition (Rauh, 2006). They also noted that although they did not see an association between childhood exposures to organophosphate pesticides, the other studies that have differed in the measurement of exposure and/or the specific outcomes found to be associated with exposure to organophosphate pesticides (Lizardi, 2008, Ruckart, 2004). The many advantages of this study, the plausibility of the findings, and that similar association have been seen in other studies support the hypothesis that prenatal exposure to OPs reduces cognitive function of children. However, to the extent that prenatal chlorpyrifos is the true exposure of interest, the assessment of non-specific organophosphate pesticide metabolites is a primary potential source of exposure measurement error. Because of the prospective design, information bias is expected to result in attenuation of the true (unobserved) associations, toward (observed) associations that are relatively smaller, or null.

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Article 15. Whyatt et al. (2007)

Within- and Between-Home Variability in Indoor-Air Insecticide Levels during Pregnancy among an Inner-City Cohort from New York City. Robin M. Whyatt, Robin Garfinkel, Lori A. Hoepner, Darrell Holmes, Mejico Borjas, Megan K. Williams, Andria Reyes, Virginia Rauh, Frederica P. Perera, and David E. Camann. Environmental Health Perspectives. 115:383–389 (2007).

Study Summary

Whyatt et al conducted a study to assess within- and between-home variability in indoor-air insecticides over the final 2 months of pregnancy among a subset of participants in the Columbia Mother's and Newborn Study, a cohort of women from New York City. Participants (n = 102) assessed in this investigation were enrolled between February, 2001 and May, 2004, after the January, 2001 implementation of a voluntary cancellation by registrants of indoor residential chlorpyrifos use. Expectant mothers were recruited during early pregnancy ($\leq 20^{th}$ week) among African-American and Dominican women age 18-35 years, and registered at New York Presbyterian Medical Center and Harlem Hospital, in New York City. Women who smoked, had a history of drug abuse, diabetes, hypertension, or HIV infection were excluded from participation in the study, as were women who resided in New York City for less than 1 year or, for this validation study, who were employed outside the home at the time of enrollment. Most of the mothers (96%) in the present study lived in residential apartment buildings; the remaining lived in multifamily housing (n = 3) or in combined residential/commercial buildings (n = 1).

Nine insecticides (the organophosphates chlorpyrifos, diazinon, malathion, and methyl parathion; the carbamates propoxur, bendiocarb, and carbofuran; and the pyrethorids cis and trans-permethrin) and piperonyl butoxide, an adjuvant that is frequently added to pyrethroid formulations, were measured in 48-hr personal air samples and 2-week integrated indoor air samples collected sequentially for 7.0 ± 2.3 weeks (n = 337 air samples). Indoor air monitoring occurred around the 32nd week of pregnancy, and air samples were collected every 2 weeks throughout the monitoring period. Personal air samples were obtained for mothers over a 48 hour period during the 32nd week of pregnancy.

Arithmetic means ± SDs were calculated for pesticides detected in greater than 45% of samples. Before statistical analyses, pesticide values were log-transformed to normalize their distributions. Values below the level of detection (LOD) were assigned a value of half of the LOD. Pearson correlation coefficients or Spearman's correlation coefficients, as appropriate, were calculated to assess the relationship between pesticide levels in indoor air and personal air samples. Analysis of variance (ANOVA) was used to test whether pesticides levels varied significantly by season and year of monitoring and among the following groups: women not using any pest control methods; women using "nonspray" methods only; and women using higher-toxicity pesticides. If levels differed significantly among the groups, the least significant difference test was used to determine which groups varied significantly. T-tests were employed to compare differences in mean pesticide levels by ethnicity. Chi-square analyses were used to test whether the proportion of women using pest control measures over the final 2 months of pregnancy was related to pest sightings in the home. Finally, a mixed-effects model was used to assess within-home and between-home variability in repeat indoor air samples, after controlling for season and year of

delivery. Except for the Spearman's rank and chi-square analyses, all statistical testing was conducted on log-transformed data.

Sixty-one percent of the participating women reported using pest control during the air samplings. Chlorpyrifos, diazinon, and propoxur were detected in 99–100% of personal and indoor samples (range, 0.4–641 ng/m^3). Piperonyl butoxide (a pyrethroid adjuvant) was detected in 45.5–68.5% (0.2–608 ng/m^3). 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 ($r = 0.7–0.9$, $p < 0.001$). Diazinon and chlorpyrifos levels declined 5-fold between 2001 and 2004 but were detected in all homes at 1.5 and 2.5 years, respectively, after implementation of a voluntary cancellation by registrants of indoor residential chlorpyrifos use. No previously unreported associations between chlorpyriphos and health outcomes were presented in this report. The authors concluded that the insecticides were persistent in the home with little variability in air concentrations over the 2 months and likely contributed to chronic maternal inhalation exposures during pregnancy.

Study Review

Although the Columbia Mother's and Newborn Study enrolled participants in years prior to the implementation of the 2001 voluntary cancellation by registrants of indoor residential chlorpyrifos use, only data for participants recruited in the post-cancellation period were considered in this validation study analysis. Explanation for this design choice was not provided, but is likely that appropriate repeated sampling was not conducted during earlier study periods. The authors noted that the mothers in the present study were comparable with women in the larger cohort in terms of maternal age, ethnicity, marital status, annual household income, and reported use of pest control methods during pregnancy. However, a greater proportion of women in the present study compared with those in the larger cohort reported having less than a high school education, which could be due to the fact that, for these analyses, the investigators excluded women who worked outside the home.

The exposure assessment in this study was well conducted using appropriate and previously published methodology. The semivolatile insecticides were readily detected in indoor air after residential use. Indoor air levels of these chlorpyrifos and the other organophosphate pesticides in the 2-week integrated indoor air samples remained remarkably stable within most homes over 6–8 weeks of sequential sampling. The two-week integrated sampling methodology was targeted towards evaluation of chronic exposure during the latter pregnancy period, and thus precludes assessment of changes in concentrations in indoor air at times scales shorter than two-weeks (*e.g.* day-to-day changes).

In this study, pesticide levels in indoor air were remarkably stable. Correspondingly, almost all (90%) of the total variance in the indoor air levels was explained by between-home variability. The degree to which this finding in the post-cancellation period informs variability in airborne pesticide levels prior to the cancellation was beyond the scope of the investigation.

The authors did not observe an association between indoor air levels of chlorpyrifos and maternal self-reported pesticide use, a finding which indicates that most of the chlorpyrifos concentrations detected in the indoor air samples likely resulted from prior, rather than current, pesticide use. However, that chlorpyrifos was detected in indoor air samples from all the homes assessed, including those evaluated 2.5 years after implementation of the voluntary cancellation of indoor residential chlorpyrifos use, demonstrates the persistence of the compound in certain indoor environments.

No associations between pesticide exposures and health outcomes were assessed in this validation study.

Article 16. Whyatt et al. (2009)

A Biomarker Validation Study of Prenatal Chlorpyrifos Exposure within an Inner-City Cohort during Pregnancy. Robin M. Whyatt, Robin Garfinkel, Lori A. Hoepner, Howard Andrews, Darrell Holmes, Megan K. Williams, Andria Reyes, Diurka Diaz, Frederica P. Perera,1 David E. Camann, and Dana B. Barr. Environal Health Perspectives. 117:559–567 (2009).

Study Summary

Whyatt et al conducted a biomarker validation study within the Columbia Mother's and Newborn Study to evaluate trends over time in multiple biomarkers of prenatal chlorpyrifos exposure. The group previously documented significant decreases in chlorpyrifos concentrations in maternal personal and indoor air samples among pregnant African-American and Dominican women from New York City after the 2001 voluntary cancellation by registrants of indoor residential chlorpyrifos use. Participants (n = 102) were enrolled between February, 2001 and May, 2004. 3,5,6-trichloro-2-pyridinol (TCPy) was measured in newborn meconium (n = 83), repeat prenatal maternal urine samples (n = 253), and postnatal urine from mothers (n = 73) and newborns (n = 59). Chlorpyrifos was directly measured in postnatal maternal (n = 92) and umbilical cord (n = 65) blood plasma and in personal and indoor air samples. No previously unreported associations between chlorpyriphos and health outcomes were presented in this report.

Study participants were recruited during early pregnancy ($\leq 20^{th}$ week) among African-American and Dominican women age 18-35 years, and registered at New York Presbyterian Medical Center and Harlem Hospital, in New York City. Women who smoked, had a history of drug abuse, diabetes, hypertension, or HIV infection were excluded from participation in the study, as were women who resided in New York City for less than 1 year or who were employed outside the home at the time of enrollment (for the purposes of this validation study). The study sample presented in this report was recruited between 2001 and 2004, after the January, 2001 implementation of a voluntary cancellation by registrants of indoor residential chlorpyrifos use. Most of the mothers (96%) in the present study lived in residential apartment buildings; the remaining lived in multifamily housing (n = 3) or in combined residential/commercial buildings (n = 1).

Indoor air monitoring occurred around the 32nd week of pregnancy using 2-week integrated indoor air samples collected every 2 weeks throughout the monitoring period. Personal air samples were obtained for mothers over a 48 hour period during the 32nd week of pregnancy.

Repeat prenatal maternal urine samples were collected from mothers at the end of each 2-week indoor air sampling period, beginning at the 34th week of pregnancy and continuing until delivery. Women who went into labor before the end of the first 2 weeks of indoor air sampling did not provide urine samples. Urine samples were collected from 97 of 102 women in the study; more than half of the women provided three or more urine samples, 29 of 97 (30%) two samples, and 15 of 97 (15%) provided one sample. An additional urine sample was obtained on the day after delivery for 73 women and 59 newborns.

An umbilical cord blood sample was collected from 65 newborns after delivery. A sample of maternal blood was obtained from 92 mothers within 2 days postpartum. Paired maternal and cord blood samples were available for 64 of the mothers and newborns.

Meconium samples were collected from 83 newborns during the postpartum hospital stay. In 53 (64%) of the births, the meconium samples were collected within 1 day of delivery.

Biologic samples were analyzed for organophosphate pesticide markers by the CDC using previously established methods using both positive and negative controls. The limit of 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 $\log_{10}$ transformed. Nonparametric statistics were used when distributions remained non-Gaussian. Spearman rank correlation coefficients were used to assess relationships between levels of chlorpyrifos or TCPy in biological samples and to examine correlations between levels of chlorpyrifos or TCPy in the biological samples and chlorpyrifos levels in air samples. Multiple linear regression was employed to evaluate the amount of variance in prenatal maternal urine TCPy levels explained by indoor air chlorpyrifos levels among subjects enrolled in 2001–2002.

Analysis of variance (ANOVA) or Kruskal–Wallis tests with post-hoc testing as appropriate, were used to assess whether pesticide levels varied by year of birth (between 2001 and 2004), or among the following groups: women not using any pest control methods; women using "nonspray" pest abatement methods only; and women using can sprays, pest bombs, or sprays by an exterminator, with or without nonspray methods. Authors performed t-tests corresponding nonparametric Mann–Whitney U-test were used to assess whether pesticide levels differed by ethnicity. Chi-square analyses were used to test whether the frequencies of detection of chlorpyrifos and TCPy in biological samples varied by year of assessment as well as to compare detection frequencies of chlorpyrifos in maternal and cord blood samples. A mixed effects model was used to evaluate the within- and between-subject variability in TCPy levels in the repeat maternal urine samples collected during pregnancy, and to calculate an intraclass correlation coefficient (ICC) for women enrolled in 2001–2002 who had three repeat urine samples (TCPy was detected in only one-third of samples among subjects enrolled after 2002). The ICC is a measure of reliability of repeated measures over time, defined as the ratio of between-subject variance to total variance.

The authors did not detect TCPy in infant urine, but all other biomarkers showed a highly significant decrease in detection frequencies ($\chi 2 = 7.8–34.0$, $p \leq 0.005$) and mean ranks ($p \leq 0.006$) among subjects enrolled in 2003–2004 compared with those enrolled in 2001–2002. Chlorpyrifos in maternal personal and indoor air declined 2- to 3-fold over the same period ($p < 0.05$). In 2001–2002 samples, TCPy levels in repeat prenatal urine samples were positively correlated ($r = 0.23–0.56$), although within-subject variability exceeded between-subject variability (intraclass correlation coefficient = 0.43); indoor air levels explained 19% of the variance in prenatal urine TCPy ($p = 0.001$). Meconium TCPy 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 ($r = 0.31$, $p < 0.01$).

Study Review

Although the Columbia Mother's and Newborn Study enrolled participants in years prior to the implementation of the 2001 voluntary cancellation by registrants of indoor residential chlorpyrifos use, only data for participants recruited in the post-cancellation period were considered in this analysis. Explanation for this design choice was not provided, but is likely that appropriate repeated sampling was not conducted during earlier study periods. The authors noted that the mothers in the present study were comparable with women in the larger cohort in terms of maternal age, ethnicity, marital status, annual household income, and reported use of pest control methods during pregnancy. However, a greater proportion of women in the present study compared with those in the larger cohort reported having less than a high school education, which could be due to the fact that, for these analyses, the investigators excluded women who worked outside the home.

The investigators' use of nonparametric, rank-based statistics is appropriate for analysis of non-normally distributed data. However, the large number of ties among marker levels due to observations below the level of detection receiving equal rank, may be problematic. When ranked values have the same numeric number (*e.g.*, half the level of detection), the rank is 0.5 times the number of ties. Whether, and at what point a large proportion of ties begins to limit the inferential power of rank based statistics is unclear, although at some point, a non-continuous classification of the data (*e.g.*, classification of observations into bins at a cut-point equal to the level of detection) seems to make more sense if the number of below the level of detection observations is large.

The exposure assessment conducted in this investigation is the most comprehensive of any epidemiologic study of chlorpyrifos to date. Strengths of the exposure assessment include the quantification of actual blood chlorpyrifos levels in addition to sensitive, but non-specific markers of organophosphate pesticide exposure in urine, the repeated sampling of urinary markers, measures in meconium and the environmental sampling. The exposure assessments were appropriately timed with respect to critical window of gestation for fetal growth and neurodevelopment outcomes which likely occurs, in part, during the third trimester of pregnancy, and appear to have been well conducted. Although inhalation exposures are likely a major route of exposure, no direct dietary assessment was included in this investigation.

The study was set during a period of rapidly declining use of chlorpyrifos, and the findings are demonstrative of decreasing exposure over time. The investigators observed a highly significant decrease in the detection frequency and mean ranks of all biomarkers among subjects enrolled during years 2001–2004, with the exception of TCPy in newborn urine, which was not detected.

Maternal and umbilical cord blood plasma chlorpyrifos levels were below the LOD in all samples collected after 2002. The results suggest that chlorpyrifos from residential use was the main contributor to the chlorpyrifos levels detected in both the maternal and umbilical cord blood among study subjects in the larger cohort (1998-2004, both before and after voluntary

cancellation of chlorpyrifos containing compounds).

While no association was observed between maternal and umbilical blood chlorpyrifos levels measured at delivery and TCPy in maternal urine samples collected through the latter third trimester, TCPy levels in meconium (an integrative measure week 13-delivery) were significantly correlated with chlorpyrifos levels in both maternal and umbilical cord blood measured at delivery, and with TCPy levels in maternal urine samples collected in third trimester. These results suggest that TCPy in meconium may provide a valid biomarker of prenatal exposure, possibly both prior to and subsequent to the cancellation period.

The finding that within-subject variability exceeded between-subject variability in maternal urine TCPy (intraclass correlation coefficient, ICC= 0.43), again emphasizes the point that a single prenatal urine sample is likely insufficient to capture 'usual' exposure. The authors noted that despite the significant within-subject variability seen in their study, the ICC observed was higher than that observed in another population (Meeker et al., 2005).

The authors concluded that their results suggest that pesticide biomarkers utilized in these studies are reliable dosimeters to differentiate between groups with prenatal chlorpyrifos exposures varying by a factor of 2 or more, and that cord blood measures are reasonable well correlated with exposure measures taken throughout pregnancy (meconium correlated with cord blood (delivery) and maternal urine (3rd trimester). Authors also support that these data illustrate the efficacy of the voluntary cancellation of indoor residential chlorpyrifos use to reduce the internal dose during pregnancy.

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Article 17. Rauh et al. (2012)

Rauh, V. A., Perera, F. P., Horton, M. K., Whyatt, R. M., Bansal, R., Hao, X., . . . Peterson, B. S. (2012).
Brain anomalies in children exposed prenatally to a common organophosphate pesticide. Proc
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Study Summary
In this study, authors with the Columbia Center for Children's Environmental Health (CCCEH) birth cohort study evaluated whether there were areas of morphological change in the pediatric brain in regions of the brain known to be associated with learning, cognition and social behavior in association with prenatal chlorpyrifos (CPF) exposure (V. A. Rauh et al., 2012). These are functional neurodevelopmental outcomes that have been adversely associated with chlorpyrifos exposure in previous observational research (V. A. Rauh et al., 2006; V. Rauh et al., 2011), as well as some experimental studies, as noted by the authors. Specifically, in this study, authors evaluated 1) whether morphological changes in the brain including cortical thickness and differences in the cerebral brain surface area and volume were related to prenatal chlorpyrifos exposure (the main effect), and, 2) whether the relation between the exposure and morphological changes in the brain were modified by either full scale intelligence quotient (FSIQ) score (evidence of effect modification by IQ) and, 3) in preliminary analyses, whether changes in the normal sexual differentiation of the brain exist by exposure-level (V. A. Rauh et al., 2012). Authors concluded that while overall brain size did not differ by prenatal chlorpyrifos exposure, certain areas of the brain were enlarged among those more highly exposed to chlorpyrifos including superior temporal, posterior middle temporal, and inferior post-central gyri, and superior frontal gyrus, gyrus rectus, cuneus, and pre-cuneus in the mesial views of the right hemisphere of the cerebrum. Further, they noted a difference exposure-response relation between cerebral surface distances and CPF exposure, by FSIQ wherein the relation between brain area enlargements and FSIQ were positively correlated in the low CPF group (greater regional brain size correlated with greater IQ), but an inverse relation among the high CPF group. In supplemental analyses, authors illustrated that areas of regional brain enlargement among those more highly exposed to chlorpyrifos were due to underlying white matter enlargements. Researchers also noted sporadic differences in cortical thickness wherein higher CPF exposure was related to reduced cortical thickness. Preliminary analyses also displayed a disruption of normal male-larger-than-female areas as well as female-larger-than-male in certain areas of the brain known to differ by child sex in normal brain. Overall, researchers concluded that differences in brain structure (regional cerebral size and thickness) exist between CPF exposure groups, and the differences (high>low CPF) in regional brain size may be due to enlargement of underlying white matter. However, authors did not speculate as to the underlying biological explanation of the observed qualitative interaction between CPF exposure and FSIQ with morphological changes in key areas of the brain linked with learning, cognition and social behavior.
Design and Methods
Children selected for this MRI study (n=40) were participants in the larger Columbia Center for Children's Environmental Health (CCCEH) birth cohort study (n=369). The study was initiated in the late 1990's to evaluate the relation between air pollution and pesticide exposure and

neurodevelopmental effects in children. The cohort is comprised of a low income, inner-city population primarily of Dominican and African American ethnicity. Mothers were recruited among those who sought prenatal care by 20 weeks of pregnancy at one of the participating study hospitals. Participants were non-smoking women, ages 18-35, with no other co-morbidities including diabetes, hypertension, known HIV infection, and no documented drug use. Therefore, the cohort represents a population of women at low-risk for adverse neurodevelopmental outcomes among their children. Participants were residents of NYC for at least one year prior to enrollment in the research study. EPA's review of all the epidemiological studies from the CCCEH (the Columbia studies) is contained in a recent presentation to the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Science Advisory Panel (FIFRA SAP), April 2012 (FIFRA Scientific Advisory Panel, 2012).

Authors measured chlorpyrifos and other organophosphate pesticides, cotinine, lead and mercury in umbilical cord blood samples taken at delivery using CDC analytic methods with published quality assurance and control procedures. Prenatal environmental tobacco smoke (ETS) was measured as maternal self-report, and verified through cotinine levels (≤15 ng/mL). Polycyclic aromatic hydrocarbon (PAH), another environmental chemical possibly related to adverse neurodevelopmental outcomes, was measured through personal air monitoring in the third trimester of pregnancy. In the current (MRI) study, high chlorpyrifos exposure was defined as the upper tertile of the exposed group, ≥4.39 pg/g, and low chlorpyrifos exposure was defined as values below the upper tertile, <4.39 pg/g, including those not exposed to CPF.

To measure intelligence among school aged children, authors 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. These indices are associated with, but distinct from, overall intelligence quotient (IQ) and are sensitive to cognitive deficits related to learning and working memory. A Full-Scale IQ score combines the four composite indices. A General Ability Index score is a summary score of general intelligence, similar to Full-Scale IQ, but excludes contributions from both Working Memory Index and Processing Speed Index. WISC-IV scores are standardized against U.S. population-based norms for English and Spanish-speaking children.

MRI

This section provides some broad detail as to the method of MRI data collection employed in this research paper; this is not a critical review of methods. Health outcomes of interest in this study were morphological changes in the brain in regions of the pediatric brain that are related to learning, cognition and social behavior, as noted in the traditional epidemiology studies. Researchers performed brain imaging studies using Magnetic Resonance Imaging (MRI) technology among a sub-sample of the cohort, and measured and compared morphological characteristics by chlorpyrifos exposure status. Details concerning the MRI imaging procedures, processing and pre-processing image modifications, methods to measure cortical thickness and methods used to identify the "template" brain against which individual child participant brain images were compared are presented in the Supplemental Information.

Briefly, researchers used a high-resolution MRI, whole body scanner, with 8-channel head scanner for this study. Authors processed MRI images using biomedical imaging software (ANALYZE 8.0) while technicians were blinded to participant characteristics, and whether left or right hemisphere of the brain was imaged. Before morphological analyses were performed,

images in the dataset were re-sliced to correct for residual head rotation, tilt, and flexion/extension by participants during imaging. MRI images were pre-processed to correct for large-scale variation in color intensity. Extra-cerebral tissues captured in the MRI brain image were manually removed from the cerebral image before analyzing differences in brain morphology among participants by chlorpyrifos exposure; authors hypothesized difference in regional brain areas of the cerebrum only, other parts of the brain were therefore excised from the image before processing. Other similar modifications to the brain image were made to enhance measurement of key/pre-identified endpoints (cerebral surface morphology and cortex thickness) (See Supplemental Information). Variation in cortical grey matter was assigned by authors as four categories of color changes across images; authors selected color categories that adequately grouped the participants as to degree of cortical grey matter (*i.e.*, creation of four-level categorical variable to measure cortical thickness) and to allow for a robust statistical analysis, particularly considering the small study sample (n=40).

To measure morphological characteristics within cerebral areas associated with learning, cognition and social behavior, authors identified a "template" brain image against which all participant images were compared and measured. Using a two-step process, authors initially identified the individual in the study sample that most closely approximated average demographic features (child age, height and weight). Using this brain image, all other participant brain image studies were co-registered, *i.e.*, similar points on the cerebral surface were matched according to the International Consortium on Brain Imaging. Researchers then measured distances of correlated points on the cerebral surface between the "template" brain image and all participant images. Using these measured distances, authors then identified a final "template" brain based upon the brain image series which most closely matched the average distances among cortical surface measures. Authors advocated that use of one "template" brain as opposed to an amalgamated "average" brain is superior because of the enhanced clarity and more well defined tissue interface with which to make the measurements for the study. All sets of brain image studies were then re-calibrated to the final "template" brain image for statistical analyses.

Statistical Analyses

Regarding the statistical analyses employed in this paper, the analyses involved comparison of the Euclidean distances measured between correlated points on the cerebral surface or cortical thickness by chlorpyrifos (high/low) exposure level, adjusting for age and sex. Supplemental information reported that thousands of statistical tests were performed. Authors used generalized linear equations and linear regression modeling to estimate average differences in morphological characteristics by change in chlorpyrifos exposure level (high/low). Because authors performed multiple statistical tests, they utilized the False Discovery Rate (FDR) methods to adjust the p-value against Type I error. Authors tested the statistical significance of interaction terms of cerebral surface measures and FSIQ, and by child sex at $p<0.05$, adjusted for multiple testing.

Study Results & Authors' Interpretation

For this study, authors selected child participants who had complete measures for CPF, PAH, ETS, and who completed the WISC IV testing. There were 70 participants who met these criteria and who also had cord blood levels in the upper tertile of 4.39 pg/g (High CPF exposure), and among these 28 met the additional criteria of having low or no exposure to PAH and ETS. Twenty of these participants completed the MRI series (20 of 28 eligible participants enrolled in

study). Similarly, there were 99 participants who met the enrollment criteria (completed the environmental exposure and IQ testing battery) and were in the lower exposure group (<4.39 pg/g) and had no or low ETS and PAH. Among this group of low CPF exposed participants, 38 were randomly selected to participate in the MRI study, and 20 participants completed the series (20 of 38 eligible participants enrolled in study).

Authors reported no significant differences between those in the high and low chlorpyrifos exposure group, or among the larger cohort according the common socioeconomic indicators including maternal income and education, maternal IQ, race, gestational age and birth weight of child, and blood lead measure (See Table 1, main text). The correlation between chlorpyrifos and blood lead was low ($\rho<0.20$) and non-significant ($p>0.20$).

Authors concluded that while overall brain size did not differ by prenatal chlorpyrifos exposure, certain areas of the brain were enlarged among those more highly exposed to chlorpyrifos including superior temporal, posterior middle temporal, and inferior post-central gyri, and superior frontal gyrus, gyrus rectus, cuneus, and pre-cuneus in the mesial views of the right hemisphere of the cerebrum. Further, they noted interaction, or a different exposure response relation between cerebral surface distances and FSIQ, by CPF exposure wherein the relation between brain area enlargements and FSIQ were positively correlated in the low CPF group (greater regional brain size equated to greater IQ), but an inverse relation among the high CPF group (greater regional brain size related to lower FSIQ scores). Authors noted statistical interactions in the superior temporal, inferior frontal, inferior pre-central, and inferior post central gyri bilaterally, and the pre-cuneus of the left hemisphere.

In supplemental analyses, authors illustrated that areas of regional brain enlargement among those more highly exposed to chlorpyrifos were due to underlying white matter enlargements. This conclusion was supported by the observation of sporadic differences in cortical thickness wherein higher CPF exposure was related to reduced cortical thickness, or thinning of the cortex in certain areas. The qualitative interaction between CPF exposure and FSIQ with cerebral brain region enlargements may be explained by size of underlying white matter of the cerebrum, but authors did not speculate as to a biological explanation of the observed qualitative interaction. Preliminary analyses also displayed a disruption of normal male-larger-than-female areas as well as female-larger-than-male in certain areas of the brain known to differ by child sex in normal brain.

Overall, researchers conclude that differences in brain structure (regional cerebral size and thickness) exist between CPF exposure groups, and the differences (high>low CPF) in regional brain size is likely due to enlargement of underlying white matter. Authors also observed an inverse relation between regional brain enlargements and IQ among those highly exposed to CPF, and positive association between regional brain size and IQ among those in the low/no exposed group. While authors do not speculate as to a possibly biological explanation of this observed statistical interaction, EPA notes that given that total brain size did not differ by exposure group, the enlargement of the white matter in certain areas could have been at the decrement of the size, quality or other morphological changes in the grey matter in other areas; neurons in grey matter of cerebrum affect learning and cognition. Observed inward deformations (reduced brain area) in the dorsal and mesial surfaces of the left superior frontal gyrus as also

reported the authors suggest may also be part of the explanation of the qualitative interaction. However, the authors indicate that additional study is needed to elucidate these possibilities.

EPA Review

This study by Rauh et al. 2012 follows upon both previous epidemiological research and experimental toxicology studies that suggest an association between chlorpyrifos exposure in the pre-natal and early life periods with adverse neurodevelopmental outcomes observed in children through school age, including intelligence deficits. Within this CCCEH cohort, authors reported links between prenatal chlorpyrifos exposure (concentration of CPF in cord blood at delivery (pg/g)) with attentional difficulties, behavioral problems and measures of intelligence particularly working memory (V. A. Rauh et al., 2006; V. Rauh et al., 2011). EPA's review of these previous epidemiology studies from the CCCEH cohort were presented to the FIFRA SAP in April 2012 (FIFRA Scientific Advisory Panel, 2012). Regarding this study, EPA notes a limited and somewhat unbalanced depiction of the available rodent experimental data was presented in the paper. A brief review of references seems to provide some support for the authors' assertion that the epidemiological findings are consistent with those in the experimental literature; however, a fuller evaluation of the data concerning animal to human concordance is needed. For example, the authors' stated support for their finding regarding enlargements in underlying white matter is not strongly supported by existing experimental data concerning CPF induced glial scarring.

In this study, authors evaluated morphological characteristics of cortical brain regions which have been linked to disrupted learning, cognition and social behavior in previous epidemiologic and experimental studies. The cerebrum has been described as the "seat of intelligence" and provides the ability to read, write, and speak; to make calculations ,and to compose music; and to remember the past, to plan for the future, and to imagine things that have never existed before (Tortora & Grabowski, 2003). However, while the cerebrum's influence upon human intelligence is generally supported, the study lacked specific hypotheses regarding particular areas of the cerebrum affected (the largest of the four components of the brain). Therefore, as noted in supplemental information, many thousands of statistical tests were performed evaluating areas of the cerebrum that have been mapped. Authors appropriately employed methods to control for Type I error, however in many ways this research requires replication, as noted by the authors. Therefore, use of these data to inform specific underlying biological mechanisms or pathways of the potential toxic action of CPF is limited.

EPA is seeking external peer review of this specific aspect of the current study to verify that the MRI methods are sound and appropriate. Briefly, however, the main text and supplemental information states that technicians (MRI image readers) were blinded as to exposure status, as well as right or left hemisphere. This design feature adds to the validity and reliability of the study. However, EPA notes authors do not state whether multiple MRI image readers were employed in identifying and measuring cortical surface thickness and cerebral surface area, etc. of interest in this study, and whether quality control statistics such as the inter-rater reliability coefficient were calculated and considered. Supplemental information notes that validation studies were performed, however does not describe the aspects of methods that were validated. EPA particularly seeks input from experts in this area as to whether these methods and validation studies, if known, are sound.

Authors appropriately described the inclusion and exclusion criteria used in this MRI study. Authors excluded participants for who measureable levels of both PAH and ETS were reported in the total cohort study. In this way, by design, authors eliminated the chance of confounding by some other environmental chemicals known to effect neurodevelopment in the relation between chlorpyrifos and brain morphology. Authors also included only those participants who provided full information regarding prenatal chlorpyrifos exposure, other environmental chemical exposure and who completed the WISC intelligence testing. Given the relatively small sample size (n=40) for this relatively expensive imaging study these decisions eliminated the issue of missing data, and the need to add degrees of freedom to statistical models for control of other measured environmental chemicals. These decisions added to the validity and statistical power of the study. Although inclusion and exclusion criteria were somewhat rigorous in this study, authors still achieved a 60% participation rate among those eligible to participant (HIGH: 20/28, LOW: 20/38). Further, Table 1 indicates adequate comparability between the exposure groups and the total cohort, although those in the high exposed group were slightly albeit not statistically significantly more likely to be in families in which maternal education and income were slightly greater than the low exposure group. Therefore, in general, EPA believes that authors were able to identify an unbiased sample of cohort, representative of population sample of low income, inner city kids. However, given the rigorous enrollment criteria, the external validity of the study findings may be limited if the organophosphate pesticide exposure-brain morphology association is modified by factors that are more, or less, prevalent in the study populations relative to the population(s) to which inference is being made, or to populations with a substantially different exposure range.

In the larger cohort, authors measured concentration of chlorpyrifos parent compound in the cord blood of infants at delivery. Generally, biomarkers are considered superior measures than questionnaire based self report or environmental monitoring. The assays used in the study were highly sensitive allowing detection of very low levels of chlorpyrifos, in the pg/g range (CDC published methods). However, these measures are comparable to those in other samples of the general population, as noted by authors. Uncertainties remain however, as to the extent the use of one biomarker measurement reflects exposure(s) over critical windows of development during pregnancy, the temporality or periodicity of exposure, over the relevant time period of gestation. These cord blood measures have been used in several studies to estimate pre-natal exposure to chlorpyrifos, *i.e.*, during the entire period of gestation. Validations studies performed within the CCCEH cohort support that use of a one-time measure is an accurate and reliable measure with which to rank participants (high/low). In this study, authors categorized chlorpyrifos exposure as high (greater than or equal to the upper tertile of 4.39 pg/g) and low (all values below 4.39 pg/g including those values below the LOD) which exposure methodology can accurately and reliably support. PAH in the ambient air was measured using stationary air monitors and ETS was measured through maternal self-report, validated by cotinine level. A noted limitation in the measurement of pesticide exposure in these studies is the lack of measurement during the post-natal period through the time period of the epidemiologic evaluations, *i.e.*, school age. Chemical exposure in the post-natal period may confound or alter the relation between prenatal chlorpyrifos and neurodevelopmental outcomes, as noted in EPA's evaluation (FIFRA Scientific Advisory Panel, 2012). In this study, child age and sex were the only co-variables considered; these variables are simple to accurately and reliably measure using linkage with birth/medical

records.

The selection of few co-variables for the statistical models used to evaluate the relation between prenatal chlorpyrifos exposure and brain morphology changes is appropriate. For another variable to be a confounder in this relation, the other variable would have to be related to both chlorpyrifos exposure and (causally) related to brain morphology (*e.g.*, surface area, cortical thickness, gyri dimensionality). Child age and sex are appropriate confounders as these factors would influence brain size, growth and differentiation. While some research has noted a link between social characteristics and brain development and possibly morphological characteristics, the authors' decision to restrict CCCEH participation to those with very similar SES characteristics reduces that possibility of confounding the association of interest in this MRI study by SES. Further, the small sample size supports the use of few additional co-variables in the statistical model to maximize statistical power. Similar, within the MRI study, authors restricted participation to those without other co-exposures such as PAH and ETS thus eliminating the need to consider co-adjustment for these variables. Authors additionally measured blood lead in this sample and the overall, total cohort and observe the correlation between chlorpyrifos and blood lead (necessary factor for positive confounding bias to occur) was very low ($\rho<0.20$) and not statistically significant ($p>0.20$).

Other biases that may be present in epidemiology studies include selection and information biases. Selection bias in prospective cohort studies may occur if participants drop out of a study and the decision to drop out was related to either the CPF exposure or neurodevelopmental outcome. In the CCCEH study the retention rate is high for a birth cohort study. The participation rate in the MRI study was good (60%). Authors illustrated that those included and excluded were comparable, and the sample and total cohort were also comparable. This ameliorates the concern. Temporal bias, a question as to whether exposure preceded disease, is not a large concern in a prospective study. The prospective nature of the cohort study also reduces that chance of differential information bias or the misclassification of exposure, confounder, or outcome information by key study factors. Non-differential exposure measurement error may have occurred: 1) the single measurement chlorpyrifos during the third trimester, 2) error arising from differences between measured biomarker levels and actual chlorpyrifos at the one-time point, 3) unmeasured time- and space- dependent patterns of chlorpyrifos exposure, 4) uncertainty regarding the critical period for chlorpyrifos effects on development, 5) missing exposure data, 6) laboratory errors, and 7) imputation of missing exposure levels. Measurement errors in the ascertainment of chlorpyrifos exposure are likely to have occurred. However, because of the prospective study design employed, the errors are unlikely to result in falsely positive findings, because the probability and magnitude of these errors are likely to be independent of the outcome status of participants (MRI characteristics). The degree to which measurement error in the imaging studies performed using MRI technology are of keen interest to EPA.

Authors employed generalized linear models to estimate the association between CPF exposure (high/low) and "voxels" or three-dimensional data reflecting morphological characteristics of the cerebral cortex, specifically cortical thickness, distances on the cerebrum, size and dimension of key gyri important to learning, cognition, and social behavioral, adjusting for child age and sex. Use of a two-level exposure variable is preferable as it conserves study power, and is supported

by the one-time measure of CPF at delivery (cord blood CPF concentration pg/g). Researchers utilized a method to correct for multiple statistical tests (FDR) which was reasonable and appropriate in this instance. Authors acknowledged low statistical power to evaluate statistical interaction was likely including gene by environment interactions. Linear modeling is a preferred method when sample size is small as the method conserves statistical power. Authors did not present a table of model results, only graphical depictions of areas of morphological differences of the cerebrum by CPF exposure, color coded to reflect the statistical significance of the differences in specific, region brain size or volume or area. Additional information reflecting model fit statistics, as well as display of evidence of effect modification in both exposure strata (high and low CPF exposure) would enhance clarity and presentation of the research results. While authors appropriately characterized the evaluation of the interaction with child sex and CPF and brain morphology as preliminary, given the number of statistical tests performed and lack of hypotheses regarding particular areas of the cerebrum and cortical surface, results require replication before firm conclusions regarding underlying biological mechanisms at play can be posited.

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 sub-serve those learning, cognition and social behavioral, supported by previous observational and experimental literature.

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Appendix 4. Detailed Summary Tables 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
Article 1: Whyatt et al. (2004) Columbia U. (N=314)	Birth length, Birth weight, head circumference	Four cord plasma chlorpyrifos exposure groups and four 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 ($p \leq 0.007$) 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.
Article 2: 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 exposure and mean indices of fetal	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
				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.
Article 3: Eskenazi et al. (2004) CHAMACOS (N=488)	Birth length, birth weight, head circumference, Gestation Length, Ponderal index	Total DAPs: median 136 nmol/L (range: 10–6,854); DEP: median 22 nmol/L	Gestational age, gestational age squared, maternal age, pregnancy weight gain, week of initiating prenatal care, parity, infant sex, mother's country of	Decreases in gestational duration associated with two measures of in utero pesticide exposure: levels of metabolites of dimethyl	Maternal urine collection averaged wks.14, 26, not creatinine-corrected	Strengths in the study design include the longitudinal design, the use of multiple exposure biomarkers, including

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
		(range: 2–680 nmol/L); TCPy: median 3.3 nmol/L (range: 0.2–56.1nmol/L) (76% >LOD)	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.	phosphate pesticide compounds and whole blood ChE.		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.
Article 4: 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, 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	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 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	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 children at age 7.	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 factor to preterm or low birth weight birth.	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 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

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
		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).	(PBDEs), polychlorinated biphenyls (PCBs), p,p'-dichlorodiphenyltrichlorethane (DDT), p,p'-dichlorodiphenyltrichlorethylene (DDE), and lead.			education, and child stimulation. The cohort had a relatively homogenous socioeconomic profile, reducing the potential for uncontrolled confounding.
Article 5: Engel et al. (2007) Mt. Sinai (N=311)	Brazelton Neonatal Behavioral Assessment Scale (BNBAS) , primitive reflexes (neurological integrity) measured before hospital discharge.	Diethylphosphates (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 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

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
						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 mi-asured) 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.
Article 6: Young et al. (2005) CHAMACOS (N=381)	Neurodevelopment, Brazelton Neonatal Behavioral AssessmentScale (BNBAS), abnormal reflexes	DAP (ave. during pregnancy): median 222nmol/L (range: 7–21,867 nmol/L); DEP (ave. pregnancy): median 21nmol/L (range: 2–680 nmol/L)	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 delivery, breastfeeding initiated after delivery, poverty level, infant sex, age in days at BNBAS, minutes since last feed at BNBAS, and BNBAS examiner.	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 = 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).	Associations seen pre-natal OP, not post-natal OP exposure, Maternal urine collection averaged wks.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 exposure during the pregnancyWeaknesses: 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.
Article 7: Rauh et al. (2006) Columbia U. (N=254)	Neurodevelopment: The Bayley Scales of Infant Development II	Exposure levels were categorized as low (≤ 6.17 pg/g) or high	Data were collected regarding lead exposure, demographics, education and occupational history, income, active and	At the 36 month milestone, the likelihood of highly exposed children developing mental delays	The authors summarize three main findings: 1) by age 3, the more highly exposed children	• Only 53% of the children reached the three year milestone with study data collected. It is unclear what

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
	(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)	(>6.17 pg/g)	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.	were 2.4 times greater (95% CI: 1.12-5.08, p = .02) and motor delays were 4.9 greater (95% CI: 1.78-13.72; p = .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 = .01).	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.	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 causality.
Article 8: Lovasi et al. 2010 Columbai U. (N=266)	Bayley scores (MDI/PDI) 12 mo., 24 mo., 36 mo.	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

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
						limitation of the study (reduced generalizability).
Article 9: Engel et al. (2011) Mt. Sinai (N=276)	Bayley scores (MDI/PDI) at 12 mo., 24 mo.	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 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.
Article 10: Eskenazi et al. (2007)	Neurodevelopment, Bayley Index (MDI, PDI),	DEP: geom. Mean in mother 18.1 nmol/L (95% CI	Psychometrician, location of assessment, exact age at assessment, sex, breast-feeding	DAP metabolite levels during pregnancy, particularly from dimethyl	No strong associations identified with DE or TCPy, Maternal urine collection	Strengths: Longitudinal design, measurement and consideration of many

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
CHAMACOS (N=372)	Maternal behavior checklist at 6, 12, and 24 months	16.7–19.7); DEP geom mean in child at 24 months 10.5 nmol/L (95% CI 8.8–12.6); TCPy median 3.54 ug/l	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	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.	averaged wks.14, 26	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.
Article 11: 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	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 mo.) $PON1_{108TT}$ –5.7 (–9.0 to –2.5) 9=0.01; Decrease PDI (24 mo.) $PON1_{108TT}$ –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 pregnancyWeaknesses: 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.

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
		U/mL (SD = 44). Mean paraoxonase activity: For infants: 256.6 U/L (SD = 165); For mothers: 989.0 U/L (SD = 616).				
Article 12: Marks et al. (2010) CHAMACOS (N=348)	CBCL; K-CPT; ADHD confidence index; Hillside behavioral rating scale; composite ADHD indicator	DAP (geom mean) pregnancy 109.0 nmol/L; DEP 17.7 nmol/L	Psychometrician, exact age at assessment, sex, maternal 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	Prenatal DAPs were non-significantly associated 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].	Marked effect modification by gender: 11-fold increase ADHD composite indicator in boys, less than 2-fold in girls, however unstable estimates; weak evidence of association DAPs at 3.5, 5 yrs. and attention	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.
Article 13: 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	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-	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,

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
		using assay-specific limit of detection (LOD) values to impute an approximate level.		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% reduction in Working Memory.		were validated.• The authors describe an elegant and 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 and how this may impact a child or his/her psychological or educational plans.

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
Article 9: 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.
Article 14: Bouchard et al. (2011) CHAMACOS (N=329)	Wechsler-IV Intelligence Scale (verbal comprehension; perceptual reasoning, working memory,	Total DAPs (quintiles): Q1 (39 nmol/L); Q2 75 nmol/L; Q3 126 nmol/L; Q4 221 nmol/L; Q5 508 nmol/L. Geometric	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	The authors observed evidence of an association between prenatal exposures to OP pesticides as measured by urinary DAP metabolites in women during pregnancy, and	Prenatal measures taken later half of pregnancy more significantly associated intelligence than early; little evidence post-natal OP exposure associated with intelligence;	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

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
	processing speed, full scale intelligence) measured at age 7 years	mean DAP 131 nmol/L	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.	decreased cognitive functioning in children at age 7.	7 point reduction in full scale intelligence DAP Q5/Q1 (SS)	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.
Article 15: 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.)
Article 16: Whyatt et al. (2009) Columbia U. (N=102)	None	The limit of 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	N/A	Meconium TCPy 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 ($r = 0.31$, $p < 0.01$).	TCPy in maternal urine samples was not reliable, but the maternal and cord blood chlorpyrifos as well as the TCPy levels in meconium were reliable measures of exposure	Comprehensive 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-

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
		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.				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 (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 limited; one time measure of pre-natal exposure

Appendix 5. Summary of OPP's ChE Policy & Use of BMD Modeling

OPP's ChE policy (USEPA, 2000[74]) describes the manner in which ChE data are used in human health risk assessment. The following text provides a brief summary of that document to provide context to points of departure selected.

AChE inhibition can be inhibited in the central or peripheral nervous tissue. Measurements of AChE or cholinesterase (ChE) inhibition in peripheral tissues (e.g., liver, diaphragm, heart, lung etc) are rare. As such, experimental laboratory studies generally measure brain (central) and blood (plasma and red blood cell, RBC) ChE. Blood measures do not represent the target tissue, per se, but are instead used as surrogate measures for peripheral toxicity in studies with laboratory animals or for peripheral and/or central toxicity in humans. In addition, RBC measures represent AChE, whereas plasma measures are predominately BuChE. Thus, RBC AChE data may provide a better representation of the inhibition in target tissues. As part of the dose response assessment, evaluations of neurobehavior and clinical signs are performed to consider the dose response linkage between AChE inhibition and apical outcomes.

Refinements to OPP's use of ChE data have come in the implementation of BMD approaches in dose response assessment. Beginning with the OP CRA, OPP has increased its use of BMD modeling to derive PoDs for AChE inhibiting compounds. Most often the decreasing exponential empirical model has been used.

OPP does not have a defined benchmark response (BMR) for OPs. However, the 10% level has been used in the majority of dose response analyses conducted to date. This 10% level represents a 10% reduction in AChE activity (i.e., inhibition) compared to background (i.e., controls). Specifically, the BMD_{10} is the estimated dose where ChE is inhibited by 10% compared to background. The $BMDL_{10}$ is the lower confidence bound on the BMD_{10}.

The use of the 10% BMR is derived from a combination of statistical and biological considerations. A power analysis was conducted by the Office of Research and Development (ORD) on over 100 brain AChE datasets across more than 25 OPs as part of the OP CRA (USEPA, 2002). This analysis demonstrated that 10% is a level that can be reliably measured in the majority of rat toxicity studies. In addition, the 10% level is generally at or near the limit of sensitivity for discerning a statistically significant decrease in ChE activity in the brain compartment and is a response level close to the background brain ChE level. With respect to biological considerations, a change in 10% brain AChE inhibition is protective for downstream cholinergic clinical signs and apical neurotoxic outcomes. With respect to RBC AChE inhibition, these data tend to be more variable than brain AChE data. OPP begins its BMD analyses using the 10% BMR for RBC AChE inhibition but BMRs up to 20% could be considered on a case by case basis as long as such PoDs are protective for brain AChE inhibition, potential peripheral inhibition, and clinical signs of cholinergic toxicity.

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[74] USEPA (2000) Office of Pesticide Programs, US Environmental Protection Agency, Washington DC 20460. August 18, 2000 Office of Pesticide Programs Science Policy of The Use of Data on Cholinesterase Inhibition for Risk Assessments of Organophosphorous and Carbamate Pesticides.

Appendix 6. Columbia Center for Children's Environmental Health (CCCEH) Epidemiology Data Acquisition "Raw Data" Request

I. ACTION REQUESTED

To fulfill identified information needs for the purposes of incorporating the Columbia Center for Children's Environmental Health (CCCEH) epidemiology data into the Human Health Risk Assessment (HHRA) for chlorpyrifos, the agency sought to obtain certain "raw data" from CCCEH researchers. Specifically, EPA requested the original analytic data file used to support analyses presented in the peer-reviewed, published epidemiology studies concerning *in utero* chlorpyrifos exposure (V. Rauh et al., 2011; V. A. Rauh et al., 2006; Whyatt et al., 2004). CCCEH researchers did not agree to provide these data, however, the researchers met with EPA and discussed the agency's questions about the data to help determine whether further review of the raw data might assist EPA in resolving uncertainties. As a result of new information gathered through an on-site meeting and other sources, EPA is no longer pursuing the request for the original analytic data file from CCCEH researchers. This memorandum details the new information gained that resolves or renders unobtainable the previously identified information needs.

II. BACKGROUND

EPA considers many different types of scientific information when performing a human health risk assessment (HHRA) of pesticide exposure in the human population. Traditionally, EPA uses toxicology, product and residue chemistry, and industrial hygiene studies as well as measured and modeled human and environmental exposure information to support assessment of environmental risks. In its preparation of the HHRA for chlorpyrifos, the agency has evaluated environmental epidemiology studies of the potential risk of long-term neurodevelopmental effects such as delayed motor skill acquisition or reduced intelligence quotient (IQ) measures among children who experienced pesticide exposure during gestational development. There are three prospective birth cohort studies in the U.S. that examine pesticide exposure (as well as other environmental toxicants) to the pregnant mother and fetus, and then measure neurological and neurodevelopmental performance in children as they grow older. EPA has provided some of the funding support for each of these studies. Authors hypothesize that *in utero* and early life

exposure may influence brain development and effect neurological functioning in children. These studies include the CHAMACOS study in the Salinas Valley, CA, the Mt. Sinai children's environmental health study (Mt. Sinai study), and the Columbia Center for Children's Environmental Health (CCCEH).

The CCCEH study is the only one of the three studies that measures maternal and fetal exposure to chlorpyrifos specifically; the other two cohorts measure exposure to organophosphate pesticides generally. Authors with the CCCEH study reported reduced birth weight and birth length among neonates more highly exposed to chlorpyrifos during gestation (as measured by cord blood concentration of chlorpyrifos) (Whyatt et al., 2004). Similarly, authors observed slower motor skill acquisition and reduced mental capacity among infants who were more highly exposed to the chemical *in utero* (V. A. Rauh et al., 2006). In 2011, authors from all three birth cohort studies concurrently reported evidence of reduced measures of intelligence (Wechslar intelligence scale scores) by increasing *in utero* chlorpyrifos and/or organophosphate exposure (M. F. Bouchard et al., 2011; Engel et al., 2011; V. Rauh et al., 2011).

Given the value of this information to the agency's HHRA for chlorpyrifos, EPA requested the FIFRA SAP to provide external peer review of the strengths and limitations of the epidemiology data for use in the chlorpyrifos HHRA (FIFRA SAP September 2008 and April 2012). The agency identified two major areas in which additional information was needed to fully incorporate these data into the HHRA: additional measures of environmental exposure to chlorpyrifos in the CCCEH cohort to discern whether acetyl cholinesterase inhibition was likely to have occurred in connection with reported adverse outcomes, and also the role of other environmental chemicals (lead, polycyclic aromatic hydrocarbon (PAH), other organophosphate pesticides) in the observed adverse neurological effects reported in relation to *in utero* chlorpyrifos exposure.

To fulfill these information needs for the purposes of incorporating the epidemiology data into the chlorpyrifos HHRA, the agency sought to obtain certain "raw data" from the Columbia Center for Children's Environmental Health (CCCEH) study. Specifically, EPA requested the original analytic data file used to support analyses presented in the peer-reviewed, published

epidemiology studies concerning *in utero* chlorpyrifos exposure (V. Rauh et al., 2011; V. A. Rauh et al., 2006; Whyatt et al., 2004). CCCEH did not agree to provide the data based upon these initial inquiries and they asserted that because EPA did not fund the pesticide exposure component of their cohort study EPA was not legally entitled to review their underlying data. CCCEH did agree, however, to meet and discuss EPA's questions about the data to help determine whether further review of the raw data might assist EPA in resolving uncertainties. As a result on April 15th, 2013, EPA scientists and CCCEH researchers held an all-day meeting at the CCCEH data center (Mailman School of Public Health, New York City, NY) to discuss EPA's information needs and whether acquisition of the full analytic data would be necessary or valuable to EPA's assessment. Addendum 1 delineates the questions EPA posed to CCCEH study staff at this all-day meeting.

III. RESOLUTION OF INFORMATION NEEDS

A. EPIDEMIOLOGY STUDY EXPOSURE CHARACTERIZATION

The primary rationale supporting EPA's request for "raw data" from the CCCEH researchers relates to the agency's need to determine whether the levels of chlorpyrifos exposure in the environment (apartments, apartment building or other outdoor environment, or dietary exposure) of CCCEH study participants were above or below levels that may elicit a greater than 10% inhibition of acetylcholinesterase enzyme levels, the current regulatory endpoint. During the April 2013 meeting, EPA learned that this type of information is neither available nor obtainable. CCCEH researchers estimated relative pesticide exposure using several different exposure methods including 48-hour air sampling with personal monitor, 2-week integrated stationary air monitoring, maternal urinary concentration of TCPy (urinary metabolite of chlopryrifos) during the last trimester of pregnancy, maternal urinary concentration of TCPy at delivery, and umbilical cord blood and meconium at delivery. To determine whether a significant change in acetyl cholinesterase levels may have occurred as a result of actual environmental exposure, temporal concordance between pesticide use and the chlorpyrifos measurement is needed, *i.e.*, exposure estimation at the time of pesticide application is optimal. The CCCEH study design did not incorporate pre- and post-pesticide use/exposure measurement in the study protocol. Therefore, this information was not collected and is not retrospectively obtainable.

In addition, EPA requested any additional information obtained by researchers as to specific pesticide products used to better understand the pattern and frequency of organophosphate pesticide use among cohort participants. This information was solicited from participants in a written questionnaire administered during a follow-up period (unpublished copy of questionnaire obtained by EPA Oct. 2012). In response to the EPA inquiry, researchers recalled that the Whyatt (2002) publication described the challenges of collecting pesticide product information in etiologic epidemiology studies, and in the on-site meeting in April 2013 confirmed that the information quality in the CCCEH written questionnaire responses is very low. This information was deemed of such poor quality by CCCEH data analysts that the data were not coded or entered into the analytic data file. Therefore, EPA learned that this specific request for "raw data" concerning pesticide product use is not available.

As a surrogate for this information, CCCEH researchers suggested EPA contact the New York City Department of Health to obtain a linked dataset of CCCEH study participant residential address and public housing pesticide usage. The linked dataset provides aggregated pesticide usage data at the cohort participant building-level only. EPA has obtained and reviewed these data (June 2013) and determined that pursuing a data reconstruction exercise is the most appropriate way to estimate environmental pesticide exposure that would have to occur among CCCEH study participants. EPA has conducted such analysis and included it in the revised human health risk assessment.

B. CO-EXPOSURE TO OTHER ENVIRONMENTAL CONTAMINENTS

A second major concern raised by EPA, FIFRA SAP peer reviewers, and public commenters is the ability of the CCCEH study authors to accurately measure and statistically model the relationship between other environmental chemicals (lead and PAH, specifically) or other pesticides (diazinon, propoxur) that may influence fetal brain development and childhood neurodevelopmental performance, and also be related to chlorpyrifos exposure (these are "potentially confounding" exposures). EPA's concern stems from the understanding that if these other exposures are not sufficiently considered in the epidemiological analysis, then an incorrect

inference and conclusion may result (*i.e.*, a potential false positive association). For example, prenatal and early life exposure to lead in the environment has been causally linked to adverse neurodevelopmental outcomes similar to those measured in the CCCEH cohort study including intelligence measures. EPA was concerned about the potential error in the CCCEH study if lead levels were not appropriately considered, *i.e.*, the apparent chlorpyrifos effect on neurodevelopment observed in the study may have been due to the lead exposure.

However, EPA has confirmed with study authors that lead levels and chlorpyrifos levels in cord blood are not statistically associated in this population. Plotting blood lead levels against cord blood chlorpyrifos levels illustrates that the two exposures are extremely weakly (linearly) correlated in this cohort ($\rho<1\%$) (V. A. Rauh et al., 2006). Further, EPA learned from unpublished, supplemental analyses performed by CCCEH researchers upon EPA request that postnatal blood lead levels and prenatal chlorpyrifos levels are also not strongly statistically associated (Andrews, January 21, 2013). This is plausible because of intensive lead abatement programs on-going in New York City during the time period of this study. According to the New York City Department of Health, the number of children with elevated blood lead levels declined 92% between 1995 and 2008.[75] Therefore, because the two exposures are not related, it is not likely that pre- or postnatal blood lead exposure could explain the observed association with chlorpyrifos.

Furthermore, during the April 2013 meeting CCCEH researchers pointed out that based upon available information it appears that lead and chlorpyrifos may affect the brain differently. It is well understood that lead affects the neurodevelopmental sub-domain leading to outward motivation and aggression; while research within the CCCEH cohort indicates chlorpyrifos may affect inward motivation, information processing and organization (V. Rauh et al., 2011; V. A. Rauh et al., 2006; Wright et al., 2008). Additionally, MRI imaging studies of lead affected persons and preliminary brain imaging studies of chlorpyrifos affected persons show different MRI patterns, grey matter as opposed to white matter compositional patterns, respectively (Brubaker, Dietrich, Lanphear, & Cecil, 2010; Brubaker et al., 2009; Cecil et al., 2008; Cecil et al., 2011; V. A. Rauh et al., 2012). Therefore, given that neither pre- nor postnatal lead levels

[75] http://www.nyc.gov/html/doh/html/data/stats-childlead.shtml

and chlorpyrifos levels are not statistically associated with one another in the CCCEH study, and the different ways through which lead and chlorpyrifos appear to influence neurodevelopmental domains EPA concludes that lead exposure did not likely confound (bias or render incorrect) the observed association between chlorpyrifos exposure and neurodevelopment in this study population.

Peer review panelists participating on the April 2012 FIFRA SAP panel identified the concern that authors had not fully considered the long-term effects of polycyclic aromatic hydrocarbon (PAH) exposure, a ubiquitous air pollutant in inner-city areas such as NYC, in the observed association between chlorpyrifos and neurodevelopmental outcomes. Specifically, panelist argued that 'a shift in environmental exposures over time' such that postnatal PAH exposure may have combined with the measured *in utero* pesticide exposure to result in the observed ND outcomes. During the April 2013 meeting, authors clarified that the study design did not include a repeat measure of exposures over time, so an analysis of postnatal PAH exposures is not possible. In the published studies, authors were able to control for the effect of prenatal PAH through statistical adjustment. In addition, authors examined the possible modifying role of prenatal PAH in this epidemiological association and did not observe any evidence of a different risk estimate between chlorpyrifos and ND among those more highly exposed to PAH. Concerning the role of postnatal environmental exposures, CCCEH researchers also stated their belief that their overall study results illustrate that it is gestational exposure, and not early life exposure, that influences neurodevelopment in the study population. They state that the longitudinal analyses of infant and child neurodevelopment in relation to *in utero* chlorpyrifos exposure illustrates a persistent effect of the prenatal environment (M. Bouchard et al., 2003; M. F. Bouchard et al., 2011; Engel et al., 2007; Engel et al., 2011; Eskenazi et al., 2004; Eskenazi et al., 2007; V. Rauh et al., 2011; V. A. Rauh et al., 2006; Whyatt et al., 2004). EPA concluded that CCCEH researchers utilized best practices in statistical analysis of epidemiological data concerning the role of prenatal PAH in neurodevelopmental outcomes, and that a study of repeated, postnatal PAH exposure was beyond the scope of the current CCCEH study, and would require a follow-up study not yet undertaken.

EPA was also interested to learn more about the co-exposure to other organophosphate pesticides

among CCCEH study participants. Specifically, EPA as well as external peer review panelists noted the uncertainty as to the degree to which exposure to multiple acetyl cholinesterase inhibiting pesticides exposures over time and/or concurrent in time may have influenced study results. CCCEH researchers agreed that a more clear understanding of the role of mixtures – exposure to multiple OP pesticides overall or concurrent in time – on these neurodevelopmental outcomes is desirable; however they also recognized that the current sample size is too small to perform this type of analysis. To better understand the role of exposure to a mixture of OP pesticides a new cohort study with a larger sample size and different design is required. Therefore, EPA concluded that co-exposure to multiple organophosphate mixtures is not currently obtainable.

For risk characterization purposes, EPA was also interested in understanding the relative contributions of various environmental exposures on ND outcomes, (*e.g.*, PAH, environmental tobacco smoke, chlorpyrifos). Researchers noted that a preliminary indication of the relative contribution of various risk factors for intelligence measures in these cohorts can be seen through examination of supplemental tables published by CCCEH researchers, *i.e.*, the beta-coefficients provided in published supplemental tables provide an indication of the relative contribution of each risk factor (V. Rauh et al., 2011). However, CCCEH researchers indicated that to gain a true reflection the causal model in the population a series of studies in other study populations is needed. EPA and CCCEH researchers agreed that these studies will likely accumulate over time, however they are not currently available.

IV. CONCLUSIONS

In the past, EPA sought to obtain the original analytic data file used to support certain epidemiological analysis of *in utero* exposure to chlorpyrifos and subsequent adverse neurodevelopmental health outcomes in children generated by the Columbia Center for Children's Environmental Health (CCCEH) to support the Human Health Risk Assessment (HHRA) of chlorpyrifos. EPA believed these data were important to both clarify the exposure-response relationship observed in the epidemiology study relative to the current regulatory endpoint (acetylcholinesterase inhibition), and also to resolve uncertainties regarding study

participants co-exposure to other environmental contaminants, among other areas of uncertainties. CCCEH researchers did not agree to provide these data, however, the researchers met with EPA and discussed the agency's questions about the data to help determine whether further review of the raw data might assist EPA in resolving uncertainties. As a result of this meeting and additional discussions with CCCEH staff, EPA concluded that access to the raw data would either not provide answers to EPA's questions or that the information EPA sought could be obtained without analyzing the raw data. Indeed, based on discussions in that meeting as well as further work conducted by agency staff, EPA has gained additional information to better clarify and characterize the major issue areas identified as uncertainties. For these reasons, EPA decided that it would not further pursue its request for the analytic data file from the CCCEH researchers.

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Appendix 6. Addendum 1:

Columbia University Epidemiology Studies

The agency is obligated to review and address peer review comments in support of regulatory decisions. The following is a list of key issues about the epidemiological studies carried out by researchers at Columbia University that were raised in peer review comments. These issues require EPA to have access to the raw data for additional analyses by the agency.

1) **Further analysis of other chemical exposures (*e.g.*, lead, PAHs, other pesticides) to address, if possible, their impact or contribution as modulating factors on the measured outcomes**

- **2012 SAP --** "it should be noted that it cannot be stated that chlorpyrifos is the sole contributor to the observed outcomes."
- **2012 SAP --** "In an earlier examination of the same cohort, Perera *et al.*(2009) reported an association between a decrease in full-scale IQ and verbal IQ in 5year-olds with prenatal polycyclic aromatic hydrocarbons (PAH) exposure rather than chlorpyrifos, thus, raising an issue of the shift in chemical exposure association with increase in age. In each of these analyses, statistical modeling showed that the exposures were independently associated with IQ, and no significant interaction was observed with the other chemical. While this is a statistically sound approach to determine independent responses, panel members noted that it is very difficult to identify the independent physiological effects of a single chemical in this type of multi-chemical exposure scenario."
- **2012 Federal Peer Review --** "even low levels of lead can impact neurodevelopment, and even that the observed neurobehavioral deficits are more pronounced at lower blood lead levels when compared with higher blood lead levels".
- **2008 SAP --** "In order to eliminate the possible causes of neurodevelopmental effects by other pesticides in the Columbia study, it is suggested that EPA should repeat the pre-post residential cancellation analysis done for chlorpyrifos using other pesticide measurements, such as malathion diacid (MDA), a specific metabolite of malathion. The outcomes from those additional analyses will either confirm or reject EPA's preliminary conclusion that chlorpyrifos is likely to play a role in the neurodevelopmental outcomes."
- **2008 SAP --** ""It would be useful to examine the results of a statistical analysis that includes all three AChE-inhibiting insecticides in the analysis model as dichotomous variables (above or below LOD) in combination with continuous measurements for these variables. This type of analysis would likely not change

the results, but it could be helpful in illustrating threshold or dose response effects."

2) **Further analysis and information to address and, if possible, better characterize uncertainty around outcome measures on learning/memory/IQ**

- **2012 SAP**-- Alternative considerations for non-quantified samples: "little use was made of techniques to integrate non-quantified samples into the statistical test…. Various methods were reviewed by the July 2010 SAP that can be applied to either normally or lognormally distributed data that include a significant (even a majority) of non-detectable sample . . . . Specifically, the use of 'probability plots' was described that can yield an estimate of the geometric mean of the distribution [GM], the geometric standard deviation [GSD], and corresponding percentiles."
- **Federal Peer Review --** "There is a scatterplot showing the raw scores for overall IQ and for each of the subtests, but it is not possible to obtain the necessary information to compare the distributions of these scores with the norms for the test or with any other study sample. Ideally, the means and standard deviations for these scores should be presented for either a non-exposed or a non-exposed combined with low exposed group and these should be compared to a moderate or high-exposed group as was done for the BSID-II in the Rauh et al., 2006 paper. Here the uncertainties stem from the assumptions that are made when regression analyses are performed. The main issue here is that outliers can greatly influence the slope of the function."
- **Federal Peer Review--**A between group analysis using inferential statistics, as was done for the Bayley Scales of Infant Development II in the Rauh et al., 2006 paper, should be performed on each variable in both studies (i.e., the Child Behavior Checklist in Rauh et al., 2006, and the full scale IQ and subscales for the WISC-IV in the Rauh et al., 2011 study). This would be the most direct and least problematic method for determining whether exposure to chlorpyrifos resulted in significant decreases in IQ or significant increases in behavioral problems "….. no information was provided regarding the qualifications of the individuals who administered and scored the tests. "

3) **Further analysis to assess, if possible, whether individual cohort members had the potential for exposure to chlorpyrifos and/or other acetylcholinesterase (AChE) inhibiting pesticides (e.g., diazinon, propoxur), prenatally and /or postnatally, at levels leading to greater than 10% AChE inhibition (the level used to derive the regulatory point of departure).**

- **2012 SAP--** recommended conducting a dose reconstruction analysis—"data on the concentration of chlorpyrifos in various media (*i.e.* house dust, air and water) while market basket data exists on the concentration of chlorpyrifos on food. These data provide the main tools for developing an effective exposure assessment and a subsequent reconstruction of potential dose." The agency has begun such analysis but the current draft analysis is limited without data on the exposure information relevant to individual women such that environmental chlorpyrifos exposure can then be linked to measures of blood chlorpyrifos.
- **2012 SAP--** recommended the agency consider issues related to multiple chemical exposure (i.e., mixtures) to chlorpyrifos and other key AChE inhibiting pesticides identified by the Columbia University studies (diazinon, propoxur). Assumptions of co-exposure will likely be grossly overestimated without access to the raw data; such raw data may enable the agency to evaluate actual co-exposure information for individuals from air monitoring samples and blood samples.

Appendix 7. Physical/Chemical Properties

Physical/Chemical Properties of Chlorpyrifos.		
Parameter	Value	Reference
Melting point/range	41.5-42.5 ºC	Chlorpyrifos IRED
pH	NR	
Density (21ºC)	1.51 g/mL	
Water solubility (25°C)	1.05 mg/L	
Solvent solubility (20°C)	Acetone >400 g/L Dichloromethane >400 g/L Methanol 250 g/L Ethyl acetate >400 g/L Toluene >400 g/L n-hexane >400 g/L	
Vapor pressure, (25°C)	1.87×10^{-5} torr[1]	
Dissociation constant, pK_a	NR	
Octanol/water partition coefficient, Log(K_{OW})	4.7	
UV/visible absorption spectrum	NR	

NR – not reported.

[1] R. Bohaty, June 2011, D368388 and D389480, *Chlorpyrifos Drinking Water Assessment for Registration Review* (CRF assessment, Oct. 16, 2009 product chemistry BC 2062713)

Appendix 8. Current U.S. Tolerances and International Residue Limits

Chlorpyrifos (059101)

Summary of US and International Tolerances and Maximum Residue Limits			
Residue Definition:			
US	**Canada**	**Mexico[2]**	**Codex[3]**
40CFR180.342 chlorpyrifos *per se* (*O,O* -diethyl *O* -(3,5,6-trichloro-2-pyridyl) phosphorothioate	*O,O*-diethyl-*O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate (apples, grapes, tomatoes) *O,O*-diethyl-*O*-(3,5,6- trichloro-2-pyridyl) phosphorothioate, including the metabolite 3,5,6-trichloro-2-pyridinol (citrus fruits; fat, kidney, and liver of cattle; kiwifruit; peppers; rutabagas; meat and meat byproducts of cattle (calculated on the fat content))		Chlorpyrifos. The residue is fat soluble.

Commodity[1,]	Tolerance (ppm) /Maximum Residue Limit (mg/kg)			
	US	Canada	Mexico[2]	Codex[3]
Alfalfa, forage	3.0			
Alfalfa, hay	13			5 alfalfa fodder
Almond	0.2			0.05
Almond, hulls	12			
Apple	0.01	0.01		1 pome fruits
Apple, wet pomace	0.02			
Banana	0.1			2
Beet, sugar, dried pulp	5.0			
Beet, sugar, molasses	15			
Beet, sugar, roots	1.0			0.05
Beet, sugar, tops	8.0			
Cattle, fat	0.3	1.0		
Cattle, meat	0.05	1.0		1 (fat)
Cattle, meat byproducts	0.05	1.0		0.01 cattle, kidney and liver
Cherry, sweet	1.0			
Cherry, tart	1.0			
Citrus, dried pulp	5.0			
Citrus, oil	20			
Corn, field, forage	8.0			
Corn, field, grain	0.05	0.05		0.05 maize
Corn, field, refined oil	0.25			0.2 maize oil, edible
Corn, field, stover	8.0			10 maize fodder (dry)
Corn, sweet, forage	8.0			
Corn, sweet, kernel plus cob with husk removed	0.05	0.05		0.01 sweet corn (corn-on-the-cob)
Corn, sweet, stover	8.0			

Summary of US and International Tolerances and Maximum Residue Limits				
Residue Definition:				
US	**Canada**		**Mexico[2]**	**Codex[3]**
Cotton, undelinted seed	0.2			0.3 cotton seed
Cranberry	1.0			1
Cucumber	0.05	0.05		
Egg	0.01			0.01 (*)
Fig	0.01			
Fruit, citrus, group 10	1.0	1.0		1
Goat, fat	0.2			
Goat, meat	0.05			
Goat, meat byproducts	0.05			
Hazelnut	0.2			
Hog, fat	0.2			
Hog, meat	0.05			0.02 (fat)
Hog, meat byproducts	0.05			0.01 (*) pig, edible offal
Horse, fat	0.25			
Horse, meat	0.25			
Horse, meat byproducts	0.25			
Kiwifruit	2.0	2.0		
Milk, fat (Reflecting 0.01 ppm in whole milk)	0.25			0.02 milk
Nectarine	0.05	0.05		
Onion, bulb	0.5			0.2
Peach	0.05	0.05		0.5
Peanut	0.2			
Peanut, refined oil	0.2			
Pear	0.05			1 pome fruits
Pecan	0.2			0.05 (*)
Pepper	1.0	1.0		2 peppers sweet including pimento or pimiento); 20 peppers chili, dried
Peppermint, tops	0.8			
Peppermint, oil	8.0			
Plum, prune, fresh	0.05			0.5 plums (including prunes)
Poultry, fat	0.1			
Poultry, meat	0.1			0.01 (fat)
Poultry, meat byproducts	0.1			0.01 (*) poultry, edible offal
Pumpkin	0.05			
Radish	2.0			
Rutabaga	0.5	0.5		
Sheep, fat	0.2			
Sheep, meat	0.05			1 (fat)

Summary of US and International Tolerances and Maximum Residue Limits				
Residue Definition:				
US	**Canada**		**Mexico[2]**	**Codex[3]**
Sheep, meat byproducts	0.05			0.01 sheep, edible offal
Spearmint, tops	0.8			
Spearmint, oil	8.0			
Sorghum, grain, forage	0.5			
Sorghum, grain, grain	0.5			0.5
Sorghum, grain, stover	2.0			2 sorghum straw and fodder, dry
Soybean, seed	0.3			0.1 soya bean (dry)
Strawberry	0.2			0.3
Sunflower, seed	0.1	0.1		
Sweet potato, roots	0.05			
Turnip, roots	1.0			
Turnip, tops	0.3			
Vegetable, brassica, leafy, group 5	1.0			2 Broccoli 1 Cabbages, head 0.05 Cauliflower 1 Chinese cabbage (type pe-tsai)
Vegetable, legume, group 6 except soybean	0.05	0.05 lentils		0.01 common bean (pods and/or immature seeds); peas (pods and succulent=immature seeds)
Walnut	0.2			0.05 (*)
Wheat, forage	3.0			
Wheat, grain	0.5			0.5
Wheat, straw	6.0			5 wheat straw and fodder, dry
MRLs with No US Equivalents				
Grapes		0.01		0.5
Tomatoes		0.01		
Carrot				0.1
Coffee beans				0.05
Cotton seed oil, crude				0.05 (*)
Cotton seed oil, edible				0.05 (*)
Dried grapes (=currants, raisins and sultanas)				0.1
Potato				2
Rice				0.5
Soya bean oil, refined				0.03
Tea, green, black (black, fermented and dried)				2

Summary of US and International Tolerances and Maximum Residue Limits				
Residue Definition:				
US	**Canada**		**Mexico[2]**	**Codex[3]**
Wheat flour				0.1

[1] Includes commodities listed in the CFR as of 4/12/11. The 40CFR 180.342 (a) (3) also stipulates that "a tolerance of 0.1 part per million is established for residues of chlorpyrifos, per se, in or on food commodities (other than those already covered by a higher tolerance as a result of use on growing crops) in food service establishments where food and food products are prepared and served, as a result of the application of chlorpyrifos in microencapsulated form."
[2] Mexico adopts US tolerances and/or Codex MRLs for its export purposes.

[3] * = absent at the limit of quantitation; Po = postharvest treatment, such as treatment of stored grains. PoP = processed postharvest treated commodity, such as processing of treated stored wheat. (fat) = to be measured on the fat portion of the sample. MRLs indicated as proposed have not been finalized by the CCPR and the CAC.

(c) *Tolerances with regional registrations.* Tolerances with regional registration, as defined in 180.1(m), are established for residues of the pesticide chlorpyrifos *per se* (*O,O* -diethyl- *O* -(3,5,6-trichloro-2-pyridyl) phosphorothioate) in or on the following food commodities:

Commodity	Parts per million	Canada	Codex
Asparagus	5.0		
Grape	0.01	0.01	0.5

In addition, the following tolerances for chlorpyrifos are recommended under registration review:

Table 8A. Recommended Tolerances for Chlorpyrifos			
Commodity	Established Tolerance (ppm)	Recommended Tolerance (ppm)	Comments Correct Commodity Definition
Aspirated grain fractions	NA	22	
Cotton, gin byproducts	NA	15	
Corn, milled byproducts	NA	0.1	
Wheat, milled byproducts	NA	1.5	

Appendix 9. Master Use Summary Document

Table 9.1. Summary of Current Chlorpyrifos Usage

Crop/Site	Residential	Agricultural	Forestry	Timing; Application Type	Method/ Equipment	Maximum Single Application Rate by Formulation[1] (lb a.i./A)	Maximum Application Rate		Maximum Application Number		PHI (days)[3]	REI (hours)[3]	MRI (days)[3]	Geographic Restrictions	Comments
							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
AGRICULT-URAL FARM PREMISES Livestock housing and holding areas (such as hog barns, empty chicken houses, dairy areas, milkrooms, calf hutches, calving pens and parlors).		✓		Indoor general surface spray	backpack sprayer; high and low sprayer (pressure or volume)	0.075 lb a.i./ 1000 ft sq 1.2 EC, ME	[14.4] NS	NA	12	NA	NA	NS	NS		Only permitted for use in poultry houses
ALFALFA		✓		At plant	groundboom	1.0 G	1.0	1.0	[1] NS	1	21	24	[10] NS	Missouri only	Lower PHI permitted for EC rates 0.33 lb a.i./A (7 d) and 0.67 lb a.i./A (14 d) e.g. Reg. No. 62719-591 Stand is in production 3-5 years. Planted ¼" to ½" deep.
		✓		Foliar	aerial or ground/	1.0 EC	[4.0] NS	4.0	[4] NS	4	21	24	10		Lower PHI permitted for

Table 9.1. Summary of Current Chlorpyrifos Usage

Crop/Site	Residential	Agricultural	Forestry	Timing; Application Type	Method/ Equipment	Maximum Single Application Rate by Formulation[1] (lb a.i./A)	Maximum Application Rate		Maximum Application Number		PHI (days)[3]	REI (hours)[3]	MRI (days)[3]	Geographic Restrictions	Comments
							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
					broadcast, chemigation										EC rates 0.33 lb a.i./A (7 d) and 0.67 lb a.i./A (14 d) *e.g.*, Reg. No. 62719-591 Multiple harvests (or cuttings) per year when used for feed/fodder and 1 harvest per year when grown for seed. Cuttings occur about every 30 days. Only 1 crop cycle per year but up to 9 cuttings, varies by geography.
				Total		**1.0**	**5.0**	**5.0**	**[5] NS**	**5**	**21**	**24**	**[10] NS**		Represents Missouri scenario otherwise 4.0 lb a.i./A per is max.
ALMOND		✓		**dormant/ delayed**	aircraft, airblast	2.0 WDG, WP	2.0	NA	1	NA	NA	24	10	Restricted use in	

Table 9.1. Summary of Current Chlorpyrifos Usage

Crop/Site	Residential	Agricultural	Forestry	Timing; Application Type	Method/ Equipment	Maximum Single Application Rate by Formulation[1] (lb a.i./A)	Maximum Application Rate		Maximum Application Number		PHI (days)[3]	REI (hours)[3]	MRI (days)[3]	Geographic Restrictions	Comments
							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
				dormant; broadcast										California.	
		✓		**foliar**; broadcast	aircraft, airblast	2.0 WDG,WP	6.0	NA	3	NA	14		10		
		✓		pre-plant, foliar; **trunk spray/drenc h or pre-plant dip**	handheld, backpack, drench/dip, handgun, and low pressure hand wand	2.5 (3.0/100 gal) WDG	2.5	NA	1	NA	14		NS		
		✓		Dormant/ delayed dormant; foliar; **orchard floors** broadcast	ground boom, handgun, chemigation	4.0 EC*	4.0	NA	2	NA	14		10	Restricted use in California. Only one dormant application can be made.	
				Total	--	**4.0**	**14.5**	**NA**	**7**	**NA**	**14**		**NS**		Excludes nursery applications (See general "Fruits" listing)
APPLE		✓		**dormant/ delayed dormant**; broadcast	aircraft, airblast	2.0 EC 2.0 WDG 1.5 WP	2	2.0	1	1	NA	24/ 4 d	10d		Reflects spray drift mitigation measures.
		✓		pre-plant, foliar;	handheld, backpack,	1.5 (1.5 lb ai/100	1.5	NA	1	1	28	4d	NS	Use permitted in	

Table 9.1. Summary of Current Chlorpyrifos Usage

Crop/Site	Residential	Agricultural	Forestry	Timing; Application Type	Method/ Equipment	Maximum Single Application Rate by Formulation[1] (lb a.i./A)	Maximum Application Rate		Maximum Application Number		PHI (days)[3]	REI (hours)[3]	MRI (days)[3]	Geographic Restrictions	Comments
							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
				trunk spray/drench or pre-plant dip; ground	drench/dip, handgun, and low pressure hand wand	gal) WDG								states east of the Rockies except Mississippi.	
				Total		**2.0**	**3.5**		**2**						
ASPARAGUS		✓		Foliar, pre-harvest; broadcast	aircraft, ground boom	1.0 EC, WDG	1.0	1.0	1	1	1	24	10		
		✓		Postharvest, broadcast	aircraft, ground boom	1.0 EC, WDG	2.0	2.0	2	1	1	24	10		
					granular soil band treatment ground boom	1.5 G	3.0	3.0	2	2	180	24	[10] NS	Permitted in California, the Midwest, and the Pacific Northwest 19713-505, 19713-521, 5481-525, 62719-34, 83222-34	Do not apply more than 3.0 lb a.i./A between harvests.
				Total		**1.5 G**	**3.0 G 2.0**	**3.0 G 2.0**	3	3	1	24	10		
BEANS		✓		Preplant; Seed	Seed Treatment	*0.016-0.348* 0.000798 lb	NS	[0.348] NS	NS	[1] NS	NS	NS	NS	ME is SLN only for ID	Italics highlight the range of

Table 9.1. Summary of Current Chlorpyrifos Usage

Crop/Site	Residential	Agricultural	Forestry	Timing; Application Type	Method/ Equipment	Maximum Single Application Rate by Formulation[1] (lb a.i./A)	Maximum Application Rate		Maximum Application Number		PHI (days)[3]	REI (hours)[3]	MRI (days)[3]	Geographic Restrictions	Comments
							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
				treatment		ai/lb seed ME *0.013-0.272* 0.000625 lb ai/lb seed WP *0.012-0.253* 0.00058 lb ai/lb seed EC									application rates depending on the number of seeds per lb and the number of seeds planted per acre. Seeding rate information provide by BEAD.[4]
BEEF/RANGE/ FEEDER CATTLE (MEAT)/ DAIRY CATTLE (NON-LACTATING)				Summer, late fall, spring; impregnated collar/tag	Animal treatment (ear tag)	0.0066 lb/animal	[0.0099] NS	NA	3	NA	NS	NS	NS		Reg. No. 39039-6 Cattle ear tags are assumed to last 4-6 months Two tags per animal at 0.0033 lb a.i./tag in the summer and one tag per animal at 0.0033 lb a.i./A.
BEETS (UNSPECIFIED; TABLE OR SUGAR) "grown for seed"		✓		At plant, soil band treatment	Ground boom	1.0 EC	NS	1	NS	1		24		Allowed in Oregon Court ordered buffer of 60 ft for ground	Minimum Incorporation: 2 inches

Table 9.1. Summary of Current Chlorpyrifos Usage

Crop/Site	Residential	Agricultural	Forestry	Timing; Application Type	Method/ Equipment	Maximum Single Application Rate by Formulation[1] (lb a.i./A)	Maximum Application Rate		Maximum Application Number		PHI (days)[3]	REI (hours)[3]	MRI (days)[3]	Geographic Restrictions	Comments
							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
														chlorpyrifos application is required for "affected waterways".	
		✓		Preplant, soil incorporated treatment	Broadcast/ ground boom	1.9 EC	NS (2.8 ID)	NS	1	NS				Allowed in Oregon and Idaho	OR-09007; 62719-591 ID-090002; 62719-591
				Total		**1.9**	**NS**	**NS**	**NS**	**NS**		**24**			One or the other type of application.
SUGAR BEETS		✓		Preplant, soil incorporated treatment	Broadcast/ ground boom	1.0 EC 2.0 G	3.0	2.0	1	1	NA	24	10		Minimum Incorporation: 1 inch
		✓		At plant, soil band treatment	Broadcast/ ground boom	1.0 EC, WDG 2.0 G	3.0	2.0	1	1	30	24	10		
		✓		Postplant, soil band	Broadcast/ ground boom	2.0 G	3.0	2.0	1	1	30	24	10		
		✓		Post-emergence band treatment; broadcast	Broadcast/ ground boom	1.0 EC, WDG	3.0	1.0	3	1	30	24	10		
		✓		broadcast	Aircraft, ground boom,	1.0 EC, WDG	3.0	1.0	3	1	30	24	10		EC is not for use in MS

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Crop/Site	Residential	Agricultural	Forestry	Timing; Application Type	Method/ Equipment	Maximum Single Application Rate by Formulation[1] (lb a.i./A)	Maximum Application Rate		Maximum Application Number		PHI (days)[3]	REI (hours)[3]	MRI (days)[3]	Geographic Restrictions	Comments
							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
					chemigation										
				Total		**1.0 EC 2.0 G**	**4.0**	**[4.0] NS**	**3**	**[3] NS**	**30**	**24**	**10**		One granular application at 2.0 a.i./A and two liquid applications at 1.0 a.i./A per year. Also assumed per crop cycle.
CARROT Grown for Seed (INCLUDING TOPS)		✓		Foliar pre-bloom broadcast	aircraft, ground boom	0.94 EC	0.94	1	1	1	7	24	NA	Oregon and Washington Court ordered buffer of 60 ft for ground and 300 ft for aerial application is required for "affected waterways".	OR090011 SLN Expires: 12/31/2018 WA090011 SNL Expires: 12/31/2016 Carrots take two years to produce seed. All commercial production of the carrot (vegetable) takes place in the first year when the plant is nowhere near blooming.

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Crop/Site	Residential	Agricultural	Forestry	Timing; Application Type	Method/ Equipment	Maximum Single Application Rate by Formulation[1] (lb a.i./A)	Maximum Application Rate		Maximum Application Number		PHI (days)[3]	REI (hours)[3]	MRI (days)[3]	Geographic Restrictions	Comments
							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
CHERRIES		✓		**dormant/ delayed dormant**; broadcast	aircraft, airblast	2.0 WDG, EC 1.5 WP	2.0	NA	1	NA	NS	24	10		
		✓		**foliar**; broadcast	airblast	4.0 EC	10.0	NA	5	NA	14	24	10		Tart cherry only
					aircraft	2.0									Reflects spray drift mitigation
		✓		Foliar, post harvest; **trunk spray/drench**	handheld, backpack, drench/dip, handgun, and low pressure hand wand	2.5 (3.0/100 gal) WDG, EC	2.5	NA	1	NA	2	24	[10] NS		Only some labels specify a 10 d MRI.
				Total	--	**4.0**	**4.5 (sweet)** **14.5 (tart only)**		**6**						Excludes nursery applications (See general "Fruits" listing) The foliar applications only apply to tart cherries, thus, sweet cherry scenarios (*e.g.*, Pacific NW) annual application rate would be 4.5 lb

Table 9.1. Summary of Current Chlorpyrifos Usage

Crop/Site	Residential	Agricultural	Forestry	Timing; Application Type	Method/ Equipment	Maximum Single Application Rate by Formulation[1] (lb a.i./A)	Maximum Application Rate		Maximum Application Number		PHI (days)[3]	REI (hours)[3]	MRI (days)[3]	Geographic Restrictions	Comments
							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
															total a.i./year.
CHRISTMAS TREE PLANTATIONS		✓		**foliar**; broadcast	helicopter, orchard blast	1.0 EC, WDG, WP	3.0	NA	3	NA	[0] NS	24	7	Aerial applications via helicopter are only permitted in Washington and Oregon.	
		✓		post harvest; **Stump Treatment**	handheld, backpack, drench/dip, handgun, and low pressure hand wand	2.5 (3.0/100 gal) EC, WDG	2.5	NA	1	NA	NA		7		
				Total		**2.5**	**5.5**		**4**						
CITRUS		✓		**foliar**; broadcast	airblast, ground boom	6.0 WP, WSP, EC	7.5	NA	2	NA	35 (21 for low rates)	5d	30 (10 for low rates)	6.0 lb a.i. /A is only permitted in California and Arizona. The max single rate in other states is restricted to 4 lb a.i./A.	
		✓			aircraft	2.3 WP, WSP, EC					21	5	10	Florida, California, and potentially	Aerial application used to control psyllid, the

Table 9.1. Summary of Current Chlorpyrifos Usage

Crop/Site	Residential	Agricultural	Forestry	Timing; Application Type	Method/ Equipment	Maximum Single Application Rate by Formulation[1] (lb a.i./A)	Maximum Application Rate		Maximum Application Number		PHI (days)[3]	REI (hours)[3]	MRI (days)[3]	Geographic Restrictions	Comments
							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
														Texas	vector for citrus greening. Reflects spray drift mitigation
		✓		foliar; **orchard floors broadcast**	ground boom, chemigation, handheld, backpack, drench/dip, handgun, and low pressure hand wand	1.0 G*, WSP, EC	3.0	NA	3	NA	28	24/ 5 d	10		
				Total	--	**6.0**	**10.5**		**5**						Registered labels permit both foliar and soil applications in the same orchard. Total excludes nursery applications (See general "Fruits" listing)
CLOVER (GROWN FOR SEED)		✓		Preplant	Ground boom	1.9 EC	1.9	1.9	1	1	NS	24	NA	Use only permitted in Oregon.	OR-0900100; master label: 62719-591
		✓		Post-Plant Foliar	aircraft and ground boom										Either a preplant or post plant

Table 9.1. Summary of Current Chlorpyrifos Usage

Crop/Site	Residential	Agricultural	Forestry	Timing; Application Type	Method/ Equipment	Maximum Single Application Rate by Formulation[1] (lb a.i./A)	Maximum Application Rate		Maximum Application Number		PHI (days)[3]	REI (hours)[3]	MRI (days)[3]	Geographic Restrictions	Comments
							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
															application is allowed.
COLE CROPS (EXCLUDES CAULIFLOWER AND BRUSSELS SPROUTS)		✓		Preplant, soil incorporated treatment	Ground boom	2.0 EC, WDG, G	4.0	2.0	2	1	30	24	10		Min. incorporation: 2 inches
		✓		At plant, soil band treatment	Ground boom					1					One granular application permitted per year.
		✓		Post plant	Ground boom					1					
		✓		Foliar Established Plantings, soil sidedress treatment	Ground boom					1					
		✓		Foliar, broadcast	Aircraft, ground boom, chemigation	1.0 EC, WDG, WP	4.0	3.0	4	3	21		10		Multiple crops per year are possible in some locations.
				Total			**8.0**	**5**	**6**	**4**					Some labels restrict the yearly application rate to 3 lb a.i./A. The maximum number of crops per year is 2.
BRUSSELS		✓		At plant, soil	Ground boom	2.0	2.0	[2.0]	2	1	21				

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Crop/Site	Residential	Agricultural	Forestry	Timing; Application Type	Method/ Equipment	Maximum Single Application Rate by Formulation[1] (lb a.i./A)	Maximum Application Rate		Maximum Application Number		PHI (days)[3]	REI (hours)[3]	MRI (days)[3]	Geographic Restrictions	Comments
							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
SPROUTS				band treatment		EC; G		NS				24	10		
		✓		Preplant, soil incorporated treatment	Ground boom										Minimum incorporation is 2 inches
		✓		Postplant, soil application	Ground boom	2.25 EC, G	2.25	[2.25] NS							
		✓		Foliar broadcast	Aircraft, Ground boom	1.0 EC	[5.3] NS	3.0	NS	3			10		83222-20, 84930-7, 86363-3 specify a 7 day MRI. All other labels specify a 10 day MRI. The PHI stated 84930-7 is conflicting [p. 4 (21 days and p. 19 (30 days)]
				Total		**2.3**	**5.3**		**NS**		**21**	**24**	**7**		Assume one application of either at plant, preplant, or postplant followed with additional foliar applications.
CAULI-FLOWER		✓		At plant, soil band	Ground boom	2.0 EC	2.0 EC	NS	[1] NS	1	21	3d	10		Only one granular

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Crop/Site	Residential	Agricultural	Forestry	Timing; Application Type	Method/ Equipment	Maximum Single Application Rate by Formulation[1] (lb a.i./A)	Maximum Application Rate		Maximum Application Number		PHI (days)[3]	REI (hours)[3]	MRI (days)[3]	Geographic Restrictions	Comments
							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
				treatment		2.3 G	2.25 G								application.
		✓		Preplant, soil incorporated treatment	Ground boom	2.3 G	2.3	NS	[1] NS	1	30, EC, 21 G				Minimum incorporation is 2 inches
		✓		Postplant, soil application	Ground boom	2.0 EC									
		✓		Foliar broadcast	aircraft, ground boom	1.0 EC	[5.3] NS	3.0	NS	3	21		10		
				Total		**2.3**	**5.3**	**[5.3] NS**	**NS**	**[4] NS**	**21**	**24**	**10**		Assume one application at either plant, preplant, or postplant followed with additional foliar applications.
COMMERCIAL /INSTITUTION-AL/ INDUSTRIAL PREMISES/ EQUIP. (INDOOR) Non-food areas of manufacturing, industrial, and food processing plants;				Broadcast	Product Container	0.4373 lb a.i./100 sq ft 190.5 G	NS	NA	NS	NA	NA	NS	NS		For treatment of fire ants
				Crack and Crevice/Void	Sprayer/ Injection	0.0625 lb a.i./1000 sq ft 2.7 ME	NS	NA	NS	NA	NA	NS	NS		499-419
				Crack and Crevice/Spot	Sprayer/ Injection	0.0424 lb/gal ME	NS	NA	NS	NA	NA	NS	7		

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Crop/Site	Residential	Agricultural	Forestry	Timing; Application Type	Method/ Equipment	Maximum Single Application Rate by Formulation[1] (lb a.i./A)	Maximum Application Rate		Maximum Application Number		PHI (days)[3]	REI (hours)[3]	MRI (days)[3]	Geographic Restrictions	Comments
							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
warehouses; ship holds; railroad boxcars.															
COMMERCIAL /INSTITUTIONAL /INDUSTRIAL PREMISES/EQUIP. (OUTDOOR) Outdoor commercial use around non-food areas of manufact-uring, industrial, and food processing plants; warehouses; ship holds; railroad boxcars				Soil broadcast	Low and High Pressure, Backpack, Handgun Sprayers	0.0247 lb a.i./1000 sq ft 1.1 ME	NS	NA	NS	NA	NA	NS	NS		
				Directed spray		0.1132 lb a.i./1000 sq ft 4.9 ME	NS	NA	NS	NA	NA	NS	NS		Specific to: Inside and outside dumpsters and other trash holding containers, trash corrals and other trash storage areas.
				Crack and Crevice/void/ general outdoor		0.0424 lb/gal ME	NS	NA	NS	NA	NA	NS	7		
CONIFERS AND DECIDUOUS TREES; PLANTATION, NURSERY		✓	?	**foliar**; broadcast	Ground boom	1.0 EC	3	NA	6	NA	7	24	7		
		✓	?	foliar; **stump treatment**	backpack, drencher, low pressure hand wand	0.3 EC	0.3	NA	1	NA	7	24	7		
				Total		**1.0**	**3**	**NA**	**6**	**NA**	**7**	**24**	**7**		The total

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Crop/Site	Residential	Agricultural	Forestry	Timing; Application Type	Method/ Equipment	Maximum Single Application Rate by Formulation[1] (lb a.i./A)	Maximum Application Rate		Maximum Application Number		PHI (days)[3]	REI (hours)[3]	MRI (days)[3]	Geographic Restrictions	Comments
							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
															number of applications assumed is either 3 foliar applications or 2 foliar applications with one stump treatment.
CORN (ALL)		✓		Preplant	ground/ **soil incorporated** conservation tillage, in furrow, broadcast, chemigation, soil band	3.0 EC 2.0 G	3.0	3.0	NS	3	NA	24/ 5 EC	10		19713-520, 19713-599, 33658-26, 34704-857, 72693-11, 83222-20 The minimum incorporation depth is 2 inches.
					soil incorporated aerial conservation tillage	2.0 EC, G									
		✓			ground/ conservation tillage, in furrow, broadcast,	1.0 EC 2.0 G	3.0	3.0	NS	3	21		10		19713-520

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Crop/Site	Residential	Agricultural	Forestry	Timing; Application Type	Method/ Equipment	Maximum Single Application Rate by Formulation[1] (lb a.i./A)	Maximum Application Rate		Maximum Application Number		PHI (days)[3]	REI (hours)[3]	MRI (days)[3]	Geographic Restrictions	Comments
							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
					chemigation, soil band										
		✓		Storage or preplant seed treatment	Seed treatment	*0.001-0.021* 0.000625 lb a.i./ lb seed WP *0.1-1.9* 0.058 lb a.i./ lb seed FC	[?] NS	[1.9] NS	[?] NS	1	NS	NS	NS		Italics highlight the range of application rates depending on the number of seeds per lb and the number of seeds planted per acre. Seeding rate information provide by BEAD.[4]
		✓		At plant	soil incorporated, conservation tillage	2.0 G	[?] NS	3.0	[?] NS	3	21	24	10		
		✓		Post emergence	Aerial or ground, broadcast, chemigation	1.5 EC 1.0 WDG	NS	3.0	NS	3	21	24/ 5d (EC	10		A brush on max single rate is permitted at 1.0 lb ai/a (72693-11)
		✓		Foliar	Aerial or ground/ broadcast, granule, seed and chemigation	1.5 EC	3.0	3.0	NS	3	21		10		

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Crop/Site	Residential	Agricultural	Forestry	Timing; Application Type	Method/ Equipment	Maximum Single Application Rate by Formulation[1] (lb a.i./A)	Maximum Application Rate		Maximum Application Number		PHI (days)[3]	REI (hours)[3]	MRI (days)[3]	Geographic Restrictions	Comments
							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
				Total		**3.0**	**8.1**	**8.1**	**NS**	**4**	**21**		**10**		Two granular applications are allowed with a maximum single rate of 1.0 lb a.i./A or one granular application at 2 lb a.i./A. Total with seed treatment PHI: 21 d except Delaware and Florida (7 d)
COTTON		✓		Storage or preplant seed treatment	Seed treatment	*0.8-2.2* 0.00116 lb/lb seed EC	[2.2] NS	[2.2] NS	[1] NS	1	NS	NS	NS		264-932 Rates in italics highlight the potential range of application rates depending on the number of seeds per lb and the number of seeds planted per acre. Seeding rate information provide by BEAD.[2]

Table 9.1. Summary of Current Chlorpyrifos Usage

Crop/Site	Residential	Agricultural	Forestry	Timing; Application Type	Method/ Equipment	Maximum Single Application Rate by Formulation[1] (lb a.i./A)	Maximum Application Rate		Maximum Application Number		PHI (days)[3]	REI (hours)[3]	MRI (days)[3]	Geographic Restrictions	Comments
							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
		✓		Foliar	aerial, chemigation, ground boom	1.0 EC, WDGP	3	3.0	3	3	14	24	10		Except MS
				Total		**1.0**	**3.2**	**3.2**	**3**	**3**	**14**	**24**	**10**		1.6 lb a.i./A is max single rate (seed treatment) **Total with seed treatment** 1 crop cycle per year assumed
CRANBERRY		✓		Foliar	aircraft, ground boom/ broadcast and chemigation	1.5 EC, WDG	3.0	NA	2	NA	60	24	10	Not for use in Mississippi.	Do not apply to bogs when flooded.
CUCUMBER		✓		Storage or preplant seed treatment	Commercial seed treatment	*0.4* 0.00058 lb/lb seed EC	NS	0.1	2	1	NS	NS	NS		Seeding rate information provide by BEAD.[2] 264-932, 62719-221, CA040004 Per registrant 2 CCs per year
FIGS		✓		**dormant/ delayed dormant**; soil application	ground boom	2.0 WDG, EC	2.0	NA	1	NA	217	4 d	NS	Use is restricted to California only.	Incorporation to 3 inches is suggested but not required following application.
FILBERTS/		✓		**dormant/**	aircraft, airblast	2.0	2.0	NA	1	NA	14	24	10		

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							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
HAZELNUT				**delayed dormant**; broadcast		WP									
		✓		**foliar**; broadcast	aircraft, airblast	2.0 WDG, WP, EC	6.0	NA	3	NA	14		10		Some labels specify a retreatment interval of 10 days.
				Total		**2.0**	**6.0**	**NS**	**3.0**	**NA**	**14**	**24**	**10**		Excludes nursery applications (See general "Fruits" listing)
FOOD PROCESSING PLANT PREMISES (NONFOOD CONTACT)				When needed, crack and crevice treatment, spot treatment		0.0424 lb/ gal ME	NS	NA	NS	NA	NA	NS	7		53883-264, 84575-3 Spot Treatment: Do not exceed two square feet per individual spot.
FOREST PLANTINGS (REFORESTATION PROGRAMS) (TREE FARMS, TREE PLANTATION, ETC.)			✓	Foliar, broadcast	ground boom	1.0 EC	6.0	NA	6	NA		24	7		
			✓	Foliar, stump treatment	direct spray, drencher	0.34 EC	6.0	NA	[18] NS	NA			7		
FOREST			✓	Foliar,	ground boom,	0.61	3.6	NA	NS	NA	24		7		

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Crop/Site	Residential	Agricultural	Forestry	Timing; Application Type	Method/ Equipment	Maximum Single Application Rate by Formulation[1] (lb a.i./A)	Maximum Application Rate		Maximum Application Number		PHI (days)[3]	REI (hours)[3]	MRI (days)[3]	Geographic Restrictions	Comments
							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
TREES (SOFTWOODS, CONIFERS)				broadcast	drencher	EC									
			✓	Foliar, stump treatment	direct spray	[3.6] 2.4 lb a.i./100 gal EC	3.6	NA	NS	NA			7		Application rate is provided as a dilution factor.
FRUITS & NUTS Non-bearing (not to bear fruit within 1 year) fruit trees in nurseries (includes: almonds, citrus, filbert, apple, cherry, nectarine, peach, pear, plum, prune).		✓		Foliar-Non-bearing nursery broadcast	High/low volume spay/ hand held sprayer/power sprayer	4.0 EC	4.0	NA	NS	NA	14	NS	7		For nectarines and peaches, the use is restricted to one application of no more than 3 lb a.i./A per cc. For apples, the max rate is 2 lb a.i./A per crop cycle and the use is restricted to 1 application (either canopy or trunk drench) per year. Example label, 62719-254
		✓		Foliar-Non-bearing nursery trunk drench	drencher, high and low pressure sprayer	2.0 WDG	2.0	NA	NS	1	14		7		
				Total		**4.0**	**6.0**								Maximum

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							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
															Single Rates: 3.0 (nectarines and peaches) 2.0 (apples) Maximum Yearly Rates: 3.0 (nectarines and peaches) 2.0 (apples)
GINSENG (MEDCINAL)		✓		Preplant, post-emergence	Ground, soil broadcast	2.0 G	2.0	NA	1	NA	365	24	NA	Permitted in Michigan and Wisconsin	MI110006,WI110003) Minimum incorporation: 4 inches Application should be followed by rainfall or overhead watering. Valid until June 29, 2016.
GOLF COURSE TURF				When needed, soil broadcast/ spot treatment	Ground, low pressure	1.0 EC	2.0	NA	2	NA		24	NS		
				Foliar, broadcast,	Ground boom, handgun, low	1.0 EC, G, B	2.0	NA	2	NA			NS		Chemigation not allowed for

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Crop/Site	Residential	Agricultural	Forestry	Timing; Application Type	Method/ Equipment	Maximum Single Application Rate by Formulation[1] (lb a.i./A)	Maximum Application Rate		Maximum Application Number		PHI (days)[3]	REI (hours)[3]	MRI (days)[3]	Geographic Restrictions	Comments
							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
					pressure and backpack										the EC formulation.
					Tractor drawn spreader, push type spreader, belly grinder	1.0 G						[24] NS	7		
				Mound treatment	Granule applicator	1.0 G	2.0	NS	2	NS		NS	7		
				Total		**2.0**	**2.0**	**NA**	**2**	**NA**	**NS**		**NS**		
GRAPES		✓		Dormant/ Delayed Dormant (pre-bloom)	Ground boom, broadcast, drench high/low spray volume	1.0 WDG, EC	1.0	1	1	NA	35		NS	East of the continental divide only.	Do not use in conjunction with soil surface applications for grape borer control.
		✓				2.0 EC	2.0	1	1	NA	35	24		Permitted in Colorado, Idaho, and Washington	CO080008, ID090004, WA090002 Master label: 62719-591
		✓		Foliar	Ground/ broadcast, basal spray and drench (soil treatment)	2.25 EC	2.25	1	1	NA	35		NS	Permitted east of the continental divide.	
		✓				1.0 EC	3.0	3	3	NA	35		NS	California	CA080010

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Crop/Site	Residential	Agricultural	Forestry	Timing; Application Type	Method/ Equipment	Maximum Single Application Rate by Formulation[1] (lb a.i./A)	Maximum Application Rate		Maximum Application Number		PHI (days)[3]	REI (hours)[3]	MRI (days)[3]	Geographic Restrictions	Comments
							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
		✓		Postharvest, dormant/ delayed dormant	Ground boom, broadcast	2.0 EC	2.0	1	1	NA	NS		NS	California	CA080009
				Total		**2.25**	**2.25**	**1**			**35**	**24**	**NS**	Permitted east of the continental divide.	
						2.0	**5.0**	**4**			**NS**		**NS**	California	
GRASS FORAGE/ FODDER/HAY		✓		Foliar, broadcast	Aircraft, ground boom, chemigation	1.0 EC	3.0	NA	3	NA	NS	24		Permitted in Nevada, Oregon, Washington, and Idaho	NV080004, NV940002, OR090009, WA090010, ID090003
GREENHOUSE		✓		early evening, aerosol, fog or fumigation	Total release fogger	0.029 0.0066 lb a.i./1000 sq. ft PL	NS	NA	NS	NA	NS	NS	2		
HOUSEHOLD/ DOMESTIC DWELLINGS INDOOR PREMISES	✓			When needed	Bait station	0.0003 lb/bait station	NS	NA	NS	NA	NA	NS	NS		9688-67
HYBRID COTTONWOOD/ POPLAR PLANTATIONS		✓		Foliar, dormant, delayed dormant; broadcast	High volume (dilute) Low volume (concentrate)	1.9 EC	[2.0] NS	6.0	[1] NS	3		24	7	Washington	WA090004 Energy wood plantations may be harvested as often as every 2-3 years;

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Crop/Site	Residential	Agricultural	Forestry	Timing; Application Type	Method/ Equipment	Maximum Single Application Rate by Formulation[1] (lb a.i./A)	Maximum Application Rate		Maximum Application Number		PHI (days)[3]	REI (hours)[3]	MRI (days)[3]	Geographic Restrictions	Comments
							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
															pulpwood 5-10 years; and saw timber 15-20 years. (Arkansas production guide). In Washington the crop takes 2-8 years
LEGUME VEGETABLES		✓		Preplant, soil treatment	Ground boom	1.0 EC, WDG	1.0	NA	1	NA	NS	24	NA		No MRI because application only once a year
		✓		At planting, soil treatment	Ground boom	1.0 EC, WDG	1.0	NA	1	NA	NS		NA		
				Total		**1.0**	**1.0**	**NA**	**1**	**NA**	**NS**	**24**	**NS**		Assumed either a preplant or an at plant treatment.
MINT/ PEPPERMINT/ SPEARMINT		✓		Preplant soil incorporated	Aerial or ground/ broadcast	2.0 EC, WDG	[2.0] NS	2.0	[1] NS	1	90	24	NA	No use in Mississippi.	19713-599, 33658-26, 34704-857, 67760-28, 84229-25, 84930-7, OR940027 MRI NA due to once per crop cycle application

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Crop/Site	Residential	Agricultural	Forestry	Timing; Application Type	Method/ Equipment	Maximum Single Application Rate by Formulation[1] (lb a.i./A)	Maximum Application Rate		Maximum Application Number		PHI (days)[3]	REI (hours)[3]	MRI (days)[3]	Geographic Restrictions	Comments
							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
		✓		Post-emergence, Postharvest, Foliar	Chemigation, ground/ airblast	2.0 EC	2.0	2.0	[1] NS	2	90		NS	No use in Mississippi.	Postharvest application retreatment not specified on some labels.
				Total		**2.0**	**4.0**	**4.0**	**2.0**	**3**	**90**	**24**	**NS**		Labels allow one growing season application including pre-plant and one post-harvest application per season.
MOSQUITO CONTROL; HOUSEHOLD/ DOMESTIC DWELLINGS OUTDOOR PREMISES; RECREATIONAL AREAS	✓			When needed; broadcast	Ultra low volume air and ground	0.01 EC	0.26	NA	26	NS	NA	NS	24 h	In Florida: Do not apply by aircraft unless approved by the Florida Dept of Ag.	Aerial applications may be made at altitudes ranging from 75-300 ft (see labels for specifics). For use by federal, state, tribal or local government officials or by persons certified in the appropriate

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Crop/Site	Residential	Agricultural	Forestry	Timing; Application Type	Method/ Equipment	Maximum Single Application Rate by Formulation[1] (lb a.i./A)	Maximum Application Rate		Maximum Application Number		PHI (days)[3]	REI (hours)[3]	MRI (days)[3]	Geographic Restrictions	Comments
							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
															category or authorized by the state or tribal lead regulatory agency.
NECTARINE		✓		**dormant/ delayed dormant** broadcast	airblast, handgun	3.0 WDG, EC	3.0	NA	1	NA	NS	24/ 4d	10		83222-20 others at 2 lb a.i./a
					Aircraft	2.0 WDG, EC									Updated to reflect spray drift mitigation.
		✓		pre-plant, foliar; **trunk spray/drench or pre-plant dip**	Handgun, low pressure backpack, dip	2.5 (3.0/100 gal) WDG, EC	2.5	NA	1	NA	14		5		There is no application retreatment interval specified on some of the label. The application rate is provided as a dilution factor.
				Total		**3.0**	**5.5**	**NA**	**2**	**NA**					Some labels limit the amount a.i./A per year. Multiple types of applications can occur such as preplant, trunk drench

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							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
															and dormant, delayed dormant applications. Excludes nursery applications (See general "Fruits" listing)
NONAGRICULTURAL OUTDOOR BUILDINGS/STRUCTURES to and around outside surfaces of nonresidential buildings and structures. Permitted areas of use include fences, pre-construction foundations, refuse dumps, outside of walls, and other areas where pests congregate or have been seen				Outdoor general surface/ Band (may be better if called perimeter)	Ground sprayer/ band sprayer	1.0 EC	NS	NA	NS	NA	NA	NS	NS		

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							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
NURSERY-STOCK: : Ornamental nursery stock annuals, perennials and woody plants being grown in the field, in ball and burlap or in containers outdoor and in greenhouses				Dormant/ Delayed Dormant	high spray	3.0 EC	3.0	NA	1	NA		24	NS		
				Preplant	Ground boom, soil incorporated	4.0 EC, WP	NS	NA	NS	NA					
				foliar, soil directed	Tractor drawn spreader, push type spreader, belly grinder, gravity fed backpack, spoon	1.1 G									
				Total		**4.0**	**CBD**		**3**						
ONIONS		✓		Post plant (seeding) Broadcast	Ground boom	1.0 EC	1.0	NS	2	NS	60	24	NS		
		✓		At plant, soil drench or basal spray	Ground boom	1.0 EC, WDG, G	1.0		1						2 inch incorporation

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							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
				Total		2.0	2.0		2		60	24	NS		
ORNAMENTAL AND/OR SHADE TREES, HERBACEOUS PLANTS		✓		Foliar broadcast	Ground boom, air blast, handgun, low and high pressure hand wands	2.0 EC, WP 1.0 G, B	2.0	NA	[2] NS	NA	NS	24	NS		Some labels include a MRI of 7 days.
		✓		Dormant /Delayed Dormant	Handgun, low pressure and backpack	3.0 EC	3.0	NA	1	NA	NS		7		Low volume spray permitted for concentrated solutions and lower rates.
ORNAMENTAL LAWNS AND TURF, SOD FARMS (TURF)		✓		When needed, broadcast, soil or spot treatment	ground boom (WP only), high pressure hand wand	3.76 EC, WP	7.52	NA	2	NA	NS	24	NS		
		✓		NS	Tractor drawn spreader, push type spreader, belly grinder	1.0 B	2.0	NA	2	NA	NS	24	NS		Bait is used for fire ant control.
ORNAMENTAL NON-FLOWERING PLANTS		✓		Foliar, broadcast, soil drench	Chemigation, ground boom, low and high pressure handwand, handgun, backpack sprayer, sprinkling can	0.007/gal ME	NS	NA	12	NA	NA	24	NS		Application rate provided as a dilution factor. Restricted use—occupational only

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							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
ORNAMENTAL WOODY SHRUBS AND VINES				Foliar broadcast	Ground boom, air blast, handgun, low and high pressure sprayer, backpack	2.0 EC, WDG 0.01 lb/gal EC	2.0 0.01 lb/gal	NA	[1] NS	NA	NS	24	NS		Several labels do not restrict the application rate in lb a.i./A. Examples include 16.5 lb/100 gal (228-625) and 1.0 lb/100 gal (829-280).
				Dormant/ delayed dormant		1.0 EC 0.005 lb/gal EC	1.0	NA	[1] NS	NA					
				Preharvest	Tractor drawn spreader, push type spreader, belly grinder	6.0 G	6.0	NA	[1] NS	NA					
				Preplant, potted, bailed-and burlapped, containerized	groundboom, handgun, low and high pressure sprayer, backpack, drench	1.0 EC	NS	1	NS	1					
				Pretransplant	groundboom	4.0 WP	[48.0] NS	4	12	4					
				Total		**6.0 G 4.0**	**CBD**		**CBD**						

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							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
						WP									
PEACH		✓		**dormant/ delayed dormant** broadcast	airblast	3.0 EC 2.0 WDG	3.0	NA	1	NA	10	24/ 4d	NS		83222-20 (all other labels restrict to 2 lb ai/a)
					aircraft,	2.0 EC 2.0 WDG							NS		Updated to reflect spray drift mitigation.
		✓		**Post-harvest** broadcast	airblast	2.5 (3.0/100 gal) EC	2.5	NA	1	NA	NA		NS	Permitted in Georgia and South Carolina	GA0400001, SC040001 SLN Expires:
					aircraft	2.0 (3.0/100 gal) EC	2.0								GA0400001, SC040001 SLN Expires: Updated to reflect spray drift mitigation
		✓		pre-plant, foliar; **trunk spray/drench or pre-plant dip**; ground	handheld, backpack, drench/dip, handgun, and low pressure hand wand	2.5 (3.0/100 gal) WDG	2.5	NA	1	NA	14	5	NS		Some labels do not specify minimum retreatment interval.
				Total		**3.0**	**5.5**	**NA**	**3**	**NA**	**NA**	**24**	**NS**		It is possible that multiple types of applications can occur such as
						3.0	**8.0**	**NA**	**3**	**NA**	**NA**	**24**	**NS**	Permitted in Georgia and South Carolina	

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							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
															soil, foliar and/or post-harvest and dormant/ delayed dormant applications. Excludes nursery applications (See general "Fruits" listing)
PEANUT		✓		Preplant	Aerial or ground/ broadcast	2.0 EC, WDG	[4.0] NS	4.0	[2] NS	2	NA	24	10	Do not apply aerial in Mississippi	Assumes one crop cycle per year.
		✓		At plant, postplant		4.0 G	[4.0] NS	4.0	2	2	21	24	10		
		✓		At pegging		2.0 G EC, WDG	[4.0] NS	4.0	2	[2] NS	21	24	10		
				Total		**4.0 G 2.0 EC, WDG**	**4.0**	**4.0**	**2**	**2**	**10**	**24**	**10**		
PEAR		✓		**dormant/ delayed dormant** broadcast	aircraft, airblast	2.0 WDG, EC	2.0	NA	1	NA	NA	24	NA	Restricted use in California.	83222-20 allows 3.0 lb a.i./ A; however, this does not match the 2001 RED.

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							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
		✓		**Post-harvest** broadcast	aircraft, airblast	2.0 WDG, EC	2.0	NA	1	NA	NA	24	NS	Permitted in California, Oregon and Washington.	
				Total		**2.0 WDG, EC**	**4.0**	**NA**	**2**	**NA**	**NA**	**24**	**NS**		Multiple types of applications may occur in within a year in California, Oregon and Washington such as a post-harvest application and a dormant, delayed dormant. Excludes nursery applications (See general "Fruits" listing)
PEAS		✓		Preplant Seed treatment	Seed Treatment	0.30 0.000625 lb/lb seed WP 0.28 0.00058 lb/lb seed	NS	NS	NS	NS	NS	NS	NS		There is a range of potential application rates depending on the number of seeds per lb and the number of seeds planted

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Crop/Site	Residential	Agricultural	Forestry	Timing; Application Type	Method/ Equipment	Maximum Single Application Rate by Formulation[1] (lb a.i./A)	Maximum Application Rate		Maximum Application Number		PHI (days)[3]	REI (hours)[3]	MRI (days)[3]	Geographic Restrictions	Comments
							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
						EC									per acre. Seeding information provide by BEAD.[2]
PECANS		✓		**dormant/ delayed dormant** broadcast	aircraft, airblast	2.0 EC, WDG	2.0	NA	1	NA	14	24	10		66222-19 and 66222-233
		✓		**foliar**; broadcast	airblast	4.3 EC, WDG	6.3	NA	3	NA	14		10		Some labels require a 28 d PHI
					aircraft	2.0 EC, WDG									Updated to reflect spray drift mitigation.
		✓		foliar; **orchard floors broadcast**	Ground boom, chemigation	4.3 EC, WDG	4.3	NA	2	NA	14		10		
				Total		**4.3**	**12.6**	**NA**	**6**	**NA**	**14**	**24**	**10**		Considers multiple type of applications (*e.g.*, dormant, foliar broadcast, and orchard floor) but excluding nursery For nursery applications

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							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
															(See general "Fruits" listing)
PEPPER		✓		Foliar	Ground broadcast	1.0 WDG	[8] NS	8.0	[8] NS	8	7	24	10	Permitted in Florida	FL040005; 1 crop cycle per year.
PINEAPPLE		✓		Postplant	Ground boom, broadcast	2.0 EC	6.0	6.0	3	NA	365	24	30	Permitted in Hawaii	HI090001 SNL Expires: March 29, 2014. Do not make applications beyond three months after planting.
PLUM/ PRUNE		✓		**dormant/ delayed dormant**; broadcast	Aircraft, airblast	2.0 EC, WDG	2.0	NA	1	NA	NA	24/ 4d	10		
		✓		foliar; **trunk spray/drenc h**	handheld, backpack, drench/dip, handgun, and low pressure hand wand	2.5 3.0/100 gal WDG	2.5	NA	1	NA	NA		10		
				Total		**2.5**	**4.5**	NA	2	NA					Excludes nursery applications (See general "Fruits" listing)
POULTRY		✓		When	Sprayer	0.07126	NS	NA	NS	NA	NA		NS		53883-264,

Table 9.1. Summary of Current Chlorpyrifos Usage

Crop/Site	Residential	Agricultural	Forestry	Timing; Application Type	Method/ Equipment	Maximum Single Application Rate by Formulation[1] (lb a.i./A)	Maximum Application Rate		Maximum Application Number		PHI (days)[3]	REI (hours)[3]	MRI (days)[3]	Geographic Restrictions	Comments
							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
LITTER				needed, animal bedding/litter treatment.		a.i./1000 sq ft 3.1 ME									84575-3
PUMPKIN		✓		Preplant Seed treatment	Seed treatment	0.3 0.00058 lb /lb seed WP	[0.3] NS	[1] NS	[1] NS	1	NS	NS	NS	California maximum single rate 0.000625 lb a.i./lb.	There is a range of potential application rates depending on the number of seeds per lb and the number of seeds planted per acre. Seeding information provide by BEAD.[4]
RADISH		✓		Foliar	Broadcast ground	1.0 EC	NS	1	NS	1	NS	24	NS	permitted in Oregon	OR090012 on radish grown for seed. Label valid until December 31, 2012. (per registrant SLN still valid)
		✓		Preplant	Soil incorporation ground	3.0 EC	12.0	3	4	1	NS	NS	10		
		✓		At plant/post-plant	In furrow drench/	3.0 EC	[15.0] NS	3	[5] NS	1	30, EC,	24	10		Only one granular

Table 9.1. Summary of Current Chlorpyrifos Usage

Crop/Site	Residential	Agricultural	Forestry	Timing; Application Type	Method/ Equipment	Maximum Single Application Rate by Formulation[1] (lb a.i./A)	Maximum Application Rate		Maximum Application Number		PHI (days)[3]	REI (hours)[3]	MRI (days)[3]	Geographic Restrictions	Comments
							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
					treatment	2.8 G					7, G				application permitted.
				Total		**3.0**	**[22.0] NS**	**2**	**[9] NS**						Only one preplant or at plant application is assumed.
RIGHTS OF WAY, ROAD MEDIANS				When needed, soil broadcast	Granular or low pressure wand	1.0 EC, G, Bait	[2.0] NS	NA	2	NA	NA	NS	7		Apply when needed
RUTABAGA		✓		Preplant	Chemigation, Groundboom	2.4 EC, WDG	[4.8] NS	2.4	[2] NS	1	30	24	10		
					Aerial	2.0 EC, WDG		2.0							Updated to reflect spray drift mitigation.
		✓		At plant/post-plant	In furrow drench/ treatment	2.4 EC, G WDG	4.8	2.4	[2] NS	1	7	24	10	Disallowed in California and Arizona.	Two crop cycles per year
				Total		**2.4**	**[9.6] NS**	**4.8**	**[4] NS**	**2**		**24**	**10**		
SEWER MANHOLE COVERS AND WALLS				When needed	Low pressure	0.31 lb/manhole RTU	NS	NA	NS	NA	NA	NA	NS		3 pints product/ manhole
SEED ORCHARD TREES		✓		**foliar**; broadcast	Ground boom	1.0 EC	3.0	3.0	NS	NA	30	24	7		62719-575, 62719-615
		✓			High volume	2.5	2.5	NS	[1]	NA	30	24	7		Cone worm

Table 9.1. Summary of Current Chlorpyrifos Usage

Crop/Site	Residential	Agricultural	Forestry	Timing; Application Type	Method/ Equipment	Maximum Single Application Rate by Formulation[1] (lb a.i./A)	Maximum Application Rate		Maximum Application Number		PHI (days)[3]	REI (hours)[3]	MRI (days)[3]	Geographic Restrictions	Comments
							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
					sprayer	0.01 a.i./tree 0.02 EC			NS						treatment (62719-575 and 62719-615) Treatment of 1000 trees per acre would results in an single application rate of 10 lb a.i./a. DAS: 1000 is a bit high, typically for orchards 312 trees per acre
		✓		foliar; **stump treatment**	backpack, drencher, low pressure hand wand,	0.3 EC	0.3	1.0	NS	NA	30	24	7		62719-575, 62719-615
				Total		**1.0**	**5.8**	**3**	**NS**	**NA**	**30**	**24**	**7**		The total number of applications assumed is either three foliar applications or two foliar applications with one stump treatment.

Table 9.1. Summary of Current Chlorpyrifos Usage

Crop/Site	Residential	Agricultural	Forestry	Timing; Application Type	Method/ Equipment	Maximum Single Application Rate by Formulation[1] (lb a.i./A)	Maximum Application Rate		Maximum Application Number		PHI (days)[3]	REI (hours)[3]	MRI (days)[3]	Geographic Restrictions	Comments
							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
SORGHUM GRAIN		✓		Seed Treatment	Seed treatment	[0.0009] 0.01-0.0024 lb ai/ 100 lbs seed EC	0.01	0.01	[1] NS	1	NA	NS	NS		264-932
		✓		Preplant Soil Directed	Ground Spreader/T Band	1.5 G	1.5	1.5	[1] NS	1	60	24	10		
		✓		Foliar/Post emergent	Ground, Aerial, Chemigation	1.0 EC, WDG	1.5	[1.5] NS	[1] NS	3	30	24	10		PHI varies across labels
				Total		**3.3 G 1.0 EC, WDG**	**3.01**	**3.01**	**[3] CBD**	**3**	30	24	10		One crop cycle per year.
SOYBEAN		✓		foliar , post-emergence soil broadcast	broadcast ground, aerial, chemigation	1.0 EC, WDG	3.0	3.0	3	3	28	24	14		One crop cycle per year.
		✓		At plant/post plant treatment; soil band	ground boom	2.2 G 1.0 EC	3.0	3.0	1 (G), 3 (EC)	1 (G), 3 (EC)	28	24	10		
				Total		**1.0 EC, WDG 2.2 G**	**3.0**	**3.0**	**3**	**3**					One crop cycle per year.
STRAW-BERRIES		✓		Pre-plant	Aerial or ground/ broadcast	2.0 EC	2.0	**NS**	1	NS	NA	24	10	No use in Mississippi	33658-26

Table 9.1. Summary of Current Chlorpyrifos Usage

Crop/Site	Residential	Agricultural	Forestry	Timing; Application Type	Method/ Equipment	Maximum Single Application Rate by Formulation[1] (lb a.i./A)	Maximum Application Rate		Maximum Application Number		PHI (days)[3]	REI (hours)[3]	MRI (days)[3]	Geographic Restrictions	Comments
							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
		✓		Foliar	Aerial or ground/ broadcast, foliar spray	1.0 EC, WDG	2.0	**NS**	2	**NS**	21	24	10		Two applications (2 lb ai) for all products per cc.
		✓		Post harvest	Ground directed spray	1.0 EC, WDG	2.0	**NS**	2	**NS**	21		14		
				Total		**2.0**	**4.0**		**3**						One preplant application and two foliar and/or postharvest application permitted per year.
SUNFLOWER		✓		At plant	Aerial/ground	2.0 G	3.0	3.0	[1] NS	1	42	24	10		Per registrant 1 cc per year
		✓		Preplant		2.0 EC, WDG	3.0	3.0	[1] NS	1	42		10		2 inches min incorporation
		✓		Post emergent or foliar		1.5 EC, WDG	3.0	3.0	[2] NS	2	42		10		
				Total		**2.0**	**5.0**	**5.0**	**3**	**3**					Assumed either an at plant or preplant application followed with two foliar applications.

Table 9.1. Summary of Current Chlorpyrifos Usage

Crop/Site	Residential	Agricultural	Forestry	Timing; Application Type	Method/ Equipment	Maximum Single Application Rate by Formulation[1] (lb a.i./A)	Maximum Application Rate		Maximum Application Number		PHI (days)[3]	REI (hours)[3]	MRI (days)[3]	Geographic Restrictions	Comments
							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
															One crop cycle per year
SWEET POTATO		✓		Preplant, soil broadcast	Aircraft, ground boom	2.1 G, EC, WDG	2.1	NS	1	1	125	24		LA090002, MS080007, NC090001 permits 60 PHI	
					Aircraft	2.0 G, EC, WDG									Updated to reflect spray drift mitigation.
TOBACCO		✓		Preplant	Aircraft, ground boom	2.0 EC, G, WDG	2.0	NS	1	1	7	24	NA		
TRITICALE		✓		Storage Commercial Slurry Seed Treatment	Seed treatment	0.003 0.0024 lb ai/ 100 lbs seed EC	[0.003] NS	[1] NS	[1] NS	[1] NS	NA	[10] NS	[10] NS		264-932 Seeding information provide by BEAD.[4] One crop cycle per year.
TURNIP		✓		Preplant	soil incorporation/ ground boom, handgun	2.3 G, WDG	[4.6] NS	2.3	[2] NS	1	30	24	10		Minimum incorporation: 2 inches.
		✓		Postplant	Soil incorporation/ ground boom, handgun	2.3 G, WDGP	[4.6] NS	2.3	[2] NS	1	30	24	10		Minimum incorporation: 2 inches.
				Total		**2.3**	**4.6**	**2.3**	**2**	**1**	**30**	**24**	**10**		Assumed either a preplant or postplant application. Two crop cycles per year

Table 9.1. Summary of Current Chlorpyrifos Usage

Crop/Site	Residential	Agricultural	Forestry	Timing; Application Type	Method/ Equipment	Maximum Single Application Rate by Formulation[1] (lb a.i./A)	Maximum Application Rate		Maximum Application Number		PHI (days)[3]	REI (hours)[3]	MRI (days)[3]	Geographic Restrictions	Comments
							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
UTILITIES For use in and around telecommunications, power, utilities and railroad systems equipment: Buried cables, cable television pedestals, cables, pad-mounted electric power transformers, telephone cables, underground vaults, telecommunications equipment, power and utilities equipment				When needed, broadcast	Product container	190.5 G 0.44 lba.i./100 sq ft (see comments)	NS	NS	NS	NS	NS	NS	NS		Applications permitted as needed. Reg. Nos. 13283-14, 13283-17 Broadcast product onto the ground covering the area of the pad location, plus a two foot perimeter around the outside of the pad location.
WALNUTS		✓		**dormant/ delayed dormant**; broadcast	Aircraft, airblast	2.0 EC, WDG	2.0	NA	1	NA	14	24	10		62719-301 (12 lb a.i./A)
		✓		**foliar**; broadcast	aircraft, airblast, chemigation	2.0 EC, WDG	4.0	NA	2	NA	14		10		Some labels do not specify retreatment interval.
		✓		foliar;	Ground boom,	4.0	4.0	NA	1	NA	14		10		

Table 9.1. Summary of Current Chlorpyrifos Usage

Crop/Site	Residential	Agricultural	Forestry	Timing; Application Type	Method/ Equipment	Maximum Single Application Rate by Formulation[1] (lb a.i./A)	Maximum Application Rate		Maximum Application Number		PHI (days)[3]	REI (hours)[3]	MRI (days)[3]	Geographic Restrictions	Comments
							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
				orchard floors broadcast	chemigation	EC, WDG									
				Total		**4.0**	**10.0**		**4**						Excluding nursery applications; includes dormant, foliar broadcast, and orchard floor. For nursery applications (See general "Fruits" listing)
WIDE AREA/ GENERAL OUTDOOR TREATMENT For ants and other misc. pests.	✓	✓		when needed, Broadcast	Ground sprayer	0.5084 lb ai/100 gal EC	[1.02] NS	NA	2	NA	NA	NS	NS		66222-19
				when needed, Drench	Drench	1	NS	NA	NS	NA	NA		NS		228-624
						[1] 8.2 lb a.i/100 gal EC	NS	NA	NS	NA	NA		NS		228-625
				Total		[1]	NS	NA	NS	NA	NA				
WHEAT		✓		Slurry Seed Treatment	Seed treatment	0.003 0.0024 lb ai/ 100 lbs seed EC	[0.006] NS	1	[2] NS	1	NA	NA	NA	Only for use in AZ, CA, CO, ID, KS, MN, MO, NE, NM, NV, ND, OK, OR,	Seeding information provide by BEAD.[4]
		✓		Foliar, soil treatment	Ground, broadcast	0.5 EC	[8.0] NS	4.0	[2] NS	1	14/ 28	24	14		PHI: 14 forage or hay, 28 grain or straw

Table 9.1. Summary of Current Chlorpyrifos Usage

Crop/Site	Residential	Agricultural	Forestry	Timing; Application Type	Method/ Equipment	Maximum Single Application Rate by Formulation[1] (lb a.i./A)	Maximum Application Rate		Maximum Application Number		PHI (days)[3]	REI (hours)[3]	MRI (days)[3]	Geographic Restrictions	Comments
							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					
		✓		Post-emergence foliar	Ground, Aerial, Chemigation	1.0 EC	[4.0] NS	2.0	[4] NS	2	14/ 28		NS	SD, TX, UT, WA and WY	Label states 1.0 lb ai/A for cereal leaf beetles and then state max rate 0.5 lb ai/A in restriction). Some labels restrict no more than 2 applications per crop/season PHI 14 forage or hay, 28 grain or straw
				Total		**[1] 4.0 EC**	[12.006]	**[6.003] 5.0**	[8] NS	[4] 2					MO otherwise 2.0 plus seed treatment
WOOD PROTECTION TREATMENT TO BUILDINGS/ PRODUCTS OUTDOOR				When needed, Wood surface treatment	Low pressure handwand, backback sprayer, paintbrush	16.65 lb/10,000 sq ft 0.17 lb a.i./gal EC	NS	NA	NS	NA	NS	NS	NS		
						0.08 lb ai/gal EC, RTU EC, ME	NS	NA	NS	NA	NS	NS	NS		Apply 1 gal per 100 sq ft of wood

Table 9.1. Summary of Current Chlorpyrifos Usage

Crop/Site	Residential	Agricultural	Forestry	Timing; Application Type	Method/ Equipment	Maximum Single Application Rate by Formulation[1] (lb a.i./A)	Maximum Application Rate		Maximum Application Number		PHI (days)[3]	REI (hours)[3]	MRI (days)[3]	Geographic Restrictions	Comments
							Per Year lb a.i./A	Per CC[2] lb a.i./A	Per Year	Per CC[2]					

1. EC - emulsifiable concentrate; WDG – water dispersible granular in water soluble packet; WP – wettable power in water soluble packet; B – bait (granular), G – granular; ME – microencapsulated; RTU – ready to use.
2. Reported as per crop cycle or per season
3. PHI – Preharvest interval; REI – reentry interval; MRI – Minimum retreatment interval
4. Becker, J.; Ratnayake, S. Acres Planted per Day and Seeding Rates of Crops Grown in the United States, U.S. EPA OPP/BEAD, 2011; example calculations provided below:
   Beans: 0.00058 lb a.i./lb seed / 960 seeds/lb seed x 418,176 seeds/A [pgs. 19, 81 (beans, succulent)]
   Corn: 0.000625 lb a.i./lb seed / 1,800 seeds/lb seed x 59,739 seeds/A [pgs. 24, 81 (corn, sweet)]
   Cotton: 0.00116 lb a.i./lb seed / 4,500 seeds/lb seed x 85,00 seeds/A [pgs. 13, 81]
   Cucumber: 0.00058 lb a.i./lb seed / 12,000 seeds/lb seed x 80,418 seeds/A [pgs. 25, 81]
   Peas: 0.000625 lb a.i./lb seed / 1,361 seeds/lb seed x 653,400 seeds/A [pgs. 34, 82]
   Pumpkin: 0.00058 lb a.i./lb seed / 1,600 seeds/lb seed x 7,260 seeds/A [pgs. 37, 82]
   Sorghum: 0.001 lb a.i./lb seed / 11,000 seeds/lb seed x 100,000 seeds/A [pgs. 16, 39]
   Triticale: 0.003 lb a.i./100 lb seed / 109 lb seed/A [pg.16]
   Wheat: 0.003 lb a.i./100 lb seed /116 lb seed/A [pg. 16]
   [ ] indicate assumptions that are made when the information is not specified but can be inferred

Appendix 10. Dose Reconstruction Analysis

Introduction

In order to better understand the possible exposure patterns in the Columbia University study cohort and their influence on the health outcomes identified in the research, an analysis was conducted to supplement the exposure metrics reported by the Columbia investigators. The goal was to predict potential exposures which could have occurred to pregnant women and young children who may have been exposed to chlorpyrifos through other pathways not addressed in the research then determine their potential for associated cholinesterase inhibition. Chlorpyrifos, at the time, was one of the most widely used insecticides for indoor pest control so there are many possible ways which exposures could have occurred as a result of these types of uses (e.g., use of a total release fogger, aerosol can sprays, or crack and crevice treatment by a pest control operator). This analysis was based on the Agency's *SOPs for Residential Exposure Assessment*[76] which describe, on a scenario basis, specific algorithms and data used to predict the potential exposures which could have occurred to individuals in the cohort. Once complete, the resulting dose estimates were used to evaluate the potential level of cholinesterase inhibition which could be attributed to them using the PBPK-PD model. The scenarios which were considered include:

- Pregnant mothers who may have purchased a consumer aerosol can product and applied it in their homes;
- Exposures to pregnant mothers who may have had contact with residues in their homes after a previous treatment which leads to exposure; and
- Exposures to young children (aged 1 to 2 years old) who may have contact with residues in their homes after a previous treatment which leads to exposure.

This appendix describes the details of those calculations.

Use Profile

In order to evaluate the chlorpyrifos exposure potential for the Columbia study cohort, it was first necessary to define what chlorpyrifos products would have been in use during the time of study conduct which spanned from 1998 to 2004. In 1997, the registrant, Dow AgroSciences, voluntarily agreed to cancel chlorpyrifos registrations for indoor broadcast use and direct pet treatments, except pet collars. In December 2001, the majority of the remaining chlorpyrifos residential products -- except for fully contained ant baits in child resistant packaging and limited public health uses -- were subject to voluntary phase out/cancellation. This analysis reconstructed the potential exposures for those in the cohort between the years 1997-2001 based on the chlorpyrifos uses that remained after the voluntary cancellation in 1997 and the phase out in 2001. The exposure questionnaire-based reported use from the Columbia publications was also used to inform this process. Per the publications, pest control measures were used by 92% of respondents, whether by housing superintendent, pest control operator (PCO), or by themselves. Of these, respondents reported that 39% were by PCO, 26% by spray can, and 5% foggers/bombs. The majority reported regular use of a pesticide product, at least once per

[76] http://www.epa.gov/pesticides/science/residential-exposure-sop.html

month. Unfortunately, while these data help inform the types of products used, they do not allow for the determination of what pesticide active ingredient(s) were used. Multiple active ingredients were in regular use during this time period including, chlorpyrifos, diazinon, and propoxur, among others. For the purpose of the dose reconstruction exercise, it was assumed that only chlorpyrifos applications occurred. Considering all the available information, the potential for chlorpyrifos exposures from use of the following chlorpyrifos product types were assessed: broadcast ready to use (RTU) sprays applied by individual themselves and not professional applicators; surface sprays applied by individuals; crack and crevice sprays applied by individuals themselves or professional pest control applicators; and foggers/bombs applied by individuals. These chlorpyrifos residential products contained either 0.5% or 1% active ingredient. The latter was assumed to ensure an assessment reflective of the highest potential exposures, or those most likely to result in the greatest % RBC AChE inhibition.

Exposure Assessment Methods

With the products and application rates defined, the 2012 *Standard Operating Procedures for Residential Pesticide Exposure Assessment*[77] were used to assess the exposures that may have occurred from these indoor residential chlorpyrifos uses. Based on the use patterns of the defined products, it is expected that exposures occurred either from the application (handling) of products by those in the cohort or after applications from being in contact with previously treated indoor environments.

A series of assumptions and exposure factors serve as the basis for completing residential handler and post-application exposure assessment:

- maximum application rates allowed by labels in its risk assessments are assumed;
- the use of personal protective equipment is not considered in residential risk assessments;
- residential assessments are based on the assumption that individuals are wearing shorts, short-sleeved shirts, socks, and shoes;
- adults are assumed to perform all pesticide applications;
- the mean female bodyweight reflective of all trimesters of pregnancy, 75 kg, was assumed for pregnant women to reflect the population of interest from the Columbia cohort being evaluated which was derived from the EPA Exposure Factors Handbook 2011 Edition[78] (adult and adult female: Tables 8-3 through 8-5; body weight of pregnant women: Table 8-29).
- the most protective index lifestage which serves as the basis for evaluating young children's indoor exposures are children 1 to < 2 years old as described in in the 2012 Residential SOPs;
- the body weight used to evaluate exposures to children 1 to < 2 years old is 11 kilograms.

For residential handler exposure assessment, the following assumptions and exposure factors apply:

- a broadcast application of an aerosol can is considered and the entire contents of the can (16 oz.) are applied at once, half the contents of the spray can are applied for crack and crevice applications, and a quarter of the can contents for a space spray application.

[77] http://www.epa.gov/pesticides/science/USEPA-OPP-HED_Residential%20SOPs_Oct2012.pdf
[78] http://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=236252

For residential post-application exposure assessment, the following assumptions and exposure factors apply:

- inhalation exposures are expected to occur up to 2 hours following application by which time the aerosolized particulate is assumed to have settled and, as a result, after that time frame exposures are assumed to be negligible;

Use of the PBPK-PD Model

Once complete, the PBPK-PD model was used to estimate the potential level of cholinesterase inhibition (% RBC AChE) attributable to the daily dose estimates that result in the highest exposure potential. In order to model the potential health outcomes in a manner reflective of these exposures, a series of inputs were used that correspond to those used to quantify the daily dose estimates. The following exposure characteristics were used: number of days exposed; body weights which vary by lifestage; daily exposure duration (hours per day, days per week); and the fraction of the dermal surface area exposed.

The dose reconstruction exercise was modeled for a subsequent 14 day post-application duration. The daily dose estimated with the 2012 Standard Operating Procedures for Residential Pesticide Exposure Assessment for the day of application was inputted into the PBPK-PD model. Dermal exposure was modeled assuming that chlorpyrifos residues dissipated at a rate of 10% per day. A 14 day period was used as the basis of the PBPK-PD modeling to ensure it adequately addressed the time it takes for the ACHE enzyme inhibition to reach a steady state.

For the adult and children 1 to < 2 years old age groups, the appropriate body weights were obtained for modeling purposes from the Exposure Factors Handbook[79] (USEPA, 2011). The mean adult female bodyweight reflective of all trimesters of pregnancy, 75 kg, was assumed for pregnant women to reflect the population of interest from the Columbia cohort being evaluated (Exposure Factors Handbook: adult and adult female: Tables 8-3 through 8-5; body weight of pregnant women: Table 8-29). For children 1 to < 2 years old, the body weight was set to 11 kg (Exposure Factors Handbook, Table 8-3, mean body weight for the 1 to < 2 year old age group). For the evaluation of adult handler exposures (dermal and inhalation) it was assumed that the application event continued for 1 hour daily and that it occurred each day of the 14 day exposure period. The 2012 Residential SOPs makes no recommendation regarding the daily exposure duration for handlers. It was assumed that 1 hour is a high end estimate of the time required to make an aerosol can application. For the evaluation of adults and children 1 < 2 years old post-application exposures (i.e., adults: dermal; children: dermal and incidental oral), the daily exposure duration was assumed to be 2 hours daily and that it occurs each day in the 14 day exposure period. This exposure duration is consistent with that recommended in the 2012 Residential SOPs for exposures on hard surfaces. For adults and children 1 to < 2 years old inhalation exposures, it was assumed that the exposure duration was 2 hours on the day of application (Day 0) and was not repeated because the exposure source (i.e., aerosols from an application event) occurred only once on the first day of the exposure period. This is consistent with the available monitoring data and consistent with the 2012 Residential SOPs, the Day 0 air monitoring measures were followed by a marked drop to negligible levels by the following day. For all dose reconstruction dermal exposures to chlorpyrifos, the fraction of skin in contact with

[79] http://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=236252

chlorpyrifos was 50% which reflected uncovered skin areas for children wearing shorts and a tee shirt. No shower (i.e., washing off the chlorpyrifos) was assumed to occur for the 14 day exposure duration modeled.

Residential Handler Exposure Scenarios

The residential handling of chlorpyrifos residential products are expected to have occurred for some percentage of the Columbia cohort population, these are anticipated to have resulted in dermal and inhalation exposures. Dermal and inhalation residential handler exposures are predicted by use of a factor known as the unit exposure. Unit exposure is the ratio, for a given formulation and application equipment, of an individual's exposure the amount of active ingredient handled (AaiH), expressed as mass active ingredient exposure per mass active ingredient handled (e.g., mg/lb ai). For the dose reconstruction assessment, unit exposure data were used as recommended in the 2012 Residential SOPs for ready-to-use (RTU) aerosol cans (dermal, 370 mg/ lb ai; inhalation, 3.0 mg/lb ai). Residential handler exposures from application of fogger formulations were not assessed which is consistent with the 2012 Residential SOPs. Fogger product labels typically direct the user to activate the device and evacuate the treated area and not to re-enter for several hours. Therefore, it is assumed that handler exposure from fogger application is not expected.

The exposure assessment for chlorpyrifos Columbia cohort handler exposures is based on the following exposure scenarios:

- Adult dermal exposure from broadcast application of a RTU spray can;
- Adult inhalation exposure from broadcast application of a RTU spray can;
- Adult dermal exposure from crack and crevice application of a RTU spray can;
- Adult inhalation exposure from crack and crevice application of a RTU spray can;
- Adult dermal exposure from space spray application of a RTU spray can;
- Adult inhalation exposure from space spray application of a RTU spray can.

Handler Exposure Algorithm

Daily dermal and inhalation exposure (mg/day) for residential pesticide handlers, for a given formulation-application method combination, is estimated by multiplying the formulation-application method-specific unit exposure by an estimate of the amount of active ingredient handled in a day, using the equation below:

$$E = UE * AR * A$$

where:

E = exposure (mg/day);
UE = unit exposure (dermal, 370 mg/ lb ai; inhalation, 3.0 mb/lb ai);
AR = application rate (e.g., lb ai/can); and
A = area treated or amount handled (broadcast, 1 can/day; crack and crevice, 0.5 can/day; space spray, 0.25 can/day).

Residential Handler Exposure/Cholinesterase Inhibition Summary

Worst-case results of this analysis are presented below representing pregnant women who potentially made a broadcast application of an aerosol spray can (Table 10.1). Peak % RBC AChE inhibition estimated for pregnant women from combined dermal and inhalation exposures occurring from product application is 0.0012%.

Table 10.1. Residential Handler (Pregnant Women in the Columbia Cohort) Estimated Exposures and Predicted % ChE Inhibition (Route-specific and Combined)

Exposure Scenario	Formulation	Amount Handled	Dermal Dose (mg/kg/day)	Dermal: Peak % ChE Inhibition	Inhalation Dose (mg/kg/day)	Inhalation: Peak % ChE Inhibition	Combined Peak % ChE Inhibition
Broadcast	Ready to Use: 1 % Aerosol Spray Can	1 can	0.049	0.0007%	0.030	0.0008%	0.0012%

a. Dermal Exposure = Dermal Unit Exposure (370 mg/lb ai) × Application Rate (0.010 lb ai/can) × Area Treated or Amount Handled (cans/day)
b. Inhalation Exposure = Inhalation Unit Exposure (3.0 mg/lb ai) × Application Rate (0.010 lb ai/can) × Area Treated or Amount Handled (cans/day)

Residential Post-application Exposure Scenarios

The use of indoor residential chlorpyrifos products are anticipated to have resulted in the potential for post-application exposures from public housing residents in the Columbia study cohort being in a previously treated environment. Based on the product use profile, residents may have been exposed via inhalation to the aerosols resulting from application or by means of contact following settling of product contents following settling on indoor surfaces or flooring. The exposure assessment for chlorpyrifos Columbia cohort post-application exposures is based on the following exposure scenarios:

- Adult dermal contact with residues deposited on flooring (carpeted and hard) from broadcast application of RTU spray can;
- Adult inhalation of aerosolized particulates following broadcast application of RTU spray can;
- Adult dermal contact with residues deposited on flooring (carpeted and hard) from space spray application of RTU spray can;
- Adult inhalation of aerosolized particulates following space spray application of RTU spray can;
- Adult dermal contact with residues deposited on flooring (carpeted and hard) from crack and crevice RTU spray can or PCO application;
- Adult inhalation of aerosolized particulates following crack and crevice application of RTU spray can;
- Children 1 to < 2 years old dermal contact with residues deposited on flooring (carpeted and hard) from broadcast application of RTU spray can;
- Children 1 to < 2 years old incidental oral (hand-to-mouth) ingestion of residues deposited on flooring (carpeted and hard) from broadcast application of RTU spray can that have transferred to children's hands;
- Children 1 to < 2 years old incidental oral (object-to-mouth) ingestion of residues deposited on toys in contact with flooring (carpeted and hard) treated by broadcast application of RTU spray can;
- Children 1 to < 2 years old inhalation of aerosolized particulate following broadcast

application of RTU spray can;
- Children 1 to < 2 years old dermal contact with residues deposited on flooring (carpeted and hard) from space spray application of RTU spray can;
- Children 1 to < 2 years old incidental oral (hand-to-mouth) ingestion of residues deposited on flooring (carpeted and hard) from space spray application of RTU spray can that have transferred to children's hands;
- Children 1 to < 2 years old incidental oral (object-to-mouth) ingestion of residues deposited on toys in contact with flooring (carpeted and hard) treated by space spray application of RTU spray can;
- Children 1 to < 2 years old inhalation of aerosolized particulate following space spray application of RTU spray can;
- Children 1 to < 2 years old dermal contact with residues deposited on flooring (carpeted and hard) from crack and crevice RTU spray can or PCO applications;
- Children 1 to < 2 years old incidental oral (hand-to-mouth) ingestion of residues deposited on flooring (carpeted and hard) from crack and crevice RTU spray can or PCO applications that have transferred to children's hands;
- Children 1 to < 2 years old incidental oral (object-to-mouth) ingestion of residues deposited on toys in contact with flooring (carpeted and hard) treated by crack and crevice RTU spray can or PCO applications;

Since all exposure routes (dermal, inhalation, and incidental oral) share a common toxicological endpoint, RBC AChE inhibition, risk estimates should been combined. The incidental oral scenarios (i.e., hand-to-mouth and object-to-mouth) should be considered inter-related and it is likely that they occur interspersed amongst each other across time. Combining these scenarios with the dermal exposure scenario would be unrealistic because of the conservative nature of each individual assessment. Therefore, the post-application exposure scenarios that were combined for children 1 < 2 years old are the dermal and hand-to-mouth scenarios. This combination should be considered a protective estimate of children's exposure to pesticides used indoors.

The lifestages selected for each post-application scenario are based on an analysis provided as an Appendix in the 2012 Residential SOPs. While not the only lifestage potentially exposed for these post-application scenarios, the lifestage that is included in the quantitative assessment is health protective for the exposures and risk estimates for any other potentially exposed lifestage.

Post-application Exposure Data

The 2012 Residential SOPs relied on exposure data from Agency submitted studies in order to establish many of the inputs necessary for estimation of post-application indoor exposures. Where chemical-specific exposure data are available, these data were relied upon as a more accurate measure of exposures from chlorpyrifos and are described below. In the absence of these data, exposure inputs are based on the use of available exposure data as recommended in the 2012 Residential SOPs.

Exposure Data Used for Dermal and Incidental Oral Exposure Assessment: No chemical-specific deposition data were available for the indoor chlorpyrifos products assessed. Therefore, as recommended by the 2012 Residential SOPs, default deposited residue values were used

based on the type of application to be made whether it be a broadcast, crack and crevice, or space spay application. For broadcast applications only, residue values were influenced by the percent spray applied (i.e., the higher the percent spray, the higher the residue values). The higher, 1% chlorpyrifos spray formulation was assumed for post-application assessment of broadcast exposures. The default values used are based on an analysis of available residue deposition data from Agency submitted studies and literature studies. A summary of the recommended values are presented in the 2012 Residential SOPs.

The transfer coefficent (TC) input used for calculation of post-application exposures is a measure of surface-to-skin residue transfer derived from concurrent measures of exposure and surface residue. Per the 2012 Residential SOPs, no studies were available that measure both exposure and surface residue with subjects performed typical indoor activities. Therefore, the transfer coefficients used for indoor scenarios are derived from information provided in three different studies: 1) two studies which measured exposure and surface residues while subjects performed a Jazzercise routine (Krieger, 2000 and Selim, 2004) and 2) a study which measured biomonitoring doses which adults performed scripted activities for 4 hours on carpets (Vaccaro, 1991). Of these studies, the Krieger study was conducted using chlorpyrifos.

As for the fraction of residue available for transfer input, a complete dataset was compiled for chlorpyrifos based on available chemical-specific data (Beamer et al., 2009). Therefore, the chlorpyrifos-specific residue fractions anticipated to transfer for both carpets and hard surfaces were used for estimation of post-application dermal and incidental oral exposures.
Exposure Data Used for Inhalation Exposure Assessment: In order to best represent the potential for inhalation exposures following use of the indoor chlorpyrifos products assessed, a review of available literature studies and registrant-submitted exposure data was conducted. The following exposure data sources were identified and used:

Indoor broadcast applications –

- Fenske, R., et al (1990). Potential Exposure and Health Risks of Infants following Indoor Residential Pesticide Applications. American Journal of Public Health. 80 (6): 689-693.
- Lu, C. and Fenske, R (1998). Air and Surface Chlorpyrifos Residues following Residential Broadcast and Aerosol Pesticide Applications. Environmental Science and Technology. 32 (10): 1386 -1390.
- EPA MRID: 42887201. Contardi, J. (1993). An Evaluation of the Appropriate Drying Time via Air Monitoring, Dislodgable Residue Determination, and Carpet Weight Loss, After Applying Dursban LO Insecticide to a Carpeted Surface: Unpublished study prepared by Dow Chemical Co., Health and Environmental Sciences. 29 pp.

Indoor crack and crevice applications –

- Byrne, S.L., Shurdut, B.A. and Saunders, D.G. (1998). Potential Chlorpyrifos Exposure to Residents following Standard Crack and Crevice Treatment. 106 (11): 725 -731.
- Hore, P. et al. (2005). Children's Residential Exposures to Chlorpyrifos: Application of CPPAES Field Measurements of Chlorpyrifos and TCPy within

MENTOR/SHEDS – Pesticides Model. Science of the Total Environment. 336 (2 -3): 525-537.

- Stout II, D.M. and Mason, M.A. (2003). The Distribution of Chlorpyrifos following a Crack and Crevice Type Application in the US EPA Indoor Air Quality Research House. Atmospheric Environment. 37 (39 -40): 5539 -5549.
- EPA MRID 44458201: Byrne, S.; Saunders, D.; Cook, W. et al. (1998) Residential Exposure to Chlorpyrifos from Reentry to Structures Treated with Crack and Crevice and Spot Applications of Dursban Pro. Unpublished study prepared by Dow AgroSciences. 133 pp.

Based on a review of the available data and consistent with the 2012 Residential SOPs, the day of application (Day 0) air monitoring measures were significant, followed by a marked drop to negligible levels by the following day. Therefore, HED made use only of day of application air monitoring measures for purpose of post-application exposure assessment. All studies referenced were based on 0.5% chlorpyrifos formulated products. In order to address the potential for use of the 1% chlorpyrifos product formulations available at the time, all air monitoring measures were doubled. The broadcast exposure data are intended for use in assessing the potential inhalation exposures/doses anticipated to occur from both broadcast and space spray applications; the crack and crevice exposure data were used only for that application type. The resulting mean day of application air monitoring measures are presented in Table 10.2 below.

Table 10.2. Airborne Concentrations of Chlorpyrifos from Open Literature and Registrant-submitted Studies Resulting from Indoor Broadcast and Crack and Crevice Applications

Study	Chlorpryrifos Concentration (mg/m^3)
Broadcast	
Fenske, R., et al (1990)	0.18
Lu, C. and Fenske, R (1998)	0.088
EPA MRID: 42887201	0.031
Average (All Studies)	0.092
Crack and Crevice	
Byrne, S.L., Shurdut, B.A. and Saunders, D.G. (1998)	0.0010
Hore, P. et al. (2005).	0.00057
Stout II, D.M. and Mason, M.A. (2003)	0.00086
EPA MRID 44458201	0.0011
Average (All Studies)	0.00089

Post-Application Algorithms for All Scenarios

Post-Application Dermal Exposure Algorithm (hard surfaces and carpets)

The algorithm to calculate exposure is as follows:

$$E = TR * TC * ET * CF1$$

where:

E = exposure (mg/day);

TR = indoor surface transferable residue (μg/cm^2);
TC = transfer coefficient (cm^2/hr);
ET = exposure time (hr/day); and
CF1 = conversion factor (0.001 mg/μg).

If chemical-specific TR data are available, this is preferred and should be used to calculate exposure. However, if chemical-specific TR data are not available, then TR can be calculated using the following formula:

$$TR = DepR * F_{ai}$$

where:
TR = indoor surface transferable residue (μg/cm^2);
DepR = deposited residue (μg/cm^2), based on (in order of preference):
(1) Chemical-specific residue deposition data (μg/cm^2),
(2) Application rate (lb ai/area), or
(3) Default residue based on type of application (μg/cm^2); and
F_{ai} = fraction of ai available for transfer from carpet or hard surface (unitless).

Absorbed dermal dose, normalized to body weight, are calculated as:

$$D = \frac{E * AF}{BW}$$

where:
D = dose (mg/kg-day);
E = exposure (mg/day);
AF = absorption factor; and
BW = body weight (kg).

Table 10.3. Indoor Environments (Hard Surfaces and Carpets) – Inputs for Residential Post-application Dermal Exposure

Algorithm Notation	Exposure Factor (units)			Point Estimate(s)
TR	Transferable residue (μg/cm^2)			Estimated: DepR * F_{ai}
DepR	Deposited residue (μg/cm^2)			Estimated based on default residue related to type of application
F_{ai}	Fraction of DepR as TR following application	Carpets		0.020[a]
		Hard surfaces		0.13[a]
TC	Transfer Coefficient (cm^2/hr)	Adult		6,800
		Children 1 < 2 years old		1,800
ET	Exposure Time (hrs/day)	Adults	Carpets	8
			Hard Surfaces	2
		Children 1 < 2	Carpets	4

Table 10.3. Indoor Environments (Hard Surfaces and Carpets) – Inputs for Residential Post-application Dermal Exposure				
Algorithm Notation	**Exposure Factor (units)**			**Point Estimate(s)**
		years old	Hard Surfaces	2
BW	Body weight (kg)	Adult		80
		Children 1 < 2 years old		11

a. Chlopyrifos-specific as identified (Table 7-8) in the 2012 Residential SOPs.

Post-application Hand-to-Mouth Exposure Algorithm

Exposure from hand-to-mouth activity is calculated as follows (based on algorithm utilized in SHEDS-Multimedia):

$$E = \left[HR * (F_M * SA_H) * (ET * N_Replen) * \left(1 - (1 - SE)^{\frac{Freq_HtM}{N_Replen}}\right)\right]$$

where:

E	= exposure (mg/day);
HR	= hand residue loading (mg/cm^2);
F_M	= fraction hand surface area mouthed / event (fraction/event);
ET	= exposure time (hr/day);
SA_H	= surface area of one hand (cm^2);
N_Replen	= number of replenishment intervals per hour (intervals/hour);
SE	= saliva extraction factor (i.e., mouthing removal efficiency); and
Freq_HtM	= number of hand-to-mouth contacts events per hour (events/hour).

and

$$HR = \frac{Fai_{hands} * DE}{SA_H * 2}$$

where:

HR	= hand residue loading (mg/cm^2);
Fai_{hands}	= fraction ai on hands compared to total surface residue from jazzercise study (unitless);
DE	= dermal exposure (mg); and
SA_H	= typical surface area of one hand (cm^2).

Table 10.4. Indoor Environments – Inputs for Residential Post-application Hand-to-Mouth Exposure				
Algorithm Notation	**Exposure Factor (units)**			**Point Estimate(s)**
Fai_{hands}	Fraction of ai on hands from jazzercise study (unitless)			0.15
DE	Dermal exposure calculated in *Section* ***Error! Reference source not found.*** (mg)			Calculated
HR	Residue available on the hands (mg/cm^2)			Calculated
SA_H	Surface area of one hand (cm^2)	Children 1 < 2 years old		150
AR	Application rate (mass active ingredient per unit area)			1% formulation
F_M	Fraction of hand mouthed per event (fraction/event)			0.13
N_Replen	Replenishment intervals per hour (intervals/hr)			4
ET	Exposure time (hours per day)	Children 1 < 2 years old	Carpets	4
			Hard Surfaces	2
SE	Saliva extraction factor (fraction)			0.48
Freq_HtM	Hand-to-mouth events per hour (events/hr)	Children 1 < 2 years old		20
BW	Body Weight (kg)	Children 1 < 2 years old		11

Post-application Object-to-Mouth Exposure Algorithm

Exposure from object-to-mouth activity is calculated as follows (based on algorithm utilized in SHEDS-Multimedia):

$$E = OR * CF1 * SAM_O * (ET * N_Replen) * \left(1 - (1 - SE)^{\frac{Freq_OtM}{N_Replen}}\right)$$

where:

E	=	exposure (mg/day);
OR	=	chemical residue loading on an object ($\mu g/cm^2$);
CF1	=	weight unit conversion factor (0.001 mg/µg);
SAM_O	=	area of the object surface that is mouthed (cm^2/event);
ET	=	exposure time (hr/day);
N_Replen	=	number of replenishment intervals per hour (intervals/hour);
SE	=	saliva extraction factor (i.e., mouthing removal efficiency); and

Freq_OtM = number of object-to-mouth contact events per hour (events/hour).

and

$$OR = DepR * F_O$$

where:

OR = chemical residue loading on the object (µg/cm^2);
DepR = deposited residue (µg/cm^2); and
F_O = fraction of residue transferred to an object (unitless).

Table 10.5. Indoor Environments – Inputs for Residential Post-application Object-to-Mouth Exposure

Algorithm Notation	Exposure Factor (units)			Point Estimate(s)
AR	Application rate (mass active ingredient per unit area)			**[input]**
F_O	Fraction of residue transferred to an object	Carpets		0.020[a]
		Hard surfaces		0.13[a]
SAM_O	Surface area of object mouthed (cm^2/event)			10
N_Replen	Replenishment intervals per hour (intervals/hour)			4
SE_O	Saliva extraction factor			0.48
ET	Exposure Time (hours per day)	Children 1 < 2 years old	Carpets	4
			Hard Surfaces	2
Freq_OtM	Object-to-mouth events per hour (events/hour)	Children 1 < 2 years old		14
BW	Body Weight (kg)	Children 1 < 2 years old		11

a. Chlopyrifos-specific as identified (Table 7-8) in the 2012 Residential SOPs.

Residential Post-Application Exposure/Cholinesterase Inhibition Summary

The results of this analysis are presented below for pregnant who were potentially exposed from contact to residues which occur in previously treated areas such as their homes (Table 10.6). Results for young children (aged 1 to < 2 years old) who are also exposed to residues which occur in previously treated areas such as their homes are presented in Table 10.7. Peak cholinesterase inhibition for pregnant women resulting from combined dermal and inhalation exposures occurring from contact with previously treated indoor areas is 0.45%. For children 1

to < 2 years old, peak cholinesterase inhibition associated with combined dermal, incidental oral, and inhalation exposures from being in previously treated areas is 2.7%.

Table 10.6. Residential Post-application To Pregnant Women in the Columbia Cohort Estimated Dermal and Inhalation Exposures and Predicted % ChE Inhibition (Route-specific and Combined)

Exposure Scenario	Formulation	Dermal Dose (mg/kg/day)	Dermal: Peak % ChE Inhibition	Airborne Concentration of Chlorpyrifos (mg/m^3)	Inhalation: Peak % ChE Inhibition	Combined: Peak % ChE Inhibition
Broadcast (Hard Surfaces)	1% PCO Application or RTU 1 16 oz can	0.71	0.45%	0.092	0.0049%	**0.45%**

* See algorithms and inputs above used to estimate post-application dermal exposures.

Table 10.7. Residential Post-application (Children 1 to < 2 Years Old in the Columbia Cohort) Estimated Dermal and Inhalation Exposures and Predicted % RBC AChE Inhibition (Route-specific and Combined)

Exposure Scenario	Formulation	Dermal Dose (mg/kg/day)	Dermal: Peak % ChE Inhibition	HTM Dose (mg/kg/day)	HTM Peak % ChE Inhibition (mg/day)	Airborne Concn. of Chlorpyrifos (mg/m3)	Inhalation: Peak % ChE Inhibition	Combined: Peak % ChE Inhibition
Broadcast (Hard Surfaces)	1% PCO Application or RTU 1 16 oz can	1.3	0.14%	0.10	2.2%	0.092	0.014%	2.7%

* See algorithms and inputs above used to estimate post-application dermal, (HTM) hand-to-mouth, and (OTM) object-to-mouth exposures/doses.

Appendix 11. New Literature on Chlorpyrifos since the 2012 FIFRA SAP Meeting

Appendix 11.	Experimental toxicology Literature search for chlorpyrifos	Revised HHRA, 2014		
Comments	**Authors**	**PubDate (Year)**	**PubDate (Month)**	**Full Citation**
Acute effects	Acker CI, Souza AC, Dos Santos MP, Mazzanti CM, Nogueira CW	2012	Sep	Acker CI, Souza AC, Dos Santos MP, Mazzanti CM, Nogueira CW.Diphenyl diselenide attenuates hepatic and hematologic toxicity induced by chlorpyrifos acute exposure in rats. Environ Sci Pollut Res Int. 2012 Sep; 19(8):3481-90.
Acute effects	Cardona D, LÃ³pez-Granero C, CaÃ±adas F, Llorens J, Flores P, Pancetti F, SÃ¡nchez-Santed F	2013		Cardona D, LÃ³pez-Granero C, CaÃ±adas F, Llorens J, Flores P, Pancetti F, SÃ¡nchez-Santed F.Dose-dependent regional brain acetylcholinesterase and acylpeptide hydrolase inhibition without cell death after chlorpyrifos administration. J Toxicol Sci. 2013; 38(2):193-203.
Acute effects	Cardona D, LÃ³pez-Granero C, CaÃ±adas F, Llorens J, Flores P, Pancetti F, SÃ¡nchez-Santed F	2013		Cardona D, LÃ³pez-Granero C, CaÃ±adas F, Llorens J, Flores P, Pancetti F, SÃ¡nchez-Santed F.Dose-dependent regional brain acetylcholinesterase and acylpeptide hydrolase inhibition without cell death after chlorpyrifos administration. J Toxicol Sci. 2013; 38(2):193-203.
Acute effects	Jayawardane P, Senanayake N, Buckley NA, Dawson AH	2012	Apr	Jayawardane P, Senanayake N, Buckley NA, Dawson AH.Electrophysiological correlates of respiratory failure in acute organophosphate poisoning: evidence for differential roles of muscarinic and nicotinic stimulation. Clin Toxicol (Phila). 2012 Apr; 50(4):250-3.
Acute effects	Li B, Eyer P, Eddleston M, Jiang W, Schopfer LM, Lockridge O	2013	Jun	Li B, Eyer P, Eddleston M, Jiang W, Schopfer LM, Lockridge O.Protein tyrosine adduct in humans self-poisoned by chlorpyrifos. Toxicol Appl Pharmacol. 2013 Jun 15; 269(3):215-25.
Acute effects	Liu J, Parsons L, Pope C	2013	Nov	Liu J, Parsons L, Pope C.Comparative effects of parathion and chlorpyrifos on extracellular endocannabinoid levels in rat hippocampus: influence on cholinergic toxicity. Toxicol Appl Pharmacol. 2013 Nov 1; 272(3):608-15.

Acute effects	Lopez-Granero C, Canadas F, Cardona D, Yu Y, Gimenez E, Lozano R, Avila DS, Aschner M, Sanchez-Santed, F.	2013	Jan	Lopez-Granero C, Canadas F, Cardona D, Yu Y, Gimenez E, Lozano R, Avila DS, Aschner M, SÃ¡nchez-Santed F.Chlorpyrifos-, diisopropylphosphorofluoridate-, and parathion-induced behavioral and oxidative stress effects: are they mediated by analogous mechanisms of action? Toxicol Sci. 2013 Jan; 131(1):206-16.
Acute effects	Lukaszewicz-Hussain A	2013		Lukaszewicz-Hussain A.[Serum glucose concentration in subacute intoxication with chlorpyrifos - organophosphate insecticide]. Med Pr. 2013; 64(4):527-31.
Acute effects	Ma P, Wu Y, Zeng Q, Gan Y, Chen J, Ye X, Yang X	2013	Aug	Ma P, Wu Y, Zeng Q, Gan Y, Chen J, Ye X, Yang X.Oxidative damage induced by chlorpyrifos in the hepatic and renal tissue of Kunming mice and the antioxidant role of vitamin E. Food Chem Toxicol. 2013 Aug; 58:177-83.
Acute effects	Montes de Oca L, Moreno M, Cardona D, Campa L, SuÃ±ol C, GalofrÃ© M, Flores P, SÃ¡nchez-Santed F	2013	Feb	Montes de Oca L, Moreno M, Cardona D, Campa L, Sunol C, Galofre© M, Flores P, Sanchez-Santed F.Long term compulsivity on the 5-choice serial reaction time task after acute Chlorpyrifos exposure. Toxicol Lett. 2013 Feb 4; 216(2-3):73-85.
Acute effects	Park JH, Lee JE, Shin IC, Koh HC	2013	Apr	Park JH, Lee JE, Shin IC, Koh HC.Autophagy regulates chlorpyrifos-induced apoptosis in SH-SY5Y cells. Toxicol Appl Pharmacol. 2013 Apr 1; 268(1):55-67.
Acute effects	Rajasekharan C, Renjith SW, Jayapal T	2012	Sep	Rajasekharan C, Renjith SW, Jayapal T.Opsoclonus and lingual myoclonus due to organophosphate poisoning: images in clinical medicine. BMJ Case Rep. 2012 Sep 21; 2012
Acute effects	Starks SE, Gerr F, Kamel F, Lynch CF, Jones MP, Alavanja MC, Sandler DP, Hoppin JA	2012	Jan-Feb	Starks SE, Gerr F, Kamel F, Lynch CF, Jones MP, Alavanja MC, Sandler DP, Hoppin JA.Neurobehavioral function and organophosphate insecticide use among pesticide applicators in the Agricultural Health Study. Neurotoxicol Teratol. 2012 Jan-Feb; 34(1):168-76.

Acute effects/Not related to neurodevelopmental effects	Mullins RJ, Xu S, Pereira EF, Mamczarz J, Albuquerque EX, Gullapalli RP	2013	May	Mullins RJ, Xu S, Pereira EF, Mamczarz J, Albuquerque EX, Gullapalli RP.Delayed hippocampal effects from a single exposure of prepubertal guinea pigs to sub-lethal dose of chlorpyrifos: a magnetic resonance imaging and spectroscopy study. Neurotoxicology. 2013 May; 36:42-8.
Added to Appendix	Ki YW, Park JH, Lee JE, Shin IC, Koh HC	2013	Apr	Ki YW, Park JH, Lee JE, Shin IC, Koh HC.JNK and p38 MAPK regulate oxidative stress and the inflammatory response in chlorpyrifos-induced apoptosis. Toxicol Lett. 2013 Apr 26; 218(3):235-45.
Added to Appendix	Ojha A, Srivastava N	2014	Feb	Ojha A, Srivastava N.In vitro studies on organophosphate pesticides induced oxidative DNA damage in rat lymphocytes. Mutat Res Genet Toxicol Environ Mutagen. 2014 Feb; 761:10-7.
Analytical Method	Papoutsis I, Nikolaou P, Spiliopoulou C, Pistos C, Stefanidou M, Athanaselis S	2012	Mar-Apr	Papoutsis I, Nikolaou P, Spiliopoulou C, Pistos C, Stefanidou M, Athanaselis S.A simple and sensitive GC/MS method for the determination of atropine during therapy of anticholinesterase poisoning in serum samples. Drug Test Anal. 2012 Mar-Apr; 4(3-4):229-34.
Appendix 1	Chen XP, Chen WZ, Wang FS, Liu JX	2012	Sep	Chen XP, Chen WZ, Wang FS, Liu JX.Selective cognitive impairments are related to selective hippocampus and prefrontal cortex deficits after prenatal chlorpyrifos exposure. Brain Res. 2012 Sep 20; 1474:19-28.
Appendix 1	Chiapella G, Flores-MartÃn J, Ridano ME, Reyna L, Magnarelli de Potas G, Panzetta-Dutari GM, Genti-Raimondi S	2013	Sep	Chiapella G, Flores-MartÃn J, Ridano ME, Reyna L, Magnarelli de Potas G, Panzetta-Dutari GM, Genti-Raimondi S.The organophosphate chlorpyrifos disturbs redox balance and triggers antioxidant defense mechanisms in JEG-3 cells. Placenta. 2013 Sep; 34(9):792-8.
Appendix 1	Cole TB, Fisher JC, Burbacher TM, Costa LG, Furlong CE	2012	May-Jun	Cole TB, Fisher JC, Burbacher TM, Costa LG, Furlong CE.Neurobehavioral assessment of mice following repeated postnatal exposure to chlorpyrifos-oxon. Neurotoxicol Teratol. 2012 May-Jun; 34(3):311-22.

Appendix 1	Lee JE, Park JH, Shin IC, Koh HC	2012	Sep	Lee JE, Park JH, Shin IC, Koh HC.Reactive oxygen species regulated mitochondria-mediated apoptosis in PC12 cells exposed to chlorpyrifos. Toxicol Appl Pharmacol. 2012 Sep 1; 263(2):148-62.
Appendix 1	Marty MS, Andrus AK, Bell MP, Passage JK, Perala AW, Brzak KA, Bartels MJ, Beck MJ, Juberg DR	2012	Jul	Marty MS, Andrus AK, Bell MP, Passage JK, Perala AW, Brzak KA, Bartels MJ, Beck MJ, Juberg DR.Cholinesterase inhibition and toxicokinetics in immature and adult rats after acute or repeated exposures to chlorpyrifos or chlorpyrifos-oxon. Regul Toxicol Pharmacol. 2012 Jul; 63(2):209-24.
Appendix 1	Mullen BR, Khialeeva E, Hoffman DB, Ghiani CA, Carpenter EM	2012	Feb	Mullen BR, Khialeeva E, Hoffman DB, Ghiani CA, Carpenter EM.Decreased reelin expression and organophosphate pesticide exposure alters mouse behaviour and brain morphology. ASN Neuro. 2012 Feb 18; 5(1):e00106.
Appendix 1	Ohishi T, Wang L, Akane H, Itahashi M, Nakamura D, Yafune A, Mitsumori K, Shibutani M	2013	Jan	Ohishi T, Wang L, Akane H, Itahashi M, Nakamura D, Yafune A, Mitsumori K, Shibutani M.Reversible effect of maternal exposure to chlorpyrifos on the intermediate granule cell progenitors in the hippocampal dentate gyrus of rat offspring. Reprod Toxicol. 2013 Jan; 35:125-36.
Appendix 1	Reiss R, Neal B, Lamb JC 4th, Juberg DR	2012	Jun	Reiss R, Neal B, Lamb JC 4th, Juberg DR.Acetylcholinesterase inhibition dose-response modeling for chlorpyrifos and chlorpyrifos-oxon. Regul Toxicol Pharmacol. 2012 Jun; 63(1):124-31.
Appendix 1	Vatanparast J, Naseh M, Baniasadi M, Haghdoost-Yazdi H	2013	Feb	Vatanparast J, Naseh M, Baniasadi M, Haghdoost-Yazdi H.Developmental exposure to chlorpyrifos and diazinon differentially affect passive avoidance performance and nitric oxide synthase-containing neurons in the basolateral complex of the amygdala. Brain Res. 2013 Feb 4; 1494:17-27.
Appendix 1	Xu F, Chang X, Lou D, Wu Q, Zhou Z	2012	May	Xu F, Chang X, Lou D, Wu Q, Zhou Z.Chlorpyrifos exposure causes alternation in dopamine metabolism in PC12 cells. Toxicol Mech Methods. 2012 May; 22(4):309-14.

Appendix 1/No body of evidence; Small number of studies without mechanistic hypothesis to outcome	Basha M, Poojary A	2012	May	Basha M, Poojary A.Cold stress offered modulation on chlorpyrifos toxicity in aging rat central nervous system. Toxicol Int. 2012 May; 19(2):173-81.
Appendix 1/No body of evidence; Small number of studies without mechanistic hypothesis to outcome	Basha PM, Poojary A	2012	Feb	Basha PM, Poojary A.Oxidative macromolecular alterations in the rat central nervous system in response to experimentally co-induced chlorpyrifos and cold stress: a comparative assessment in aging rats. Neurochem Res. 2012 Feb; 37(2):335-48.
Appendix 1/No body of evidence; Small number of studies without mechanistic hypothesis to outcome	Poojary A, Basha PM	2012	Feb	Poojary A, Basha PM.Cold stress interaction on organophosphate insecticide poisoning: age-related assessment in rat cerebral cortex. Indian J Exp Biol. 2012 Feb; 50(2):110-6.
Appendix 1/Review Article	Carr RL, Adams AL, Kepler DR, Ward AB, Ross MK	2013	Sep	Carr RL, Adams AL, Kepler DR, Ward AB, Ross MK.Induction of endocannabinoid levels in juvenile rat brain following developmental chlorpyrifos exposure. Toxicol Sci. 2013 Sep; 135(1):193-201.
Appendix 1/Review Article	Flaskos, J.	2012	Feb	Flaskos J.The developmental neurotoxicity of organophosphorus insecticides: a direct role for the oxon metabolites. Toxicol Lett. 2012 Feb 25; 209(1):86-93.
Appendix 1/Review Article	Poet, Torka S., Timchalk, C., Hotchkiss, Jon A., Bartels, M. J.	2014	April	Poet, Torka S., Timchalk, C., Hotchkiss, Jon A., Bartels, M. J.Chlorpyrifos PBPK/PD model for multiple routes of exposure. Xenobioticam 2014; 44(10): 868-881

Biomonitoring Method	Schmidt L, Muller J, Goen T	2013	Feb	Schmidt L, Muller J, Goen T.Simultaneous monitoring of seven phenolic metabolites of endocrine disrupting compounds (EDC) in human urine using gas chromatography with tandem mass spectrometry. Anal Bioanal Chem. 2013 Feb; 405(6):2019-29.
Biosensor	van der Schans MJ, Hulst AG, van der Riet-van Oeveren D, Noort D, Benschop HP, Dishovsky Ch	2013	Mar	van der Schans MJ, Hulst AG, van der Riet-van Oeveren D, Noort D, Benschop HP, Dishovsky Ch.New tools in diagnosis and biomonitoring of intoxications with organophosphorothioates: case studies with chlorpyrifos and diazinon. Chem Biol Interact. 2013 Mar 25; 203(1):96-102.
Biosensor Method	Scognamiglio V, Pezzotti I, Pezzotti G, Cano J, Manfredonia I, Buonasera K, Arduini F, Moscone D, Palleschi G, Giardi MT	2012	Nov	Scognamiglio V, Pezzotti I, Pezzotti G, Cano J, Manfredonia I, Buonasera K, Arduini F, Moscone D, Palleschi G, Giardi MT.Towards an integrated biosensor array for simultaneous and rapid multi-analysis of endocrine disrupting chemicals. Anal Chim Acta. 2012 Nov 2; 751:161-70.
Biosensor Method	Smith JN, Wang J, Lin Y, Klohe EM, Timchalk C	2012	Dec	Smith JN, Wang J, Lin Y, Klohe EM, Timchalk C.Pharmacokinetics and pharmacodynamics of chlorpyrifos and 3,5,6-trichloro-2-pyridinol in rat saliva after chlorpyrifos administration. Toxicol Sci. 2012 Dec; 130(2):245-56.
Case Report	Chaou CH, Lin CC, Chen HY, Lee CH, Chen TH	2013	Jun	Chaou CH, Lin CC, Chen HY, Lee CH, Chen TH.Chlorpyrifos is associated with slower serum cholinesterase recovery in acute organophosphate-poisoned patients. Clin Toxicol (Phila). 2013 Jun; 51(5):402-8.
Cumulative risk assessment	Wason SC, Smith TJ, Perry MJ, Levy JI	2012	May	Wason SC, Smith TJ, Perry MJ, Levy JI.Using physiologically-based pharmacokinetic models to incorporate chemical and non-chemical stressors into cumulative risk assessment: a case study of pesticide exposures. Int J Environ Res Public Health. 2012 May; 9(5):1971-83.
Editorial	Biello D	2012	Jul	Biello D.Bad for bugs and brains? A common pesticide may interfere with a child's brain development. Sci Am. 2012 Jul; 307(1):22.

Editorial	Juberg DR	2012	Aug	Juberg DR.Differentiating experimental animal doses from human exposures to chlorpyrifos. Proc Natl Acad Sci U S A. 2012 Aug 14; 109(33):E2195; author reply E2196.
Editorial	Saunders M, Magnanti BL, Correia Carreira S, Yang A, Alamo-HernÃ¡ndez U, Riojas-Rodriguez H, Calamandrei G, Koppe JG, Krayer von Krauss M, Keune H, Bartonova A	2012	Jun	Saunders M, Magnanti BL, Correia Carreira S, Yang A, Alamo-HernÃ¡ndez U, Riojas-Rodriguez H, Calamandrei G, Koppe JG, Krayer von Krauss M, Keune H, Bartonova A.Chlorpyrifos and neurodevelopmental effects: a literature review and expert elicitation on research and policy. Environ Health. 2012 Jun 28; 11 Suppl 1:S5.
Epidemiology	Cecchi, A., Rovedatti, M.G. , Sabino, G. , Magnarelli, G.G.	2012	March	Cecchi, A. , Rovedatti, M.G. , Sabino, G. , Magnarelli, G.G. Environmental exposure to organophosphate pesticides: Assessment of endocrine disruption and heptatoxicity in pregnant women. Ecotoxicology and Environmental Safety 80 (2012) 280-287.
Epidemiology	Lee PC, Rhodes SL, Sinsheimer JS, Bronstein J, Ritz B	2013	Jun	Lee PC, Rhodes SL, Sinsheimer JS, Bronstein J, Ritz B.Functional paraoxonase 1 variants modify the risk of Parkinson's disease due to organophosphate exposure. Environ Int. 2013 Jun; 56:42-7.
Epidemiology	Peter JV, Thomas L, Graham PL, Moran JL, Abhilash KP, Jasmine S, Iyyadurai R	2013	Nov	Peter JV, Thomas L, Graham PL, Moran JL, Abhilash KP, Jasmine S, Iyyadurai R.Performance of clinical scoring systems in acute organophosphate poisoning. Clin Toxicol (Phila). 2013 Nov; 51(9):850-4.
Epidemiology	Thivakaran T, Gamage R, Gunarathne KS, Gooneratne IK	2012	Jul	Thivakaran T, Gamage R, Gunarathne KS, Gooneratne IK.Chlorpyrifos-induced delayed myelopathy and pure motor neuropathy: a case report. Neurologist. 2012 Jul; 18(4):226-8.
Epidemiology/Exposure	Cavari Y, Lifshitz M, Leibson T, Shorer Z, Rubinstein M, Sofer S	2013	Jul	Cavari Y, Lifshitz M, Leibson T, Shorer Z, Rubinstein M, Sofer S.[Severe and uncommon complications of anticholinesterase intoxication in children]. Harefuah. 2013 Jul; 152(7):391-4, 434.

Epidemiology/Exposure	Crane AL, Abdel Rasoul G, Ismail AA, Hendy O, Bonner MR, Lasarev MR, Al-Batanony M, Singleton ST, Khan K, Olson JR, Rohlman DS	2013	Jul	Crane AL, Abdel Rasoul G, Ismail AA, Hendy O, Bonner MR, Lasarev MR, Al-Batanony M, Singleton ST, Khan K, Olson JR, Rohlman DS.Longitudinal assessment of chlorpyrifos exposure and effect biomarkers in adolescent Egyptian agricultural workers. J Expo Sci Environ Epidemiol. 2013 Jul; 23(4):356-62.
Epidemiology/Exposure	Dalvie MA, Sosan MB, Africa A, Cairncross E, London L	2014	Jan	Dalvie MA, Sosan MB, Africa A, Cairncross E, London L.Environmental monitoring of pesticide residues from farms at a neighbouring primary and pre-school in the Western Cape in South Africa. Sci Total Environ. 2014 Jan 1; 466-467:1078-84.
Epidemiology/Exposure	de Cock M, Maas YG, van de Bor M	2012	Aug	de Cock M, Maas YG, van de Bor M.Does perinatal exposure to endocrine disruptors induce autism spectrum and attention deficit hyperactivity disorders? Review. Acta Paediatr. 2012 Aug; 101(8):811-8.
Epidemiology/Exposure	Ellison CA, Abou El-Ella SS, Tawfik M, Lein PJ, Olson JR	2012		Ellison CA, Abou El-Ella SS, Tawfik M, Lein PJ, Olson JR.Allele and genotype frequencies of CYP2B6 and CYP2C19 polymorphisms in Egyptian agricultural workers. J Toxicol Environ Health A. 2012; 75(4):232-41.
Epidemiology/Exposure	Ellison CA, Crane AL, Bonner MR, Knaak JB, Browne RW, Lein PJ, Olson JR	2012	Dec	Ellison CA, Crane AL, Bonner MR, Knaak JB, Browne RW, Lein PJ, Olson JR.PON1 status does not influence cholinesterase activity in Egyptian agricultural workers exposed to chlorpyrifos. Toxicol Appl Pharmacol. 2012 Dec 15; 265(3):308-15.
Epidemiology/Exposure	Fenske RA, Farahat FM, Galvin K, Fenske EK, Olson JR	2012	Jul-Sep	Fenske RA, Farahat FM, Galvin K, Fenske EK, Olson JR.Contributions of inhalation and dermal exposure to chlorpyrifos dose in Egyptian cotton field workers. Int J Occup Environ Health. 2012 Jul-Sep; 18(3):198-209.
Epidemiology/Exposure	Fenske RA, Lu C, Negrete M, Galvin K	2013	Sep	Fenske RA, Lu C, Negrete M, Galvin K.Breaking the take home pesticide exposure pathway for agricultural families: workplace predictors of residential contamination. Am J Ind Med. 2013 Sep; 56(9):1063-71.

Epidemiology/Exposure	Freire C, Koifman S	2012	Oct	Freire C, Koifman S.Pesticide exposure and Parkinson's disease: epidemiological evidence of association. Neurotoxicology. 2012 Oct; 33(5):947-71.
Epidemiology/Exposure	Freire C, Koifman S	2013	Jul	Freire C, Koifman S.Pesticides, depression and suicide: a systematic review of the epidemiological evidence. Int J Hyg Environ Health. 2013 Jul; 216(4):445-60.
Epidemiology/Exposure	Goodman JE, Prueitt RL, Rhomberg LR	2013		Goodman JE, Prueitt RL, Rhomberg LR.Incorporating Low-dose Epidemiology Data in a Chlorpyrifos Risk Assessment. Dose Response. 2013; 11(2):207-19.
Epidemiology/Exposure	Hengel M, Lee P	2014	Mar	Hengel M, Lee P.Community air monitoring for pesticides-part 2: multiresidue determination of pesticides in air by gas chromatography, gas chromatography-mass spectrometry, and liquid chromatography-mass spectrometry. Environ Monit Assess. 2014 Mar; 186(3):1343-53.
Epidemiology/Exposure	Hoppin JA, Long S, Umbach DM, Lubin JH, Starks SE, Gerr F, Thomas K, Hines CJ, Weichenthal S, Kamel F, Koutros S, Alavanja M, Beane Freeman LE, Sandler DP	2012	Nov	Hoppin JA, Long S, Umbach DM, Lubin JH, Starks SE, Gerr F, Thomas K, Hines CJ, Weichenthal S, Kamel F, Koutros S, Alavanja M, Beane Freeman LE, Sandler DP.Lifetime organophosphorous insecticide use among private pesticide applicators in the Agricultural Health Study. J Expo Sci Environ Epidemiol. 2012 Nov; 22(6):584-92.
Epidemiology/Exposure	Horton MK, Kahn LG, Perera F, Barr DB, Rauh V	2012	Sep-Oct	Horton MK, Kahn LG, Perera F, Barr DB, Rauh V.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. 2012 Sep-Oct; 34(5):534-41.
Epidemiology/Exposure	Huen K, Bradman A, Harley K, Yousefi P, Boyd Barr D, Eskenazi B, Holland N	2012	Aug	Huen K, Bradman A, Harley K, Yousefi P, Boyd Barr D, Eskenazi B, Holland N.Organophosphate pesticide levels in blood and urine of women and newborns living in an agricultural community. Environ Res. 2012 Aug; 117:8-16.

Epidemiology/Exposure	Karunanayake CP, Spinelli JJ, McLaughlin JR, Dosman JA, Pahwa P, McDuffie HH	2012	Jan	Karunanayake CP, Spinelli JJ, McLaughlin JR, Dosman JA, Pahwa P, McDuffie HH.Hodgkin lymphoma and pesticides exposure in men: a Canadian case-control study. J Agromedicine. 2012 Jan; 17(1):30-9.
Epidemiology/Exposure	Khan K, Ismail AA, Abdel Rasoul G, Bonner MR, Lasarev MR, Hendy O, Al-Batanony M, Crane AL, Singleton ST, Olson JR, Rohlman DS	2014	Mar	Khan K, Ismail AA, Abdel Rasoul G, Bonner MR, Lasarev MR, Hendy O, Al-Batanony M, Crane AL, Singleton ST, Olson JR, Rohlman DS.Longitudinal assessment of chlorpyrifos exposure and self-reported neurological symptoms in adolescent pesticide applicators. BMJ Open. 2014 Mar 4; 4(3):e004177.
Epidemiology/Exposure	Kim HH, Lim YW, Yang JY, Shin DC, Ham HS, Choi BS, Lee JY	2013	Feb	Kim HH, Lim YW, Yang JY, Shin DC, Ham HS, Choi BS, Lee JY.Health risk assessment of exposure to chlorpyrifos and dichlorvos in children at childcare facilities. Sci Total Environ. 2013 Feb 1; 444:441-50.
Epidemiology/Exposure	Lein PJ, Bonner MR, Farahat FM, Olson JR, Rohlman DS, Fenske RA, Lattal KM, Lasarev MR, Galvin K, Farahat TM, Anger WK	2012	Aug	Lein PJ, Bonner MR, Farahat FM, Olson JR, Rohlman DS, Fenske RA, Lattal KM, Lasarev MR, Galvin K, Farahat TM, Anger WK.Experimental strategy for translational studies of organophosphorus pesticide neurotoxicity based on real-world occupational exposures to chlorpyrifos. Neurotoxicology. 2012 Aug; 33(4):660-8.
Epidemiology/Exposure	Lurati AR	2013	Jun	Lurati AR.Organophosphate exposure with pseudocholinesterase deficiency. Workplace Health Saf. 2013 Jun; 61(6):243-5.
Epidemiology/Exposure	Ostrea EM Jr, Reyes A, Villanueva-Uy E, Pacifico R, Benitez B, Ramos E, Bernardo RC, Bielawski DM, Delaney-Black V, Chiodo L, Janisse JJ, Ager JW	2012	Aug	Ostrea EM Jr, Reyes A, Villanueva-Uy E, Pacifico R, Benitez B, Ramos E, Bernardo RC, Bielawski DM, Delaney-Black V, Chiodo L, Janisse JJ, Ager JW.Fetal exposure to propoxur and abnormal child neurodevelopment at 2 years of age. Neurotoxicology. 2012 Aug; 33(4):669-75.
Epidemiology/Exposure	Phung DT, Connell D, Yu Q, Chu C	2013	Sep	Phung DT, Connell D, Yu Q, Chu C.Health risk characterization of chlorpyrifos using epidemiological dose-response data and probabilistic techniques: a case study with rice farmers in Vietnam. Risk Anal. 2013 Sep; 33(9):1596-607.

Epidemiology/Exposure	QuirÃ³s-AlcalÃ¡ L, Bradman A, Smith K, Weerasekera G, Odetokun M, Barr DB, Nishioka M, Castorina R, Hubbard AE, Nicas M, Hammond SK, McKone TE, Eskenazi B	2012	Nov	QuirÃ³s-AlcalÃ¡ L, Bradman A, Smith K, Weerasekera G, Odetokun M, Barr DB, Nishioka M, Castorina R, Hubbard AE, Nicas M, Hammond SK, McKone TE, Eskenazi B.Organophosphorous pesticide breakdown products in house dust and children's urine. J Expo Sci Environ Epidemiol. 2012 Nov; 22(6):559-68.
Epidemiology/Exposure	Rauh VA, Perera FP, Horton MK, Whyatt RM, Bansal R, Hao X, Liu J, Barr DB, Slotkin TA, Peterson BS	2012	May	Rauh VA, Perera FP, Horton MK, Whyatt RM, Bansal R, Hao X, Liu J, Barr DB, Slotkin TA, Peterson BS.Brain anomalies in children exposed prenatally to a common organophosphate pesticide. Proc Natl Acad Sci U S A. 2012 May 15; 109(20):7871-6.
Epidemiology/Exposure	Wang N, Yi L, Shi L, Kong D, Cai D, Wang D, Shan Z	2012		Wang N, Yi L, Shi L, Kong D, Cai D, Wang D, Shan Z.Pollution level and human health risk assessment of some pesticides and polychlorinated biphenyls in Nantong of Southeast China. J Environ Sci (China). 2012; 24(10):1854-60.
Epidemiology/Exposure	Wu N, Hao F, Yu X	2012	Jul	Wu N, Hao F, Yu X.Peripheral nerve and skin damage associated with working in a STCP factory: report of four cases. Clin Toxicol (Phila). 2012 Jul; 50(6):514-7.
Epidemiology/Exposure	Costa LG, Giordano G, Cole TB, Marsillach J, Furlong CE	2013	May	Costa LG, Giordano G, Cole TB, Marsillach J, Furlong CE.Paraoxonase 1 (PON1) as a genetic determinant of susceptibility to organophosphate toxicity. Toxicology. 2013 May 10; 307:115-22.
Exposure	Abdulra'uf LB, Tan GH	2013	Dec	Abdulra'uf LB, Tan GH.Multivariate study of parameters in the determination of pesticide residues in apple by headspace solid phase microextraction coupled to gas chromatography-mass spectrometry using experimental factorial design. Food Chem. 2013 Dec 15; 141(4):4344-8.
Exposure	Affam AC, Chaudhuri M	2013	Nov	Affam AC, Chaudhuri M.Degradation of pesticides chlorpyrifos, cypermethrin and chlorothalonil in aqueous solution by TiO2 photocatalysis. J Environ Manage. 2013 Nov 30; 130:160-5.

Exposure	Agbo SO, KeinÃ¤nen M, Keski-Saari S, Lemmetyinen J, Akkanen J, LeppÃ¤nen MT, Mayer P, Kukkonen JV	2013	Sep	Agbo SO, KeinÃ¤nen M, Keski-Saari S, Lemmetyinen J, Akkanen J, LeppÃ¤nen MT, Mayer P, Kukkonen JV.Changes in Lumbriculus variegatus metabolites under hypoxic exposure to benzo(a)pyrene, chlorpyrifos and pentachlorophenol: consequences on biotransformation. Chemosphere. 2013 Sep; 93(2):302-10.
Exposure	Agostini MG, Kacoliris F, Demetrio P, Natale GS, Bonetto C, Ronco AE	2013	May	Agostini MG, Kacoliris F, Demetrio P, Natale GS, Bonetto C, Ronco AE.Abnormalities in amphibian populations inhabiting agroecosystems in Northeastern Buenos Aires Province, Argentina. Dis Aquat Organ. 2013 May 27; 104(2):163-71.
Exposure	Agudelo C RM, Jaramillo ML, PeÃ±uela G	2012	Aug	Agudelo C RM, Jaramillo ML, PeÃ±uela G.Comparison of the removal of chlorpyrifos and dissolved organic carbon in horizontal sub-surface and surface flow wetlands. Sci Total Environ. 2012 Aug 1; 431:271-7.
Exposure	Aguilera-Luiz MM, Romero-GonzÃ¡lez R, Plaza-BolaÃ±os P, MartÃnez Vidal JL, Garrido Frenich A	2013	Aug	Aguilera-Luiz MM, Romero-GonzÃ¡lez R, Plaza-BolaÃ±os P, MartÃnez Vidal JL, Garrido Frenich A.Wide-scope analysis of veterinary drug and pesticide residues in animal feed by liquid chromatography coupled to quadrupole-time-of-flight mass spectrometry. Anal Bioanal Chem. 2013 Aug; 405(20):6543-53.
Exposure	Akkad R, Schwack W	2012	Sep-Oct	Akkad R, Schwack W.Determination of organophosphorus and carbamate insecticides in fresh fruits and vegetables by high-performance thin-layer chromatography-multienzyme inhibition assay. J AOAC Int. 2012 Sep-Oct; 95(5):1371-7.
Exposure	AlcÃ¡ntara-ConcepciÃ³n V, Cram S, Gibson R, Ponce de LeÃ³n C, Mazari-Hiriart M	2013	Jul-Aug	AlcÃ¡ntara-ConcepciÃ³n V, Cram S, Gibson R, Ponce de LeÃ³n C, Mazari-Hiriart M.Method development and validation for the simultaneous determination of organochlorine and organophosphorus pesticides in a complex sediment matrix. J AOAC Int. 2013 Jul-Aug; 96(4):854-63.

Exposure	Alves AC, GonÃ§alves MM, Bernardo MM, Mendes BS	2012	Oct	Alves AC, GonÃ§alves MM, Bernardo MM, Mendes BS.Dispersive liquid-liquid microextraction of organophosphorous pesticides using nonhalogenated solvents. J Sep Sci. 2012 Oct; 35(19):2653-8.
Exposure	Anderson B, Phillips B, Hunt J, Siegler K, Voorhees J, Smalling K, Kuivila K, Hamilton M, Ranasinghe JA, Tjeerdema R	2014	Mar	Anderson B, Phillips B, Hunt J, Siegler K, Voorhees J, Smalling K, Kuivila K, Hamilton M, Ranasinghe JA, Tjeerdema R.Impacts of pesticides in a Central California estuary. Environ Monit Assess. 2014 Mar; 186(3):1801-14.
Exposure	Armstrong JL, Fenske RA, Yost MG, Tchong-French M, Yu J	2013	Jul	Armstrong JL, Fenske RA, Yost MG, Tchong-French M, Yu J.Comparison of polyurethane foam and XAD-2 sampling matrices to measure airborne organophosphorus pesticides and their oxygen analogs in an agricultural community. Chemosphere. 2013 Jul; 92(4):451-7.
Exposure	Arora S, Mukherjee I, Kumar A, Garg DK	2014	Jan	Arora S, Mukherjee I, Kumar A, Garg DK.Comparative assessment of pesticide residues in grain, soil, and water from IPM and non-IPM trials of basmati rice. Environ Monit Assess. 2014 Jan; 186(1):361-6.
Exposure	Babina K, Dollard M, Pilotto L, Edwards JW	2012	Nov	Babina K, Dollard M, Pilotto L, Edwards JW.Environmental exposure to organophosphorus and pyrethroid pesticides in South Australian preschool children: a cross sectional study. Environ Int. 2012 Nov 1; 48:109-20.
Exposure	Bagheri H, Es'haghi A, Es-haghi A, Mesbahi N	2012	Aug	Bagheri H, Es'haghi A, Es-haghi A, Mesbahi N.A high-throughput approach for the determination of pesticide residues in cucumber samples using solid-phase microextraction on 96-well plate. Anal Chim Acta. 2012 Aug 31; 740:36-42.
Exposure	Baldim IM, Souza MC, Souza JC, Figueiredo EC, Martins I	2012	Oct	Baldim IM, Souza MC, Souza JC, Figueiredo EC, Martins I.Application of the molecularly imprinted solid-phase extraction to the organophosphate residues determination in strawberries. Anal Bioanal Chem. 2012 Oct; 404(6-7):1959-66.

Exposure	Beamer PI, Canales RA, Ferguson AC, Leckie JO, Bradman A	2012	Jan	Beamer PI, Canales RA, Ferguson AC, Leckie JO, Bradman A.Relative pesticide and exposure route contribution to aggregate and cumulative dose in young farmworker children. Int J Environ Res Public Health. 2012 Jan; 9(1):73-96.
Exposure	Bedi JS, Gill JP, Aulakh RS, Kaur P, Sharma A, Pooni PA	2013	Oct	Bedi JS, Gill JP, Aulakh RS, Kaur P, Sharma A, Pooni PA.Pesticide residues in human breast milk: risk assessment for infants from Punjab, India. Sci Total Environ. 2013 Oct 1; 463-464:720-6.
Exposure	Bhattacharjee S, Fakhruddin AN, Chowdhury MA, Rahman MA, Alam MK	2012	Aug	Bhattacharjee S, Fakhruddin AN, Chowdhury MA, Rahman MA, Alam MK.Monitoring of selected pesticides residue levels in water samples of paddy fields and removal of cypermethrin and chlorpyrifos residues from water using rice bran. Bull Environ Contam Toxicol. 2012 Aug; 89(2):348-53.
Exposure	Bootharaju MS, Pradeep T	2012	Feb	Bootharaju MS, Pradeep T.Understanding the degradation pathway of the pesticide, chlorpyrifos by noble metal nanoparticles. Langmuir. 2012 Feb 7; 28(5):2671-9.
Exposure	Brun EM, Puchades R, Maquieira Ã	2013	Apr	Brun EM, Puchades R, Maquieira Ã .Gold, carbon, and aluminum low-reflectivity compact discs as microassaying platforms. Anal Chem. 2013 Apr 16; 85(8):4178-86.
Exposure	Cai D, Wang L, Zhang G, Zhang X, Wu Z	2013	Sep	Cai D, Wang L, Zhang G, Zhang X, Wu Z.Controlling pesticide loss by natural porous micro/nano composites: straw ash-based biochar and biosilica. ACS Appl Mater Interfaces. 2013 Sep 25; 5(18):9212-6.
Exposure	Carlson JC, Challis JK, Hanson ML, Wong CS	2013	Feb	Carlson JC, Challis JK, Hanson ML, Wong CS.Stability of pharmaceuticals and other polar organic compounds stored on polar organic chemical integrative samplers and solid-phase extraction cartridges. Environ Toxicol Chem. 2013 Feb; 32(2):337-44.

Exposure	Catalina Rodriguez D, Carvajal S, Penuela G	2013	Jul	Catalina Rodriguez D, Carvajal S, Penuela G.Effect of chlorpyrifos on the inhibition of the enzyme acetylcholinesterase by cross-linking in water-supply samples and milk from dairy cattle. Talanta. 2013 Jul 15; 111:1-7.
Exposure	Cavalcante RM, Lima DM, Fernandes GM, DuavÃ WC	2012	May	Cavalcante RM, Lima DM, Fernandes GM, DuavÃ WC.Relation factor: a new strategy for quality control in the determination of pesticides in environmental aqueous matrices. Talanta. 2012 May 15; 93:212-8.
Exposure	Cavari Y, Landau D, Sofer S, Leibson T, Lazar I	2013	May	Cavari Y, Landau D, Sofer S, Leibson T, Lazar I.Organophosphate poisoning-induced acute renal failure. Pediatr Emerg Care. 2013 May; 29(5):646-7.
Exposure	Chai LK, Wong MH, Bruun Hansen HC	2013	Aug	Chai LK, Wong MH, Bruun Hansen HC.Degradation of chlorpyrifos in humid tropical soils. J Environ Manage. 2013 Aug 15; 125:28-32.
Exposure	Chen C, Qian Y, Liu X, Tao C, Liang Y, Li Y	2012	Feb	Chen C, Qian Y, Liu X, Tao C, Liang Y, Li Y.Risk assessment of chlorpyrifos on rice and cabbage in China. Regul Toxicol Pharmacol. 2012 Feb; 62(1):125-30.
Exposure	Chishti Z, Hussain S, Arshad KR, Khalid A, Arshad M	2013	Jan	Chishti Z, Hussain S, Arshad KR, Khalid A, Arshad M.Microbial degradation of chlorpyrifos in liquid media and soil. J Environ Manage. 2013 Jan 15; 114:372-80.
Exposure	Chowdhury MA, Banik S, Uddin B, Moniruzzaman M, Karim N, Gan SH	2012	Sep	Chowdhury MA, Banik S, Uddin B, Moniruzzaman M, Karim N, Gan SH.Organophosphorus and carbamate pesticide residues detected in water samples collected from paddy and vegetable fields of the Savar and Dhamrai Upazilas in Bangladesh. Int J Environ Res Public Health. 2012 Sep 11; 9(9):3318-29.
Exposure	Clifton MS, Wargo JP, Weathers WS, ColÃ³n M, Bennett DH, Tulve NS	2013	Jan	Clifton MS, Wargo JP, Weathers WS, ColÃ³n M, Bennett DH, Tulve NS.Quantitative analysis of organophosphate and pyrethroid insecticides, pyrethroid transformation products, polybrominated diphenyl ethers and bisphenol A in residential surface wipe samples. J Chromatogr A. 2013 Jan 18; 1273:1-11.

Exposure	Dabrowski JM, Balderacchi M	2013	Nov	Dabrowski JM, Balderacchi M.Development and field validation of an indicator to assess the relative mobility and risk of pesticides in the Lourens River catchment, South Africa. Chemosphere. 2013 Nov; 93(10):2433-43.
Exposure	Deme P, Azmeera T, Prabhavathi Devi BL, Jonnalagadda PR, Prasad RB, Vijaya Sarathi UV	2014	Jan	Deme P, Azmeera T, Prabhavathi Devi BL, Jonnalagadda PR, Prasad RB, Vijaya Sarathi UV.An improved dispersive solid-phase extraction clean-up method for the gas chromatography-negative chemical ionisation tandem mass spectrometric determination of multiclass pesticide residues in edible oils. Food Chem. 2014 Jan 1; 142:144-51.
Exposure	Du D, Wang J, Wang L, Lu D, Lin Y	2012	Feb	Du D, Wang J, Wang L, Lu D, Lin Y.Integrated lateral flow test strip with electrochemical sensor for quantification of phosphorylated cholinesterase: biomarker of exposure to organophosphorus agents. Anal Chem. 2012 Feb 7; 84(3):1380-5.
Exposure	Dubey KK, Fulekar MH	2012	Apr	Dubey KK, Fulekar MH.Chlorpyrifos bioremediation in Pennisetum rhizosphere by a novel potential degrader Stenotrophomonas maltophilia MHF ENV20. World J Microbiol Biotechnol. 2012 Apr; 28(4):1715-25.
Exposure	Duffner A, Ingwersen J, Hugenschmidt C, Streck T	2012	Jul-Aug	Duffner A, Ingwersen J, Hugenschmidt C, Streck T.Pesticide transport pathways from a sloped Litchi orchard to an adjacent tropical stream as identified by hydrograph separation. J Environ Qual. 2012 Jul-Aug; 41(4):1315-23.
Exposure	EstÃ©vez E, Cabrera Mdel C, Molina-DÃaz A, Robles-Molina J, Palacios-DÃaz Mdel P	2012	Sep	EstÃ©vez E, Cabrera Mdel C, Molina-DÃaz A, Robles-Molina J, Palacios-DÃaz Mdel P.Screening of emerging contaminants and priority substances (2008/105/EC) in reclaimed water for irrigation and groundwater in a volcanic aquifer (Gran Canaria, Canary Islands, Spain). Sci Total Environ. 2012 Sep 1; 433:538-46.

Exposure	Fan S, Deng K, Yu C, Zhao P, Bai A, Li Y, Pan C, Li X	2013	Sep	Fan S, Deng K, Yu C, Zhao P, Bai A, Li Y, Pan C, Li X.Influence of different planting seasons of six leaf vegetables on residues of five pesticides. J Agric Food Chem. 2013 Sep 25; 61(38):9036-44.
Exposure	Fan S, Zhang F, Deng K, Yu C, Liu S, Zhao P, Pan C	2013	Mar	Fan S, Zhang F, Deng K, Yu C, Liu S, Zhao P, Pan C.Spinach or amaranth contains highest residue of metalaxyl, fluazifop-P-butyl, chlorpyrifos, and lambda-cyhalothrin on six leaf vegetables upon open field application. J Agric Food Chem. 2013 Mar 6; 61(9):2039-44.
Exposure	Femia J, Mariani M, Zalazar C, Tiscornia I	2013		Femia J, Mariani M, Zalazar C, Tiscornia I.Photodegradation of chlorpyrifos in water by UV/H2O2 treatment: toxicity evaluation. Water Sci Technol. 2013; 68(10):2279-86.
Exposure	Fuentes MS, BriceÃ±o GE, Saez JM, Benimeli CS, Diez MC, Amoroso MJ	2013		Fuentes MS, BriceÃ±o GE, Saez JM, Benimeli CS, Diez MC, Amoroso MJ.Enhanced removal of a pesticides mixture by single cultures and consortia of free and immobilized Streptomyces strains. Biomed Res Int. 2013; 2013:392573.
Exposure	Garrison VH, Majewski MS, Foreman WT, Genualdi SA, Mohammed A, Massey Simonich SL	2014	Jan	Garrison VH, Majewski MS, Foreman WT, Genualdi SA, Mohammed A, Massey Simonich SL.Persistent organic contaminants in Saharan dust air masses in West Africa, Cape Verde and the eastern Caribbean. Sci Total Environ. 2014 Jan 15; 468-469:530-43.
Exposure	Gebremariam SY, Beutel MW, Flury M, Harsh JB, Yonge DR	2012	Jan	Gebremariam SY, Beutel MW, Flury M, Harsh JB, Yonge DR.Nonsingular adsorption/desorption of chlorpyrifos in soils and sediments: experimental results and modeling. Environ Sci Technol. 2012 Jan 17; 46(2):869-75.
Exposure	Gebremariam SY, Beutel MW, Yonge DR, Flury M, Harsh JB	2012		Gebremariam SY, Beutel MW, Yonge DR, Flury M, Harsh JB.Adsorption and desorption of chlorpyrifos to soils and sediments. Rev Environ Contam Toxicol. 2012; 215:123-75.
Exposure	Ghoshdastidar AJ, Saunders JE, Brown KH, Tong AZ	2012		Ghoshdastidar AJ, Saunders JE, Brown KH, Tong AZ.Membrane bioreactor treatment of commonly used organophosphate pesticides. J Environ Sci Health B. 2012; 47(7):742-50.

Exposure	GonzÃ¡lez-Curbelo MÃ , HernÃ¡ndez-Borges J, Borges-Miquel TM, RodrÃguez-Delgado MÃ	2012		GonzÃ¡lez-Curbelo MÃ , HernÃ¡ndez-Borges J, Borges-Miquel TM, RodrÃguez-Delgado MÃ .Determination of pesticides and their metabolites in processed cereal samples. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2012; 29(1):104-16.
Exposure	GonzÃ¡lez-Curbelo MÃ , Herrera-Herrera AV, HernÃ¡ndez-Borges J, RodrÃ-guez-Delgado MÃ	2013	Feb	GonzÃ¡lez-Curbelo MÃ , Herrera-Herrera AV, HernÃ¡ndez-Borges J, RodrÃguez-Delgado MÃ .Analysis of pesticides residues in environmental water samples using multiwalled carbon nanotubes dispersive solid-phase extraction. J Sep Sci. 2013 Feb; 36(3):556-63.
Exposure	Gu S, Lu Y, Ding Y, Li L, Zhang F, Wu Q	2013	Sep	Gu S, Lu Y, Ding Y, Li L, Zhang F, Wu Q.Droplet-based microfluidics for dose-response assay of enzyme inhibitors by electrochemical method. Anal Chim Acta. 2013 Sep 24; 796:68-74.
Exposure	Hall L Jr, Anderson RD	2012		Hall L Jr, Anderson RD.Historical trends analysis of 2004 to 2009 toxicity and pesticide data for California's central valley. J Environ Sci Health A Tox Hazard Subst Environ Eng. 2012; 47(6):801-11.
Exposure	Harnpicharnchai K, Chaiear N, Charerntanyarak L	2013	Nov	Harnpicharnchai K, Chaiear N, Charerntanyarak L.Residues of organophosphate pesticides used in vegetable cultivation in ambient air, surface water and soil in Bueng Niam Subdistrict, Khon Kaen, Thailand. Southeast Asian J Trop Med Public Health. 2013 Nov; 44(6):1088-97.
Exposure	Harwood AD, Landrum PF, Weston DP, Lydy MJ	2013	Feb	Harwood AD, Landrum PF, Weston DP, Lydy MJ.Using SPME fibers and Tenax to predict the bioavailability of pyrethroids and chlorpyrifos in field sediments. Environ Pollut. 2013 Feb; 173:47-51.

Exposure	Hayward DG, Wong JW, Shi F, Zhang K, Lee NS, DiBenedetto AL, Hengel MJ	2013	May	Hayward DG, Wong JW, Shi F, Zhang K, Lee NS, DiBenedetto AL, Hengel MJ.Multiresidue pesticide analysis of botanical dietary supplements using salt-out acetonitrile extraction, solid-phase extraction cleanup column, and gas chromatography-triple quadrupole mass spectrometry. Anal Chem. 2013 May 7; 85(9):4686-93.
Exposure	Ismail M, Khan HM, Sayed M, Cooper WJ	2013	Oct	Ismail M, Khan HM, Sayed M, Cooper WJ.Advanced oxidation for the treatment of chlorpyrifos in aqueous solution. Chemosphere. 2013 Oct; 93(4):645-51.
Exposure	Jyot G, Mandal K, Battu RS, Singh B	2013	Jul	Jyot G, Mandal K, Battu RS, Singh B.Estimation of chlorpyriphos and cypermethrin residues in chilli (Capsicum annuum L.) by gas-liquid chromatography. Environ Monit Assess. 2013 Jul; 185(7):5703-14.
Exposure	KÃ¶ck-Schulmeyer M, Olmos M, LÃ³pez de Alda M, BarcelÃ³ D	2013	Aug	KÃ¶ck-Schulmeyer M, Olmos M, LÃ³pez de Alda M, BarcelÃ³ D.Development of a multiresidue method for analysis of pesticides in sediments based on isotope dilution and liquid chromatography-electrospray-tandem mass spectrometry. J Chromatogr A. 2013 Aug 30; 1305:176-87.
Exposure	Karanasios EC, Tsiropoulos NG, Karpouzas DG	2013	Sep	Karanasios EC, Tsiropoulos NG, Karpouzas DG.Quantitative and qualitative differences in the metabolism of pesticides in biobed substrates and soil. Chemosphere. 2013 Sep; 93(1):20-8.
Exposure	Karpuzcu ME, Sedlak DL, Stringfellow WT	2013	Jan	Karpuzcu ME, Sedlak DL, Stringfellow WT.Biotransformation of chlorpyrifos in riparian wetlands in agricultural watersheds: implications for wetland management. J Hazard Mater. 2013 Jan 15; 244-245:111-20.
Exposure	Kong Z, Shan W, Dong F, Liu X, Xu J, Li M, Zheng Y	2012	Aug	Kong Z, Shan W, Dong F, Liu X, Xu J, Li M, Zheng Y.Effect of home processing on the distribution and reduction of pesticide residues in apples. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2012 Aug; 29(8):1280-7.

Exposure	Korba K, Pelit L, Pelit FO, Ozdokur KV, ErtaÅŸ H, EroÄŸlu AE, ErtaÅŸ FN	2013	Jun	Korba K, Pelit L, Pelit FO, Ozdokur KV, ErtaÅŸ H, EroÄŸlu AE, ErtaÅŸ FN.Preparation and characterization of sodium dodecyl sulfate doped polypyrrole solid phase micro extraction fiber and its application to endocrine disruptor pesticide analysis. J Chromatogr B Analyt Technol Biomed Life Sci. 2013 Jun 15; 929:90-6.
Exposure	Kouzayha A, Al Ashi A, Al Akoum R, Al Iskandarani M, Budzinski H, Jaber F	2013	Nov	Kouzayha A, Al Ashi A, Al Akoum R, Al Iskandarani M, Budzinski H, Jaber F.Occurrence of pesticide residues in Lebanon's water resources. Bull Environ Contam Toxicol. 2013 Nov; 91(5):503-9.
Exposure	Kuang Y, Qiu F, Kong W, Luo J, Cheng H, Yang M	2013	Nov	Kuang Y, Qiu F, Kong W, Luo J, Cheng H, Yang M.Simultaneous quantification of mycotoxins and pesticide residues in ginseng with one-step extraction using ultra-high performance liquid chromatography-electrospray ionization tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2013 Nov 15; 939:98-107.
Exposure	Kumar A, Nayak AK, Shukla AK, Panda BB, Raja R, Shahid M, Tripathi R, Mohanty S, Rath PC	2012	Apr	Kumar A, Nayak AK, Shukla AK, Panda BB, Raja R, Shahid M, Tripathi R, Mohanty S, Rath PC.Microbial biomass and carbon mineralization in agricultural soils as affected by pesticide addition. Bull Environ Contam Toxicol. 2012 Apr; 88(4):538-42.
Exposure	Kusvuran E, Yildirim D, Mavruk F, Ceyhan M	2012	Nov	Kusvuran E, Yildirim D, Mavruk F, Ceyhan M.Removal of chloropyrifos ethyl, tetradifon and chlorothalonil pesticide residues from citrus by using ozone. J Hazard Mater. 2012 Nov 30; 241-242:287-300.
Exposure	Lari SZ, Khan NA, Gandhi KN, Meshram TS, Thacker NP	2014	Jan	Lari SZ, Khan NA, Gandhi KN, Meshram TS, Thacker NP.Comparison of pesticide residues in surface water and ground water of agriculture intensive areas. J Environ Health Sci Eng. 2014 Jan 7; 12(1):11.

Exposure	Lavin KS, Hageman KJ, Marx SK, Dillingham PW, Kamber BS	2012	Jan	Lavin KS, Hageman KJ, Marx SK, Dillingham PW, Kamber BS.Using trace elements in particulate matter to identify the sources of semivolatile organic contaminants in air at an alpine site. Environ Sci Technol. 2012 Jan 3; 46(1):268-76.
Exposure	Lee KG, Lee SK	2012	Dec	Lee KG, Lee SK.Monitoring and risk assessment of pesticide residues in yuza fruits (Citrus junos Sieb. ex Tanaka) and yuza tea samples produced in Korea. Food Chem. 2012 Dec 15; 135(4):2930-3.
Exposure	Li H, Wei Y, Lydy MJ, You J	2014	Jul	Li H, Wei Y, Lydy MJ, You J.Inter-compartmental transport of organophosphate and pyrethroid pesticides in South China: implications for a regional risk assessment. Environ Pollut. 2014 Jul; 190:19-26.
Exposure	Linhares AG, Assis CR, Siqueira MT, Bezerra RS, Carvalho LB Jr	2013	Aug	Linhares AG, Assis CR, Siqueira MT, Bezerra RS, Carvalho LB Jr.Development of a method for extraction and assay of human erythrocyte acetylcholinesterase and pesticide inhibition. Hum Exp Toxicol. 2013 Aug; 32(8):837-45.
Exposure	Lu D, Qiu X, Feng C, Jin Y, Lin Y, Xiong L, Wen Y, Wang D, Wang G	2012	May	Lu D, Qiu X, Feng C, Jin Y, Lin Y, Xiong L, Wen Y, Wang D, Wang G.Simultaneous determination of 45 pesticides in fruit and vegetable using an improved QuEChERS method and on-line gel permeation chromatography-gas chromatography/mass spectrometer. J Chromatogr B Analyt Technol Biomed Life Sci. 2012 May 1; 895-896:17-24.
Exposure	Lu HY, Shen Y, Sun X, Zhu H, Liu XJ	2013	Sep	Lu HY, Shen Y, Sun X, Zhu H, Liu XJ.Washing effects of limonene on pesticide residues in green peppers. J Sci Food Agric. 2013 Sep; 93(12):2917-21.
Exposure	Maliyekkal SM, Sreeprasad TS, Krishnan D, Kouser S, Mishra AK, Waghmare UV, Pradeep T	2013	Jan	Maliyekkal SM, Sreeprasad TS, Krishnan D, Kouser S, Mishra AK, Waghmare UV, Pradeep T.Graphene: a reusable substrate for unprecedented adsorption of pesticides. Small. 2013 Jan 28; 9(2):273-83.
Exposure	Mast MA, Alvarez DA, Zaugg SD	2012	Mar	Mast MA, Alvarez DA, Zaugg SD.Deposition and accumulation of airborne organic contaminants in Yosemite National Park, California. Environ Toxicol Chem. 2012 Mar; 31(3):524-33.

Exposure	Mishra RK, Istamboulie G, Bhand S, Marty JL	2012	Oct	Mishra RK, Istamboulie G, Bhand S, Marty JL.Detoxification of organophosphate residues using phosphotriesterase and their evaluation using flow based biosensor. Anal Chim Acta. 2012 Oct 1; 745:64-9.
Exposure	Moreno-GonzÃ¡lez R, Campillo JA, GarcÃa V, LeÃ³n VM	2013	Jul	Moreno-GonzÃ¡lez R, Campillo JA, GarcÃa V, LeÃ³n VM.Seasonal input of regulated and emerging organic pollutants through surface watercourses to a Mediterranean coastal lagoon. Chemosphere. 2013 Jul; 92(3):247-57.
Exposure	Moreno-GonzÃ¡lez R, Campillo JA, LeÃ³n VM	2013	Dec	Moreno-GonzÃ¡lez R, Campillo JA, LeÃ³n VM.Influence of an intensive agricultural drainage basin on the seasonal distribution of organic pollutants in seawater from a Mediterranean coastal lagoon (Mar Menor, SE Spain). Mar Pollut Bull. 2013 Dec 15; 77(1-2):400-11.
Exposure	Moscoso F, Teijiz I, Deive FJ, SanromÃ¡n MA	2013	Sep	Moscoso F, Teijiz I, Deive FJ, SanromÃ¡n MA.Approaching chlorpyrifos bioelimination at bench scale bioreactor. Bioprocess Biosyst Eng. 2013 Sep; 36(9):1303-9.
Exposure	Moussaoui Y, Tuduri L, Kerchich Y, Meklati BY, Eppe G	2012	Jul	Moussaoui Y, Tuduri L, Kerchich Y, Meklati BY, Eppe G.Atmospheric concentrations of PCDD/Fs, dl-PCBs and some pesticides in northern Algeria using passive air sampling. Chemosphere. 2012 Jul; 88(3):270-7.
Exposure	Mugni H, Demetrio P, Paracampo A, Pardi M, Bulus G, Bonetto C	2012	Jul	Mugni H, Demetrio P, Paracampo A, Pardi M, Bulus G, Bonetto C.Toxicity persistence in runoff water and soil in experimental soybean plots following chlorpyrifos application. Bull Environ Contam Toxicol. 2012 Jul; 89(1):208-12.
Exposure	Muhammad F, Awais MM, Akhtar M, Anwar MI	2013	Jan	Muhammad F, Awais MM, Akhtar M, Anwar MI.Quantitative structure activity relationship and risk analysis of some pesticides in the goat milk. Iranian J Environ Health Sci Eng. 2013 Jan 4; 10(1):4.

Exposure	Munoz-Quezada MT, Iglesias V, Lucero B, Steenland K, Barr DB, Levy K, Ryan PB, Alvarado S, Concha C	2012	Oct	Munoz-Quezada MT, Iglesias V, Lucero B, Steenland K, Barr DB, Levy K, Ryan PB, Alvarado S, Concha C.Predictors of exposure to organophosphate pesticides in schoolchildren in the Province of Talca, Chile. Environ Int. 2012 Oct 15; 47:28-36.
Exposure	Nagarajan G, Khan ZS, Utture SC, Dasgupta S, Banerjee K	2013	Nov	Nagarajan G, Khan ZS, Utture SC, Dasgupta S, Banerjee K.Ensuring selectivity and sensitivity by timed- and ultra-selective reaction monitoring during gas chromatography-tandem mass spectrometric determination of pesticides. J Chromatogr A. 2013 Nov 29; 1318:226-33.
Exposure	Navarro P, Morais S, GabaldÃ³n JA, PÃ©rez AJ, Puchades R, Maquieira A	2013	Jun	Navarro P, Morais S, GabaldÃ³n JA, PÃ©rez AJ, Puchades R, Maquieira A.Arrays on disc for screening and quantification of pollutants. Anal Chim Acta. 2013 Jun 19; 784:59-64.
Exposure	Navarro P, PÃ©rez AJ, GabaldÃ³n JA, NÃºÃ±ez-Delicado E, Puchades R, Maquieira A, Morais S	2013	Nov	Navarro P, PÃ©rez AJ, GabaldÃ³n JA, NÃºÃ±ez-Delicado E, Puchades R, Maquieira A, Morais S.Detection of chemical residues in tangerine juices by a duplex immunoassay. Talanta. 2013 Nov 15; 116:33-8.
Exposure	NougadÃ¨re A, Sirot V, Kadar A, Fastier A, Truchot E, Vergnet C, Hommet F, BaylÃ© J, Gros P, Leblanc JC	2012	Sep	NougadÃ¨re A, Sirot V, Kadar A, Fastier A, Truchot E, Vergnet C, Hommet F, BaylÃ© J, Gros P, Leblanc JC.Total diet study on pesticide residues in France: levels in food as consumed and chronic dietary risk to consumers. Environ Int. 2012 Sep 15; 45:135-50.
Exposure	Odabasi M, Cetin B	2012	Dec	Odabasi M, Cetin B.Determination of octanol-air partition coefficients of organochlorine pesticides (OCPs) as a function of temperature: application to air-soil exchange. J Environ Manage. 2012 Dec 30; 113:432-9.
Exposure	Oellig C, Schwack W	2012	Oct	Oellig C, Schwack W.Planar solid phase extraction clean-up for pesticide residue analysis in tea by liquid chromatography-mass spectrometry. J Chromatogr A. 2012 Oct 19; 1260:42-53.

Exposure	Oliver DP, Pan YF, Anderson JS, Lin TF, Kookana RS, Douglas GB, Wendling LA	2013	Jan	Oliver DP, Pan YF, Anderson JS, Lin TF, Kookana RS, Douglas GB, Wendling LA.Sorption of pesticides by a mineral sand mining by-product, neutralised used acid (NUA). Sci Total Environ. 2013 Jan 1; 442:255-62.
Exposure	Omeroglu PY, Ambrus A, Boyacioglu D	2013		Omeroglu PY, Ambrus A, Boyacioglu D.Estimation of the uncertainties of extraction and clean-up steps in pesticide residue analysis of plant commodities. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2013; 30(2):308-20.
Exposure	Osman R, Saim N, Juahir H, Abdullah MP	2012	Jan	Osman R, Saim N, Juahir H, Abdullah MP.Chemometric application in identifying sources of organic contaminants in Langat river basin. Environ Monit Assess. 2012 Jan; 184(2):1001-14.
Exposure	Otieno PO, Owuor PO, Lalah JO, Pfister G, Schramm KW	2013	Mar	Otieno PO, Owuor PO, Lalah JO, Pfister G, Schramm KW.Impacts of climate-induced changes on the distribution of pesticides residues in water and sediment of Lake Naivasha, Kenya. Environ Monit Assess. 2013 Mar; 185(3):2723-33.
Exposure	Otieno PO, Owuor PO, Lalah JO, Pfister G, Schramm KW	2013	Dec	Otieno PO, Owuor PO, Lalah JO, Pfister G, Schramm KW.Comparative evaluation of ELISA kit and HPLC DAD for the determination of chlorpyrifos ethyl residues in water and sediments. Talanta. 2013 Dec 15; 117:250-7.
Exposure	Otieno PO, Schramm KW, Pfister G, Lalah JO, Ojwach SO, Virani M	2012	Apr	Otieno PO, Schramm KW, Pfister G, Lalah JO, Ojwach SO, Virani M.Spatial distribution and temporal trend in concentration of carbofuran, diazinon and chlorpyrifos ethyl residues in sediment and water in Lake Naivasha, Kenya. Bull Environ Contam Toxicol. 2012 Apr; 88(4):526-32.
Exposure	Pelit FO, ErtaÅŸ H, Seyrani I, ErtaÅŸ FN	2013	May	Pelit FO, ErtaÅŸ H, Seyrani I, ErtaÅŸ FN.Assessment of DFG-S19 method for the determination of common endocrine disruptor pesticides in wine samples with an estimation of the uncertainty of the analytical results. Food Chem. 2013 May 1; 138(1):54-61.

Exposure	Pelit FO, Pelit L, ErtaÅŸ H, Nil ErtaÅŸ F	2012	Sep	Pelit FO, Pelit L, ErtaÅŸ H, Nil ErtaÅŸ F.Development of a gas chromatographic method for the determination of Chlorpyrifos and its metabolite Chlorpyrifos-oxon in wine matrix. J Chromatogr B Analyt Technol Biomed Life Sci. 2012 Sep 1; 904:35-41.
Exposure	Pelit L, DizdaÅŸ TN	2013	Oct	Pelit L, DizdaÅŸ TN.Preparation and application of a polythiophene solid-phase microextraction fiber for the determination of endocrine-disruptor pesticides in well waters. J Sep Sci. 2013 Oct; 36(19):3234-41.
Exposure	Phung DT, Connell D, Miller G, Chu C	2012	Jul	Phung DT, Connell D, Miller G, Chu C.Probabilistic assessment of chlorpyrifos exposure to rice farmers in Viet Nam. J Expo Sci Environ Epidemiol. 2012 Jul; 22(4):417-23.
Exposure	Phung DT, Connell D, Miller G, Hodge M, Patel R, Cheng R, Abeyewardene M, Chu C	2012	Apr	Phung DT, Connell D, Miller G, Hodge M, Patel R, Cheng R, Abeyewardene M, Chu C.Biological monitoring of chlorpyrifos exposure to rice farmers in Vietnam. Chemosphere. 2012 Apr; 87(4):294-300.
Exposure	Querejeta GA, Ramos LM, Flores AP, Hughes EA, Zalts A, Montserrat JM	2012	Apr	Querejeta GA, Ramos LM, Flores AP, Hughes EA, Zalts A, Montserrat JM.Environmental pesticide distribution in horticultural and floricultural periurban production units. Chemosphere. 2012 Apr; 87(5):566-72.
Exposure	Rani M, Saini S, Kumari B	2013	Sep	Rani M, Saini S, Kumari B.Persistence and effect of processing on chlorpyriphos residues in tomato (Lycopersicon esculantum Mill.). Ecotoxicol Environ Saf. 2013 Sep; 95:247-52.
Exposure	Rani M, Saini S, Kumari B	2014	Jan	Rani M, Saini S, Kumari B.Leaching behaviour of chlorpyriphos and cypermethrin in sandy loam soil. Environ Monit Assess. 2014 Jan; 186(1):175-82.

Exposure	Robles-Molina J, MartÃn de Vidales MJ, GarcÃa-Reyes JF, CaÃ±izares P, SÃ¡ez C, Rodrigo MA, Molina-DÃaz A	2012	Nov	Robles-Molina J, MartÃn de Vidales MJ, GarcÃa-Reyes JF, CaÃ±izares P, SÃ¡ez C, Rodrigo MA, Molina-DÃaz A.Conductive-diamond electrochemical oxidation of chlorpyrifos in wastewater and identification of its main degradation products by LC-TOFMS. Chemosphere. 2012 Nov; 89(10):1169-76.
Exposure	Roca M, Miralles-Marco A, FerrÃ© J, PÃ©rez R, YusÃ V	2014	May	Roca M, Miralles-Marco A, FerrÃ© J, PÃ©rez R, YusÃ V.Biomonitoring exposure assessment to contemporary pesticides in a school children population of Spain. Environ Res. 2014 May; 131:77-85.
Exposure	RodrÃguez T, van Wendel de Joode B, Lindh CH, Rojas M, Lundberg I, Wesseling C	2012	Feb	Rodriguez T, van Wendel de Joode B, Lindh CH, Rojas M, Lundberg I, Wesseling C.Assessment of long-term and recent pesticide exposure among rural school children in Nicaragua. Occup Environ Med. 2012 Feb; 69(2):119-25.
Exposure	Rojas R, Morillo J, Usero J, Delgado-Moreno L, Gan J	2013	Aug	Rojas R, Morillo J, Usero J, Delgado-Moreno L, Gan J.Enhancing soil sorption capacity of an agricultural soil by addition of three different organic wastes. Sci Total Environ. 2013 Aug 1; 458-460:614-23.
Exposure	Rotariu L, Zamfir LG, Bala C	2012	Oct	Rotariu L, Zamfir LG, Bala C.A rational design of the multiwalled carbon nanotube-7,7,8,8-tetracyanoquinodimethan sensor for sensitive detection of acetylcholinesterase inhibitors. Anal Chim Acta. 2012 Oct 20; 748:81-8.
Exposure	Samadi S, Sereshti H, Assadi Y	2012	Jan	Samadi S, Sereshti H, Assadi Y.Ultra-preconcentration and determination of thirteen organophosphorus pesticides in water samples using solid-phase extraction followed by dispersive liquid-liquid microextraction and gas chromatography with flame photometric detection. J Chromatogr A. 2012 Jan 6; 1219:61-5.

Exposure	Sangchan W, Bannwarth M, Ingwersen J, Hugenschmidt C, Schwadorf K, Thavornyutikarn P, Pansombat K, Streck T	2014	Feb	Sangchan W, Bannwarth M, Ingwersen J, Hugenschmidt C, Schwadorf K, Thavornyutikarn P, Pansombat K, Streck T.Monitoring and risk assessment of pesticides in a tropical river of an agricultural watershed in northern Thailand. Environ Monit Assess. 2014 Feb; 186(2):1083-99.
Exposure	Sarkouhi M, Shamsipur M, Hassan J	2012	Dec	Sarkouhi M, Shamsipur M, Hassan J.Â³Â¹P-NMR evaluation of organophosphorus pesticides degradation through metal ion promoted hydrolysis. Environ Monit Assess. 2012 Dec; 184(12):7383-93.
Exposure	Sasikala C, Jiwal S, Rout P, Ramya M	2012	Mar	Sasikala C, Jiwal S, Rout P, Ramya M.Biodegradation of chlorpyrifos by bacterial consortium isolated from agriculture soil. World J Microbiol Biotechnol. 2012 Mar; 28(3):1301-8.
Exposure	Seebunrueng K, Santaladchaiyakit Y, Srijaranai S	2012	Sep	Seebunrueng K, Santaladchaiyakit Y, Srijaranai S.Study on the effect of chain-length compatibility of mixed anionic-cationic surfactants on the cloud-point extraction of selected organophosphorus pesticides. Anal Bioanal Chem. 2012 Sep; 404(5):1539-48.
Exposure	Shahpoury P, Hageman KJ, Matthaei CD, Magbanua FS	2013	Oct	Shahpoury P, Hageman KJ, Matthaei CD, Magbanua FS.Chlorinated pesticides in stream sediments from organic, integrated and conventional farms. Environ Pollut. 2013 Oct; 181:219-25.
Exposure	Sharma I, Bhardwaj R, Pati PK	2012	Nov	Sharma I, Bhardwaj R, Pati PK.Mitigation of adverse effects of chlorpyrifos by 24-epibrassinolide and analysis of stress markers in a rice variety Pusa Basmati-1. Ecotoxicol Environ Saf. 2012 Nov; 85:72-81.
Exposure	Shi H, Zhao G, Liu M, Fan L, Cao T	2013	Sep	Shi H, Zhao G, Liu M, Fan L, Cao T.Aptamer-based colorimetric sensing of acetamiprid in soil samples: sensitivity, selectivity and mechanism. J Hazard Mater. 2013 Sep 15; 260:754-61.

Exposure	Shin HM, McKone TE, Tulve NS, Clifton MS, Bennett DH	2013	Jan	Shin HM, McKone TE, Tulve NS, Clifton MS, Bennett DH.Indoor residence times of semivolatile organic compounds: model estimation and field evaluation. Environ Sci Technol. 2013 Jan 15; 47(2):859-67.
Exposure	Shing WL, Heng LY, Surif S	2013	May	Shing WL, Heng LY, Surif S.Performance of a cyanobacteria whole cell-based fluorescence biosensor for heavy metal and pesticide detection. Sensors (Basel). 2013 May 14; 13(5):6394-404.
Exposure	Silva E, Pereira AC, Estalagem SP, Moreira-Santos M, Ribeiro R, Cerejeira MJ	2012	Sep-Oct	Silva E, Pereira AC, Estalagem SP, Moreira-Santos M, Ribeiro R, Cerejeira MJ.Assessing the quality of freshwaters in a protected area within the Tagus River basin district (central Portugal). J Environ Qual. 2012 Sep-Oct; 41(5):1413-26.
Exposure	Smalling KL, Kuivila KM, Orlando JL, Phillips BM, Anderson BS, Siegler K, Hunt JW, Hamilton M	2013	Aug	Smalling KL, Kuivila KM, Orlando JL, Phillips BM, Anderson BS, Siegler K, Hunt JW, Hamilton M.Environmental fate of fungicides and other current-use pesticides in a central California estuary. Mar Pollut Bull. 2013 Aug 15; 73(1):144-53.
Exposure	Sreeprasad TS, Gupta SS, Maliyekkal SM, Pradeep T	2013	Feb	Sreeprasad TS, Gupta SS, Maliyekkal SM, Pradeep T.Immobilized graphene-based composite from asphalt: facile synthesis and application in water purification. J Hazard Mater. 2013 Feb 15; 246-247:213-20.
Exposure	Srinivas P S, Banerjee K, Jadhav MR, Ghaste MS, Lawande KE	2012		Srinivas P S, Banerjee K, Jadhav MR, Ghaste MS, Lawande KE.Bioefficacy, dissipation kinetics and safety evaluation of selected insecticides in Allium cepa L. J Environ Sci Health B. 2012; 47(7):700-9.
Exposure	Starner K, Goh KS	2013	Sep	Starner K, Goh KS.Chlorpyrifos-treated crops in the vicinity of surface water contamination in the San Joaquin Valley, California, USA. Bull Environ Contam Toxicol. 2013 Sep; 91(3):287-91.
Exposure	Suciu NA, Ferrari F, Vasileiadis S, Merli A, Capri E, Trevisan M	2013		Suciu NA, Ferrari F, Vasileiadis S, Merli A, Capri E, Trevisan M.Pesticides water decontamination in oxygen-limited conditions. J Environ Sci Health B. 2013; 48(9):793-9.

Exposure	Sun X, Cao Y, Gong Z, Wang X, Zhang Y, Gao J	2012	Dec	Sun X, Cao Y, Gong Z, Wang X, Zhang Y, Gao J.An amperometric immunosensor based on multi-walled carbon nanotubes-thionine-chitosan nanocomposite film for chlorpyrifos detection. Sensors (Basel). 2012 Dec 13; 12(12):17247-61.
Exposure	Sun X, Zhai C, Wang X	2013	Mar	Sun X, Zhai C, Wang X.A novel and highly sensitive acetyl-cholinesterase biosensor modified with hollow gold nanospheres. Bioprocess Biosyst Eng. 2013 Mar; 36(3):273-83.
Exposure	Swarnam TP, Velmurugan A	2013	Jul	Swarnam TP, Velmurugan A.Pesticide residues in vegetable samples from the Andaman Islands, India. Environ Monit Assess. 2013 Jul; 185(7):6119-27.
Exposure	Tiwari MK, Guha S	2013	Oct	Tiwari MK, Guha S.Simultaneous analysis of endosulfan, chlorpyrifos, and their metabolites in natural soil and water samples using gas chromatography-tandem mass spectrometry. Environ Monit Assess. 2013 Oct; 185(10):8451-63.
Exposure	Tortella GR, Rubilar O, Castillo Md, Cea M, Mella-Herrera R, Diez MC	2012	Jun	Tortella GR, Rubilar O, Castillo Md, Cea M, Mella-Herrera R, Diez MC.Chlorpyrifos degradation in a biomixture of biobed at different maturity stages. Chemosphere. 2012 Jun; 88(2):224-8.
Exposure	Urrutia C, Rubilar O, Tortella GR, Diez MC	2013	Aug	Urrutia C, Rubilar O, Tortella GR, Diez MC.Degradation of pesticide mixture on modified matrix of a biopurification system with alternatives lignocellulosic wastes. Chemosphere. 2013 Aug; 92(10):1361-6.
Exposure	van Wendel de Joode B, Barraza D, Ruepert C, Mora AM, CÃ³rdoba L, Oberg M, Wesseling C, Mergler D, Lindh CH	2012	Aug	van Wendel de Joode B, Barraza D, Ruepert C, Mora AM, CÃ³rdoba L, Oberg M, Wesseling C, Mergler D, Lindh CH.Indigenous children living nearby plantations with chlorpyrifos-treated bags have elevated 3,5,6-trichloro-2-pyridinol (TCPy) urinary concentrations. Environ Res. 2012 Aug; 117:17-26.

Exposure	Vogt R, Bennett D, Cassady D, Frost J, Ritz B, Hertz-Picciotto I	2012	Nov	Vogt R, Bennett D, Cassady D, Frost J, Ritz B, Hertz-Picciotto I.Cancer and non-cancer health effects from food contaminant exposures for children and adults in California: a risk assessment. Environ Health. 2012 Nov 9; 11:83.
Exposure	Walorczyk S, DroÅ¼dÅ¼yÅ„ski D	2012	Aug	Walorczyk S, DroÅ¼dÅ¼yÅ„ski D.Improvement and extension to new analytes of a multi-residue method for the determination of pesticides in cereals and dry animal feed using gas chromatography-tandem quadrupole mass spectrometry revisited. J Chromatogr A. 2012 Aug 17; 1251:219-31.
Exposure	Wang C, Zhou Q, Zhang L, Zhang Y, Xiao E, Wu Z	2013		Wang C, Zhou Q, Zhang L, Zhang Y, Xiao E, Wu Z.Variation characteristics of chlorpyrifos in nonsterile wetland plant hydroponic system. Int J Phytoremediation. 2013; 15(6):550-60.
Exposure	Wang P, Dai W, Ge L, Yan M, Ge S, Yu J	2013	Feb	Wang P, Dai W, Ge L, Yan M, Ge S, Yu J.Visible light photoelectrochemical sensor based on Au nanoparticles and molecularly imprinted poly(o-phenylenediamine)-modified TiO2 nanotubes for specific and sensitive detection chlorpyrifos. Analyst. 2013 Feb 21; 138(3):939-45.
Exposure	Wang Q, Yang J, Li C, Xiao B, Que X	2013		Wang Q, Yang J, Li C, Xiao B, Que X.Influence of initial pesticide concentrations in water on chlorpyrifos toxicity and removal by Iris pseudacorus. Water Sci Technol. 2013; 67(9):1908-15.
Exposure	Wang S, Wang Z, Zhang Y, Wang J, Guo R	2013	Jun	Wang S, Wang Z, Zhang Y, Wang J, Guo R.Pesticide residues in market foods in Shaanxi Province of China in 2010. Food Chem. 2013 Jun 1; 138(2-3):2016-25.
Exposure	Wang Z, Huang J, Chen J, Li F	2013	Aug	Wang Z, Huang J, Chen J, Li F.Effectiveness of dishwashing liquids in removing chlorothalonil and chlorpyrifos residues from cherry tomatoes. Chemosphere. 2013 Aug; 92(8):1022-8.

Exposure	Wang ZW, Huang J, Chen JY, Li FL	2013	Jul	Wang ZW, Huang J, Chen JY, Li FL.Time-dependent movement and distribution of chlorothalonil and chlorpyrifos in tomatoes. Ecotoxicol Environ Saf. 2013 Jul; 93:107-11.
Exposure	Wason SC, Julien R, Perry MJ, Smith TJ, Levy JI	2013	Jul	Wason SC, Julien R, Perry MJ, Smith TJ, Levy JI.Modeling exposures to organophosphates and pyrethroids for children living in an urban low-income environment. Environ Res. 2013 Jul; 124:13-22.
Exposure	Wei W, Zong X, Wang X, Yin L, Pu Y, Liu S	2012	Dec	Wei W, Zong X, Wang X, Yin L, Pu Y, Liu S.A disposable amperometric immunosensor for chlorpyrifos-methyl based on immunogen/platinum doped silica sol-gel film modified screen-printed carbon electrode. Food Chem. 2012 Dec 1; 135(3):888-92.
Exposure	Weschler CJ, Nazaroff WW	2012	Oct	Weschler CJ, Nazaroff WW.SVOC exposure indoors: fresh look at dermal pathways. Indoor Air. 2012 Oct; 22(5):356-77.
Exposure	Weston DP, Ding Y, Zhang M, Lydy MJ	2013	Jan	Weston DP, Ding Y, Zhang M, Lydy MJ.Identifying the cause of sediment toxicity in agricultural sediments: the role of pyrethroids and nine seldom-measured hydrophobic pesticides. Chemosphere. 2013 Jan; 90(3):958-64.
Exposure	Wofford P, Segawa R, Schreider J, Federighi V, Neal R, Brattesani M	2014	Mar	Wofford P, Segawa R, Schreider J, Federighi V, Neal R, Brattesani M.Community air monitoring for pesticides. Part 3: using health-based screening levels to evaluate results collected for a year. Environ Monit Assess. 2014 Mar; 186(3):1355-70.
Exposure	Yang L, Wang G, Liu Y, Wang M	2013	Sep	Yang L, Wang G, Liu Y, Wang M.Development of a biosensor based on immobilization of acetylcholinesterase on NiO nanoparticles-carboxylic graphene-nafion modified electrode for detection of pesticides. Talanta. 2013 Sep 15; 113:135-41.
Exposure	Yang L, Wang GC, Liu YJ, An JJ, Wang M	2013	Mar	Yang L, Wang GC, Liu YJ, An JJ, Wang M.Development of a stable biosensor based on a SiO2 nanosheet-Nafion-modified glassy carbon electrode for sensitive detection of pesticides. Anal Bioanal Chem. 2013 Mar; 405(8):2545-52.

Exposure	Yao GH, Liang RP, Huang CF, Wang Y, Qiu JD	2013	Dec	Yao GH, Liang RP, Huang CF, Wang Y, Qiu JD.Surface plasmon resonance sensor based on magnetic molecularly imprinted polymers amplification for pesticide recognition. Anal Chem. 2013 Dec 17; 85(24):11944-51.
Exposure	Zhai C, Sun X, Zhao W, Gong Z, Wang X	2013	Apr	Zhai C, Sun X, Zhao W, Gong Z, Wang X.Acetylcholinesterase biosensor based on chitosan/prussian blue/multiwall carbon nanotubes/hollow gold nanospheres nanocomposite film by one-step electrodeposition. Biosens Bioelectron. 2013 Apr 15; 42:124-30.
Exposure	Zhan Y, Zhang M	2012	Aug	Zhan Y, Zhang M.PURE: a web-based decision support system to evaluate pesticide environmental risk for sustainable pest management practices in California. Ecotoxicol Environ Saf. 2012 Aug; 82:104-13.
Exposure	Zhang Q, Wang B, Cao Z, Yu Y	2012	Jun	Zhang Q, Wang B, Cao Z, Yu Y.Plasmid-mediated bioaugmentation for the degradation of chlorpyrifos in soil. J Hazard Mater. 2012 Jun 30; 221-222:178-84.
Exposure	Zhang W, Tang Y, Du D, Smith J, Timchalk C, Liu D, Lin Y	2013	Sep	Zhang W, Tang Y, Du D, Smith J, Timchalk C, Liu D, Lin Y.Direct analysis of trichloropyridinol in human saliva using an Au nanoparticles-based immunochromatographic test strip for biomonitoring of exposure to chlorpyrifos. Talanta. 2013 Sep 30; 114:261-7.
Exposure	Zhang X, Shen Y, Yu XY, Liu XJ	2012	Apr	Zhang X, Shen Y, Yu XY, Liu XJ.Dissipation of chlorpyrifos and residue analysis in rice, soil and water under paddy field conditions. Ecotoxicol Environ Saf. 2012 Apr; 78:276-80.
Exposure	Zhang X, Starner K, Spurlock F	2012	Nov	Zhang X, Starner K, Spurlock F.Analysis of chlorpyrifos agricultural use in regions of frequent surface water detections in California, USA. Bull Environ Contam Toxicol. 2012 Nov; 89(5):978-84.

Exposure	Zhang Y, An J, Ye W, Yang G, Qian ZG, Chen HF, Cui L, Feng Y	2012	Sep	Zhang Y, An J, Ye W, Yang G, Qian ZG, Chen HF, Cui L, Feng Y.Enhancing the promiscuous phosphotriesterase activity of a thermostable lactonase (GkaP) for the efficient degradation of organophosphate pesticides. Appl Environ Microbiol. 2012 Sep; 78(18):6647-55.
Exposure	Zhao X, Wu C, Wang Y, Cang T, Chen L, Yu R, Wang Q	2012	Feb	Zhao X, Wu C, Wang Y, Cang T, Chen L, Yu R, Wang Q.Assessment of toxicity risk of insecticides used in rice ecosystem on Trichogramma japonicum, an egg parasitoid of rice lepidopterans. J Econ Entomol. 2012 Feb; 105(1):92-101.
Exposure	Zhao Y, Wang C, Wendling LA, Pei Y	2013	Aug	Zhao Y, Wang C, Wendling LA, Pei Y.Feasibility of using drinking water treatment residuals as a novel chlorpyrifos adsorbent. J Agric Food Chem. 2013 Aug 7; 61(31):7446-52.
Exposure	Zhong G, Xie Z, Cai M, MÃ¶ller A, Sturm R, Tang J, Zhang G, He J, Ebinghaus R	2012	Jan	Zhong G, Xie Z, Cai M, MÃ¶ller A, Sturm R, Tang J, Zhang G, He J, Ebinghaus R.Distribution and air-sea exchange of current-use pesticides (CUPs) from East Asia to the high Arctic Ocean. Environ Sci Technol. 2012 Jan 3; 46(1):259-67.
Exposure	Zhou J, Lu Q, Tong Y, Wei W, Liu S	2012	Sep	Zhou J, Lu Q, Tong Y, Wei W, Liu S.Detection of DNA damage by using hairpin molecular beacon probes and graphene oxide. Talanta. 2012 Sep 15; 99:625-30.
Exposure	GonzÃ¡lez-Curbelo MA, Asensio-Ramos M, Herrera-Herrera AV, HernÃ¡ndez-Borges J	2012	Jul	GonzÃ¡lez-Curbelo MA, Asensio-Ramos M, Herrera-Herrera AV, HernÃ¡ndez-Borges J.Pesticide residue analysis in cereal-based baby foods using multi-walled carbon nanotubes dispersive solid-phase extraction. Anal Bioanal Chem. 2012 Jul; 404(1):183-96.
Exposure	Lin YZ, Zeng GM, Zhang Y, Chen M, Jiang M, Zhang JC, Lu LH, Liu LF	2012	Mar	Lin YZ, Zeng GM, Zhang Y, Chen M, Jiang M, Zhang JC, Lu LH, Liu LF.[Biodegradation mechanism of DDT and chlorpyrifos using molecular simulation]. Huan Jing Ke Xue. 2012 Mar; 33(3):1015-9.
Exposure/Biomarkers	Adolfsson-Erici M, Ã…Kerman G, McLachlan MS	2012	Jun	Adolfsson-Erici M, Ã…Kerman G, McLachlan MS.In-vivo passive sampling to measure elimination kinetics in bioaccumulation tests. Chemosphere. 2012 Jun; 88(1):62-8.

Exposure/Biomarkers	Ding Y, Landrum PF, You J, Harwood AD, Lydy MJ	2012	Sep	Ding Y, Landrum PF, You J, Harwood AD, Lydy MJ.Use of solid phase microextraction to estimate toxicity: relating fiber concentrations to toxicity--part I. Environ Toxicol Chem. 2012 Sep; 31(9):2159-67.
Exposure/Biomarkers	Sun H, Si C, Bian Q, Chen X, Chen L, Wang X	2012	Aug	Sun H, Si C, Bian Q, Chen X, Chen L, Wang X.Developing in vitro reporter gene assays to assess the hormone receptor activities of chemicals frequently detected in drinking water. J Appl Toxicol. 2012 Aug; 32(8):635-41.
High Dose	Nutter TJ, Cooper BY	2014	Jun	Nutter TJ, Cooper BY.Persistent modification of Nav1.9 following chronic exposure to insecticides and pyridostigmine bromide. Toxicol Appl Pharmacol. 2014 Jun 15; 277(3):298-309.
High Dose	Yildirim, E., Baydan E, Kanbur M, Kul O, CÄ±nar M, Ekici H, Atmaca N	2013		Yildirim, E., Baydan E, Kanbur M, Kul O, Cinar M, Ekici H, Atmaca N.The effect of chlorpyrifos on isolated thoracic aorta in rats. Biomed Res Int. 2013; 2013:376051.
High Dose/Mixture	Nutter TJ, Jiang N, Cooper BY	2013	Dec	Nutter TJ, Jiang N, Cooper BY.Persistent Na+ and K+ channel dysfunctions after chronic exposure to insecticides and pyridostigmine bromide. Neurotoxicology. 2013 Dec; 39:72-83.
High Dose/Mixture	Ojha A, Srivastava N	2012	Jan	Ojha A, Srivastava N.Redox imbalance in rat tissues exposed with organophosphate pesticides and therapeutic potential of antioxidant vitamins. Ecotoxicol Environ Saf. 2012 Jan; 75(1):230-41.
High Doses/Acute/Adults	Sandhu MA, Saeed AA, Khilji MS, Ahmed A, Latif MS, Khalid N	2013		Sandhu MA, Saeed AA, Khilji MS, Ahmed A, Latif MS, Khalid N.Genotoxicity evaluation of chlorpyrifos: a gender related approach in regular toxicity testing. J Toxicol Sci. 2013; 38(2):237-44.

Methods Paper (Biosensor and Biomonitor Approach)	Camann DE, Schultz ST, Yau AY, Heilbrun LP, Zuniga MM, Palmer RF, Miller CS	2013	Mar	Camann DE, Schultz ST, Yau AY, Heilbrun LP, Zuniga MM, Palmer RF, Miller CS.Acetaminophen, pesticide, and diethylhexyl phthalate metabolites, anandamide, and fatty acids in deciduous molars: potential biomarkers of perinatal exposure. J Expo Sci Environ Epidemiol. 2013 Mar; 23(2):190-6.
Methods Paper (Biosensor and Biomonitor Approach)	Henderson JD, Glucksman G, Leong B, Tigyi A, Ankirskaia A, Siddique I, Lam H, DePeters E, Wilson BW	2012	Jan	Henderson JD, Glucksman G, Leong B, Tigyi A, Ankirskaia A, Siddique I, Lam H, DePeters E, Wilson BW.Pyridostigmine bromide protection against acetylcholinesterase inhibition by pesticides. J Biochem Mol Toxicol. 2012 Jan; 26(1):31-4.
Methods Paper (Biosensor and Biomonitor Approach)	Jiang W, Duysen EG, Lockridge O	2012	May	Jiang W, Duysen EG, Lockridge O.Mice treated with a nontoxic dose of chlorpyrifos oxon have diethoxyphosphotyrosine labeled proteins in blood up to 4 days post exposure, detected by mass spectrometry. Toxicology. 2012 May 16; 295(1-3):15-22.
Methods Paper (Biosensor and Biomonitor Approach)	Joly C, Gay-QuÃ©heillard J, LÃ©kÃ© A, Chardon K, Delanaud S, Bach V, Khorsi-Cauet H	2013	May	Joly C, Gay-QuÃ©heillard J, LÃ©kÃ© A, Chardon K, Delanaud S, Bach V, Khorsi-Cauet H.Impact of chronic exposure to low doses of chlorpyrifos on the intestinal microbiota in the Simulator of the Human Intestinal Microbial Ecosystem (SHIME) and in the rat. Environ Sci Pollut Res Int. 2013 May; 20(5):2726-34.
Methods Paper (Biosensor and Biomonitor Approach)	Jones OA, Swain SC, Svendsen C, Griffin JL, Sturzenbaum SR, Spurgeon DJ	2012	Feb	Jones OA, Swain SC, Svendsen C, Griffin JL, Sturzenbaum SR, Spurgeon DJ.Potential new method of mixture effects testing using metabolomics and Caenorhabditis elegans. J Proteome Res. 2012 Feb 3; 11(2):1446-53.
Methods Paper (Biosensor and Biomonitor Approach)	Maravgakis G, Tzatzarakis MN, Alegakis AK, Stivaktakis PD, Tsatsakis AM	2012	May	Maravgakis G, Tzatzarakis MN, Alegakis AK, Stivaktakis PD, Tsatsakis AM.Diethyl phosphates accumulation in rabbits' hair as an indicator of long term exposure to diazinon and chlorpyrifos. Forensic Sci Int. 2012 May 10; 218(1-3):106-10.

Methods Paper (Biosensor and Biomonitor Approach)	Multari RA, Cremers DA, Scott T, Kendrick P	2013	Mar	Multari RA, Cremers DA, Scott T, Kendrick P.Detection of pesticides and dioxins in tissue fats and rendering oils using laser-induced breakdown spectroscopy (LIBS). J Agric Food Chem. 2013 Mar 13; 61(10):2348-57.
Methods Paper (Biosensor and Biomonitor Approach)	Papoutsis I, Mendonis M, Nikolaou P, Athanaselis S, Pistos C, Maravelias C, Spiliopoulou C	2012	May	Papoutsis I, Mendonis M, Nikolaou P, Athanaselis S, Pistos C, Maravelias C, Spiliopoulou C.Development and validation of a simple GC-MS method for the simultaneous determination of 11 anticholinesterase pesticides in blood--clinical and forensic toxicology applications. J Forensic Sci. 2012 May; 57(3):806-12.
Methods Paper (Biosensor and Biomonitor Approach)	Schopfer LM, Lockridge O	2012	Mar-Apr	Schopfer LM, Lockridge O.Analytical approaches for monitoring exposure to organophosphorus and carbamate agents through analysis of protein adducts. Drug Test Anal. 2012 Mar-Apr; 4(3-4):246-61.
Methods Paper for Dermal Absorption	Mircioiu C, Voicu VA, Ionescu M, Miron DS, Radulescu FS, Nicolescu AC	2013	May	Mircioiu C, Voicu VA, Ionescu M, Miron DS, Radulescu FS, Nicolescu AC.Evaluation of in vitro absorption, decontamination and desorption of organophosphorous compounds from skin and synthetic membranes. Toxicol Lett. 2013 May 23; 219(2):99-106.
Methods Paper/Alternatives for neurodevelopment	Visan A, Hayess K, Sittner D, Pohl EE, Riebeling C, Slawik B, Gulich K, OelgeschlÃ¤ger M, Luch A, Seiler AE	2012	Oct	Visan A, Hayess K, Sittner D, Pohl EE, Riebeling C, Slawik B, Gulich K, OelgeschlÃ¤ger M, Luch A, Seiler AE.Neural differentiation of mouse embryonic stem cells as a tool to assess developmental neurotoxicity in vitro. Neurotoxicology. 2012 Oct; 33(5):1135-46.
Methods Paper/Alternatives for neurodevelopment/Not ready to establish AOP	Amaroli A, Aluigi MG, Falugi C, Chessa MG	2013	Feb	Amaroli A, Aluigi MG, Falugi C, Chessa MG.Effects of the neurotoxic thionophosphate pesticide chlorpyrifos on differentiating alternative models. Chemosphere. 2013 Feb; 90(7):2115-22.

Methods Paper/Alternatives for neurodevelopment/Not ready to establish AOP	Culbreth ME, Harrill JA, Freudenrich TM, Mundy WR, Shafer TJ	2012	Dec	Culbreth ME, Harrill JA, Freudenrich TM, Mundy WR, Shafer TJ.Comparison of chemical-induced changes in proliferation and apoptosis in human and mouse neuroprogenitor cells. Neurotoxicology. 2012 Dec; 33(6):1499-510.
Methods Paper/Alternatives for neurodevelopment/Not ready to establish AOP	Estevan C, Vilanova E, Sogorb MA	2013	Feb	Estevan C, Vilanova E, Sogorb MA.Chlorpyrifos and its metabolites alter gene expression at non-cytotoxic concentrations in D3 mouse embryonic stem cells under in vitro differentiation: considerations for embryotoxic risk assessment. Toxicol Lett. 2013 Feb 13; 217(1):14-22.
Methods Paper/Alternatives for neurodevelopment/Not ready to establish AOP	GuiÃ±azÃº N, Rena V, Genti-Raimondi S, Rivero V, Magnarelli G	2012	Apr	Guinaza, N, Rena V, Genti-Raimondi S, Rivero V, Magnarelli G.Effects of the organophosphate insecticides phosmet and chlorpyrifos on trophoblast JEG-3 cell death, proliferation and inflammatory molecule production. Toxicol In Vitro. 2012 Apr; 26(3):406-13.
Microbial	Abraham J, Shanker A, Silambarasan S	2013	Dec	Abraham J, Shanker A, Silambarasan S.Role of Gordonia sp JAAS1 in biodegradation of chlorpyrifos and its hydrolysing metabolite 3,5,6-trichloro-2-pyridinol. Lett Appl Microbiol. 2013 Dec; 57(6):510-6.
Mixtures	Cole, T.B. , Jansen, Karen, Park, Sarah , Li, Wan-Fen, Furlong, Clement E. , Costa, Lucio G.			Cole, T.B. , Jansen, Karen, Park, Sarah , Li, Wan-Fen, Furlong, Clement E. , Costa, Lucio G.The Toxicity of Mixtures of Specific Organophosphate Compounds is Modulated by Paraoxonase 1 Status. S.T. Reddy (ed.), Paraoxonase in Inflammation, Infection, and Toxicology, Advances in Experimental Medicine and Biology 660, DOI 10.1007/978-1-60761-350-3_6, Humana Press, LLC 2010

Mixtures	Slotkin TA, Card J, Seidler FJ	2013	Jul	Slotkin TA, Card J, Seidler FJ.Adverse benzo[a]pyrene effects on neurodifferentiation are altered by other neurotoxicant coexposures: interactions with dexamethasone, chlorpyrifos, or nicotine in PC12 cells. Environ Health Perspect. 2013 Jul; 121(7):825-31.
Mixtures/Acute effects	Wilson BW, Rusli FJ, Yan Tam MK, DePeters E, Henderson JD	2012	Dec	Wilson BW, Rusli FJ, Yan Tam MK, DePeters E, Henderson JD.Carbamate protection of AChE against inhibition by agricultural chemicals. J Biochem Mol Toxicol. 2012 Dec; 26(12):506-9.
No body of evidence; Small number of studies without mechanistic hypothesis to outcome	Li W, Ehrich M	2013	Oct	Li W, Ehrich M.Transient alterations of the blood-brain barrier tight junction and receptor potential channel gene expression by chlorpyrifos. J Appl Toxicol. 2013 Oct; 33(10):1187-91.
No body of evidence; Small number of studies without mechanistic hypothesis to outcome	Potera C	2012	Jul	Potera C.Newly discovered mechanism for chlorpyrifos effects on neurodevelopment. Environ Health Perspect. 2012 Jul; 120(7):a270-1.
No body of evidence; Small number of studies without mechanistic hypothesis to outcome	Ridano ME, Racca AC, Flores-MartÃn J, Camolotto SA, de Potas GM, Genti-Raimondi S, Panzetta-Dutari GM	2012	Jun	Ridano ME, Racca AC, Flores-MartÃn J, Camolotto SA, de Potas GM, Genti-Raimondi S, Panzetta-Dutari GM.Chlorpyrifos modifies the expression of genes involved in human placental function. Reprod Toxicol. 2012 Jun; 33(3):331-8.

No body of evidence; Small number of studies without mechanistic hypothesis to outcome	Vera, Berta, Santa Cruz, Silvia, Magnarelli, Gladis	2012	April	Vera, Berta , Santa Cruz, Silvia , Magnarelli, Gladis Plasma cholinesterase and carboxylesterase activities and nuclear and mitochondrial lipid composition of human placenta associated with maternal exposure to pesticides. Reproductive Toxicology 34 (2012) 402-407.
Not in English	Å ukaszewicz-Hussain A	2012		Å ukaszewicz-Hussain A.[Paraoxonase activity and lipid peroxides concentration in serum of rats subchronically intoxicated with chlorpyrifos--organophosphate insecticide]. Med Pr. 2012; 63(5):559-64.
Not in English	Tan QT, Bai HS, Liu W	2012	Dec	Tan QT, Bai HS, Liu W.[Detection of chlorpyrifos in air of workplace with HPLC]. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi. 2012 Dec; 30(12):953-5.
Not related to neurodevelopmental	Ambali SF, Ayo JO	2012	May	Ambali SF, Ayo JO.Vitamin C Attenuates Chronic Chlorpyrifos-induced Alteration of Neurobehavioral Parameters in Wistar Rats. Toxicol Int. 2012 May; 19(2):144-52.
Not related to neurodevelopmental	Dutta AL, Sahu CR	2013		Dutta AL, Sahu CR.Emblica officinalis Garten fruits extract ameliorates reproductive injury and oxidative testicular toxicity induced by chlorpyrifos in male rats. Springerplus. 2013; 2:541.
Not related to neurodevelopmental	Juberg DR, Gehen SC, Coady KK, LeBaron MJ, Kramer VJ, Lu H, Marty MS	2013	Aug	Juberg DR, Gehen SC, Coady KK, LeBaron MJ, Kramer VJ, Lu H, Marty MS.Chlorpyrifos: weight of evidence evaluation of potential interaction with the estrogen, androgen, or thyroid pathways. Regul Toxicol Pharmacol. 2013 Aug; 66(3):249-63.
Not related to neurodevelopmental	Mandal TK, Das NS	2012	Apr	Mandal TK, Das NS.Testicular gametogenic and steroidogenic activities in chlorpyrifos insecticide-treated rats: a correlation study with testicular oxidative stress and role of antioxidant enzyme defence systems in Sprague-Dawley rats. Andrologia. 2012 Apr; 44(2):102-15.

Not related to neurodevelopmental	Noro Y, Tomizawa M, Ito Y, Suzuki H, Abe K, Kamijima M	2013	Oct	Noro Y, Tomizawa M, Ito Y, Suzuki H, Abe K, Kamijima M.Anticholinesterase insecticide action at the murine male reproductive system. Bioorg Med Chem Lett. 2013 Oct 1; 23(19):5434-6.
Not related to neurodevelopmental	Rich JD, Gabriel SM, Schultz-Norton JR	2012		Rich JD, Gabriel SM, Schultz-Norton JR.In vitro effects of herbicides and insecticides on human breast cells. ISRN Toxicol. 2012; 2012:232461.
Not related to neurodevelopmental	Slotkin TA, Cooper EM, Stapleton HM, Seidler FJ	2013	Sep	Slotkin TA, Cooper EM, Stapleton HM, Seidler FJ.Does thyroid disruption contribute to the developmental neurotoxicity of chlorpyrifos? Environ Toxicol Pharmacol. 2013 Sep; 36(2):284-7.
Not related to neurodevelopmental	Svensson RU, Bannick NL, Marin MJ, Robertson LW, Lynch CF, Henry MD	2013		Svensson RU, Bannick NL, Marin MJ, Robertson LW, Lynch CF, Henry MD.Chronic chlorpyrifos exposure does not promote prostate cancer in prostate specific PTEN mutant mice. J Environ Pathol Toxicol Oncol. 2013; 32(1):29-39.
Not related to neurodevelopmental	Tripathi S, Suzuki N, Srivastav AK	2013	Jul	Tripathi S, Suzuki N, Srivastav AK.Response of serum minerals (calcium, phosphate, and magnesium) and endocrine glands (calcitonin cells and parathyroid gland) of Wistar rat after chlorpyrifos administration. Microsc Res Tech. 2013 Jul; 76(7):673-8.
Not related to neurodevelopmental	Yaduvanshi SK, Srivastava N, Marotta F, Jain S, Yadav H	2012	Sep	Yaduvanshi SK, Srivastava N, Marotta F, Jain S, Yadav H.Evaluation of micronuclei induction capacity and mutagenicity of organochlorine and organophosphate pesticides. Drug Metab Lett. 2012 Sep 1; 6(3):187-97.
Not related to neurodevelopmental effects	Å ozowicka B	2013	Nov	Å ozowicka B.The development, validation and application of a GC-dual detector (NPD-ECD) multi-pesticide residue method for monitoring bee poisoning incidents. Ecotoxicol Environ Saf. 2013 Nov; 97:210-22.
Not related to neurodevelopmental effects	Acker CI, Nogueira CW	2012	Oct	Acker CI, Nogueira CW.Chlorpyrifos acute exposure induces hyperglycemia and hyperlipidemia in rats. Chemosphere. 2012 Oct; 89(5):602-8.

Not related to neurodevelopmental effects	Ahmad F, Iqbal S, Anwar S, Afzal M, Islam E, Mustafa T, Khan QM	2012	Oct	Ahmad F, Iqbal S, Anwar S, Afzal M, Islam E, Mustafa T, Khan QM.Enhanced remediation of chlorpyrifos from soil using ryegrass (Lollium multiflorum) and chlorpyrifos-degrading bacterium Bacillus pumilus C2A1. J Hazard Mater. 2012 Oct 30; 237-238:110-5.
Not related to neurodevelopmental effects	Ahmad M, Akhtar S	2013	Apr	Ahmad M, Akhtar S.Development of insecticide resistance in field populations of Brevicoryne brassicae (Hemiptera: Aphididae) in Pakistan. J Econ Entomol. 2013 Apr; 106(2):954-8.
Not related to neurodevelopmental effects	Alasbahi RH, Melzig MF	2012	Jan	Alasbahi RH, Melzig MF.Forskolin and derivatives as tools for studying the role of cAMP. Pharmazie. 2012 Jan; 67(1):5-13.
Not related to neurodevelopmental effects	Alvi AH, Sayyed AH, Naeem M, Ali M	2012		Alvi AH, Sayyed AH, Naeem M, Ali M.Field evolved resistance in Helicoverpa armigera (Lepidoptera: Noctuidae) to Bacillus thuringiensis toxin Cry1Ac in Pakistan. PLoS One. 2012; 7(10):e47309.
Not related to neurodevelopmental effects	Ansari MA, Butt TM	2012	Oct	Ansari MA, Butt TM.Evaluation of entomopathogenic fungi and a nematode against the soil-dwelling stages of the crane fly Tipula paludosa. Pest Manag Sci. 2012 Oct; 68(10):1337-44.
Not related to neurodevelopmental effects	Arain MS, Hu XX, Li GQ	2014	Feb	Arain MS, Hu XX, Li GQ.Assessment of toxicity and potential risk of butene-fipronil using Drosophila melanogaster, in comparison to nine conventional insecticides. Bull Environ Contam Toxicol. 2014 Feb; 92(2):190-5.
Not related to neurodevelopmental effects	Asselman J, Janssen CR, Smagghe G, De Schamphelaere KA	2014	May	Asselman J, Janssen CR, Smagghe G, De Schamphelaere KA.Ecotoxicity of binary mixtures of Microcystis aeruginosa and insecticides to Daphnia pulex. Environ Pollut. 2014 May; 188:56-63.

Not related to neurodevelopmental effects	Bajracharya NS, Opit GP, Talley J, Jones CL	2013	Oct	Bajracharya NS, Opit GP, Talley J, Jones CL.Efficacies of spinosad and a combination of chlorpyrifos-methyl and deltamethrin against phosphine-resistant Rhyzopertha dominica (Coleoptera: Bostrichidae) and Tribolium castaneum (Coleoptera: Tenebrionidae) on wheat. J Econ Entomol. 2013 Oct; 106(5):2208-15.
Not related to neurodevelopmental effects	Ban L, Zhang S, Huang Z, He Y, Peng Y, Gao C	2012	Dec	Ban L, Zhang S, Huang Z, He Y, Peng Y, Gao C.Resistance monitoring and assessment of resistance risk to pymetrozine in Laodelphax striatellus (Hemiptera: Delphacidae). J Econ Entomol. 2012 Dec; 105(6):2129-35.
Not related to neurodevelopmental effects	Barata C, FernÃ¡ndez-San Juan M, Feo ML, Eljarrrat E, Soares AM, BarcelÃ³ D, Baird DJ	2012	Sep	Barata C, FernÃ¡ndez-San Juan M, Feo ML, Eljarrrat E, Soares AM, BarcelÃ³ D, Baird DJ.Population growth rate responses of Ceriodaphnia dubia to ternary mixtures of specific acting chemicals: pharmacological versus ecotoxicological modes of action. Environ Sci Technol. 2012 Sep 4; 46(17):9663-72.
Not related to neurodevelopmental effects	Barmaz S, Vaj C, Ippolito A, Vighi M	2012	Nov	Barmaz S, Vaj C, Ippolito A, Vighi M.Exposure of pollinators to plant protection products. Ecotoxicology. 2012 Nov; 21(8):2177-85.
Not related to neurodevelopmental effects	Bhinder P, Chaudhry A	2013	Sep	Bhinder P, Chaudhry A.Mutagenicity Assessment of Organophosphates using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism Assay. Toxicol Int. 2013 Sep; 20(3):254-60.
Not related to neurodevelopmental effects	Bhinder P, Chaudhry A	2013	May	Bhinder P, Chaudhry A.Evaluation of toxic potential of acephate and chlorpyrifos by dominant lethal test on Culex quinquefasciatus. J Environ Biol. 2013 May; 34(3):573-7.
Not related to neurodevelopmental effects	Bisset JA, MarÃn R, RodrÃguez MM, Severson DW, Ricardo Y, French L, DÃaz M, PÃ©rez O	2013	Mar	Bisset JA, MarÃn R, RodrÃguez MM, Severson DW, Ricardo Y, French L, DÃaz M, PÃ©rez O.Insecticide resistance in two Aedes aegypti (Diptera: Culicidae) strains from Costa Rica. J Med Entomol. 2013 Mar; 50(2):352-61.

Not related to neurodevelopmental effects	Boatti L, Robotti E, Marengo E, Viarengo A, Marsano F	2012	Nov	Boatti L, Robotti E, Marengo E, Viarengo A, Marsano F.Effects of Nickel, Chlorpyrifos and Their Mixture on the Dictyostelium discoideum Proteome. Int J Mol Sci. 2012 Nov 23; 13(12):15679-705.
Not related to neurodevelopmental effects	Bousova, I, Skalova, L	2012	Dec	Bousova, I, Skalova, L.Inhibition and induction of glutathione S-transferases by flavonoids: possible pharmacological and toxicological consequences. Drug Metab Rev. 2012 Dec; 44(4):267-86.
Not related to neurodevelopmental effects	Carrasco-Letelier L, Mendoza-Spina Y, Branchiccela MB	2012	Jul	Carrasco-Letelier L, Mendoza-Spina Y, Branchiccela MB.Acute contact toxicity test of insecticides (Cipermetrina 25, Lorsban 48E, Thionex 35) on honeybees in the southwestern zone of Uruguay. Chemosphere. 2012 Jul; 88(4):439-44.
Not related to neurodevelopmental effects	Carrillo D, Crane JH, PeÃ±a JE	2013	Dec	Carrillo D, Crane JH, PeÃ±a JE.Potential of contact insecticides to control Xyleborus glabratus (Coleoptera: Curculionidae), a vector of laurel wilt disease in avocados. J Econ Entomol. 2013 Dec; 106(6):2286-95.
Not related to neurodevelopmental effects	Chatterjee S, Basak P, Chaklader M, Das P, Pereira JA, Chaudhuri S, Law S	2013	Mar	Chatterjee S, Basak P, Chaklader M, Das P, Pereira JA, Chaudhuri S, Law S.Pesticide induced marrow toxicity and effects on marrow cell population and on hematopoietic stroma. Exp Toxicol Pathol. 2013 Mar; 65(3):287-95.
Not related to neurodevelopmental effects	Che W, Shi T, Wu Y, Yang Y	2013	Aug	Che W, Shi T, Wu Y, Yang Y.Insecticide resistance status of field populations of Spodoptera exigua (Lepidoptera: Noctuidae) from China. J Econ Entomol. 2013 Aug; 106(4):1855-62.
Not related to neurodevelopmental effects	Chen WQ, Ma H, Bian JM, Zhang YZ, Li J	2012	Oct	Chen WQ, Ma H, Bian JM, Zhang YZ, Li J.Hyper-phosphorylation of GSK-3Î²: possible roles in chlorpyrifos-induced behavioral alterations in animal model of depression. Neurosci Lett. 2012 Oct 24; 528(2):148-52.
Not related to neurodevelopmental effects	Chiba S, Ikawa T, Takeshita H, Kanno S, Nagai T, Takada M, Mukai T, Wempe MF	2013	Aug	Chiba S, Ikawa T, Takeshita H, Kanno S, Nagai T, Takada M, Mukai T, Wempe MF.Human organic cation transporter 2 (hOCT2): Inhibitor studies using S2-hOCT2 cells. Toxicology. 2013 Aug 9; 310:98-103.

Not related to neurodevelopmental effects	Clark BW, Di Giulio RT	2012	Mar	Clark BW, Di Giulio RT.Fundulus heteroclitus adapted to PAHs are cross-resistant to multiple insecticides. Ecotoxicology. 2012 Mar; 21(2):465-74.
Not related to neurodevelopmental effects	CycoÅ„ M, Å»mijowska A, WÃ³jcik M, Piotrowska-Seget Z	2013	Mar	CycoÅ„ M, Å»mijowska A, WÃ³jcik M, Piotrowska-Seget Z.Biodegradation and bioremediation potential of diazinon-degrading Serratia marcescens to remove other organophosphorus pesticides from soils. J Environ Manage. 2013 Mar 15; 117:7-16.
Not related to neurodevelopmental effects	Elsharkawy EE, Yahia D, El-Nisr NA	2013	Mar	Elsharkawy EE, Yahia D, El-Nisr NA.Sub-chronic exposure to chlorpyrifos induces hematological, metabolic disorders and oxidative stress in rat: attenuation by glutathione. Environ Toxicol Pharmacol. 2013 Mar; 35(2):218-27.
Not related to neurodevelopmental effects	FÃ¼hrer E, Rudolph A, Espinoza C, DÃaz R, Gajardo M, CamaÃ±o N	2012		FÃ¼hrer E, Rudolph A, Espinoza C, DÃaz R, Gajardo M, CamaÃ±o N.Integrated Use of Biomarkers (Oâ€‰:â€‰N Ratio and Acetylcholinesterase Inhibition) on Aulacomya ater (Molina, 1782) (Bivalvia: Mytilidae) as a Criteria for Effects of Organophosphate Pesticide Exposition. J Toxicol. 2012; 2012:951568.
Not related to neurodevelopmental effects	Fortenberry GZ, Hu H, Turyk M, Barr DB, Meeker JD	2012	May	Fortenberry GZ, Hu H, Turyk M, Barr DB, Meeker JD.Association between urinary 3, 5, 6-trichloro-2-pyridinol, a metabolite of chlorpyrifos and chlorpyrifos-methyl, and serum T4 and TSH in NHANES 1999-2002. Sci Total Environ. 2012 May 1; 424:351-5.
Not related to neurodevelopmental effects	Gao Y, Chen S, Hu M, Hu Q, Luo J, Li Y	2012		Gao Y, Chen S, Hu M, Hu Q, Luo J, Li Y.Purification and characterization of a novel chlorpyrifos hydrolase from Cladosporium cladosporioides Hu-01. PLoS One. 2012; 7(6):e38137.

Not related to neurodevelopmental effects	Garcia-Reyero N, Escalon BL, Prats E, Stanley JK, Thienpont B, Melby NL, BarÃ³n E, Eljarrat E, BarcelÃ³ D, Mestres J, Babin PJ, Perkins EJ, RaldÃºa D	2014	Feb	Garcia-Reyero N, Escalon BL, Prats E, Stanley JK, Thienpont B, Melby NL, BarÃ³n E, Eljarrat E, BarcelÃ³ D, Mestres J, Babin PJ, Perkins EJ, RaldÃºa D.Effects of BDE-209 contaminated sediments on zebrafish development and potential implications to human health. Environ Int. 2014 Feb; 63:216-23.
Not related to neurodevelopmental effects	Guillade AC, Folgarait PJ	2014	Feb	Guillade AC, Folgarait PJ.Natural enemies of Atta vollenweideri (Hymenoptera: Formicidae) leaf-cutter ants negatively affected by synthetic pesticides, chlorpyrifos and fipronil. J Econ Entomol. 2014 Feb; 107(1):105-14.
Not related to neurodevelopmental effects	Gupta S, Gupta R, Sharma S	2013	Dec	Gupta S, Gupta R, Sharma S.Impact of chemical- and bio-pesticides on bacterial diversity in rhizosphere of Vigna radiata. Ecotoxicology. 2013 Dec; 22(10):1479-89.
Not related to neurodevelopmental effects	He G, Sun Y, Li F	2012	Feb	He G, Sun Y, Li F.RNA interference of two acetylcholinesterase genes in Plutella xylostella reveals their different functions. Arch Insect Biochem Physiol. 2012 Feb; 79(2):75-86.
Not related to neurodevelopmental effects	He Y, Zhang J, Gao C, Su J, Chen J, Shen J	2013	Aug	He Y, Zhang J, Gao C, Su J, Chen J, Shen J.Regression analysis of dynamics of insecticide resistance in field populations of Chilo suppressalis (Lepidoptera: Crambidae) during 2002-2011 in China. J Econ Entomol. 2013 Aug; 106(4):1832-7.
Not related to neurodevelopmental effects	He Y, Zhao J, Zheng Y, Weng Q, Biondi A, Desneux N, Wu K	2013		He Y, Zhao J, Zheng Y, Weng Q, Biondi A, Desneux N, Wu K.Assessment of potential sublethal effects of various insecticides on key biological traits of the tobacco whitefly, Bemisia tabaci. Int J Biol Sci. 2013; 9(3):246-55.
Not related to neurodevelopmental effects	Hieu TT, Kim SI, Ahn YJ	2012	Sep	Hieu TT, Kim SI, Ahn YJ.Toxicity of Zanthoxylum piperitum and Zanthoxylum armatum oil constituents and related compounds to Stomoxys calcitrans (Diptera: Muscidae). J Med Entomol. 2012 Sep; 49(5):1084-91.

Not related to neurodevelopmental effects	JanakiDevi V, Nagarani N, YokeshBabu M, Kumaraguru AK, Ramakritinan CM	2013	Jan	JanakiDevi V, Nagarani N, YokeshBabu M, Kumaraguru AK, Ramakritinan CM.A study of proteotoxicity and genotoxicity induced by the pesticide and fungicide on marine invertebrate (Donax faba). Chemosphere. 2013 Jan; 90(3):1158-66.
Not related to neurodevelopmental effects	Janssens L, Stoks R	2013	Jun	Janssens L, Stoks R.Exposure to a widespread non-pathogenic bacterium magnifies sublethal pesticide effects in the damselfly Enallagma cyathigerum: from the suborganismal level to fitness-related traits. Environ Pollut. 2013 Jun; 177:143-9.
Not related to neurodevelopmental effects	Juan-Blasco M, Sabatier-MuÃ±oz B, ArgilÃ©s R, Jacas JA, Ortego F, Urbaneja A	2013	Jun	Juan-Blasco M, Sabatier-MuÃ±oz B, ArgilÃ©s R, Jacas JA, Ortego F, Urbaneja A.Effects of pesticides used on citrus grown in Spain on the mortality of Ceratitis capitata (Diptera: Tephritidae) Vienna-8 strain sterile males. J Econ Entomol. 2013 Jun; 106(3):1226-33.
Not related to neurodevelopmental effects	Kadian N, Malik A, Satya S, Dureja P	2012	Mar	Kadian N, Malik A, Satya S, Dureja P.Effect of organic amendments on microbial activity in chlorpyrifos contaminated soil. J Environ Manage. 2012 Mar; 95 Suppl:S199-202.
Not related to neurodevelopmental effects	Kalender Y, Kaya S, Durak D, Uzun FG, Demir F	2012	Mar	Kalender Y, Kaya S, Durak D, Uzun FG, Demir F.Protective effects of catechin and quercetin on antioxidant status, lipid peroxidation and testis-histoarchitecture induced by chlorpyrifos in male rats. Environ Toxicol Pharmacol. 2012 Mar; 33(2):141-8.
Not related to neurodevelopmental effects	Kang S, Song B, Wu J, He M, Hu D, Jin L, Zeng S, Xue W, Yang S	2013	Sep	Kang S, Song B, Wu J, He M, Hu D, Jin L, Zeng S, Xue W, Yang S.Design, synthesis and insecticidal activities of novel acetamido derivatives containing N-pyridylpyrazole carboxamides. Eur J Med Chem. 2013 Sep; 67:14-8.
Not related to neurodevelopmental effects	Karimullina E, Li Y, Ginjupalli GK, Baldwin WS	2012	Jul	Karimullina E, Li Y, Ginjupalli GK, Baldwin WS.Daphnia HR96 is a promiscuous xenobiotic and endobiotic nuclear receptor. Aquat Toxicol. 2012 Jul 15; 116-117:69-78.

Not related to neurodevelopmental effects	Khan HA, Akram W, Shad SA	2013	Apr	Khan HA, Akram W, Shad SA.Resistance to conventional insecticides in Pakistani populations of Musca domestica L. (Diptera: Muscidae): a potential ectoparasite of dairy animals. Ecotoxicology. 2013 Apr; 22(3):522-7.
Not related to neurodevelopmental effects	Khan HA, Akram W, Shad SA, Lee JJ	2013		Khan HA, Akram W, Shad SA, Lee JJ.Insecticide mixtures could enhance the toxicity of insecticides in a resistant dairy population of Musca domestica L. PLoS One. 2013; 8(4):e60929.
Not related to neurodevelopmental effects	Khodi S, Latifi AM, Saadati M, Mirzaei M, Aghamollaei H	2012	Feb	Khodi S, Latifi AM, Saadati M, Mirzaei M, Aghamollaei H.Surface display of organophosphorus hydrolase on E. coli using N-terminal domain of ice nucleation protein InaV. J Microbiol Biotechnol. 2012 Feb; 22(2):234-8.
Not related to neurodevelopmental effects	Koenig S, GuillÃ©n K, SolÃ© M	2013	May	Koenig S, GuillÃ©n K, SolÃ© M.Comparative xenobiotic metabolism capacities and pesticide sensitivity in adults of Solea solea and Solea senegalensis. Comp Biochem Physiol C Toxicol Pharmacol. 2013 May; 157(4):329-36.
Not related to neurodevelopmental effects	Krishnan K, Mitra NK, Yee LS, Yang HM	2012	Mar	Krishnan K, Mitra NK, Yee LS, Yang HM.A comparison of neurotoxicity in cerebellum produced by dermal application of chlorpyrifos in young and adult mice. J Neural Transm. 2012 Mar; 119(3):345-52.
Not related to neurodevelopmental effects	LÃ³pez-Granero C, Cardona D, GimÃ©nez E, Lozano R, Barril J, SÃ¡nchez-Santed F, CaÃ±adas F	2013	Jun	LÃ³pez-Granero C, Cardona D, GimÃ©nez E, Lozano R, Barril J, SÃ¡nchez-Santed F, CaÃ±adas F.Chronic dietary exposure to chlorpyrifos causes behavioral impairments, low activity of brain membrane-bound acetylcholinesterase, and increased brain acetylcholinesterase-R mRNA. Toxicology. 2013 Jun 7; 308:41-9.
Not related to neurodevelopmental effects	LeBlanc HM, Culp JM, Baird DJ, Alexander AC, Cessna AJ	2012	Oct	LeBlanc HM, Culp JM, Baird DJ, Alexander AC, Cessna AJ.Single versus combined lethal effects of three agricultural insecticides on larvae of the freshwater insect Chironomus dilutus. Arch Environ Contam Toxicol. 2012 Oct; 63(3):378-90.

Not related to neurodevelopmental effects	Leong SC, Abang F, Beattie A, Kueh RJ, Wong SK	2012		Leong SC, Abang F, Beattie A, Kueh RJ, Wong SK.Impacts of horticultural mineral oils and two insecticide practices on population fluctuation of Diaphorina citri and spread of Huanglongbing in a citrus orchard in Sarawak. ScientificWorldJournal. 2012; 2012:651416.
Not related to neurodevelopmental effects	Leskey TC, Lee DH, Short BD, Wright SE	2012	Oct	Leskey TC, Lee DH, Short BD, Wright SE.Impact of insecticides on the invasive Halyomorpha halys (Hemiptera: Pentatomidae): analysis of insecticide lethality. J Econ Entomol. 2012 Oct; 105(5):1726-35.
Not related to neurodevelopmental effects	Liang P, Tian YA, Biondi A, Desneux N, Gao XW	2012	Oct	Liang P, Tian YA, Biondi A, Desneux N, Gao XW.Short-term and transgenerational effects of the neonicotinoid nitenpyram on susceptibility to insecticides in two whitefly species. Ecotoxicology. 2012 Oct; 21(7):1889-98.
Not related to neurodevelopmental effects	Licznar P, List O, Goven D, Nna RN, Lapied B, Apaire-Marchais V	2014	Jan	Licznar P, List O, Goven D, Nna RN, Lapied B, Apaire-Marchais V.A novel method using Autographa californica multiple nucleopolyhedrovirus for increasing the sensitivity of insecticide through calcium influx in insect cell line. J Virol Methods. 2014 Jan; 195:72-5.
Not related to neurodevelopmental effects	Lima DB, Melo JW, Guedes RN, Siqueira HA, Pallini A, Gondim MG Jr	2013	Jul	Lima DB, Melo JW, Guedes RN, Siqueira HA, Pallini A, Gondim MG Jr.Survival and behavioural response to acaricides of the coconut mite predator Neoseiulus baraki. Exp Appl Acarol. 2013 Jul; 60(3):381-93.
Not related to neurodevelopmental effects	Limoee M, Davari B, Moosa-Kazemi SH	2012	Dec	Limoee M, Davari B, Moosa-Kazemi SH.Toxicity of Pyrethroid and Organophosphorous Insecticides against Two Field Collected Strains of the German Cockroach Blattella germanica (Blattaria: Blattellidae). J Arthropod Borne Dis. 2012 Dec; 6(2):112-8.
Not related to neurodevelopmental effects	Ling S, Zhang H	2013	Dec	Ling S, Zhang H.Influences of chlorpyrifos on antioxidant enzyme activities of Nilaparvata lugens. Ecotoxicol Environ Saf. 2013 Dec; 98:187-90.

Not related to neurodevelopmental effects	Liu H, Yuan B, Li S	2012	Jun	Liu H, Yuan B, Li S.Altered quantities and in vivo activities of cholinesterase from Daphnia magna in sub-lethal exposure to organophosphorus insecticides. Ecotoxicol Environ Saf. 2012 Jun; 80:118-25.
Not related to neurodevelopmental effects	Lopez B, Ponce G, Gonzalez JA, Gutierrez SM, Villanueva OK, Gonzalez G, Bobadilla C, Rodriguez IP, Black WC 4th, Flores AE	2014	May	Lopez B, Ponce G, Gonzalez JA, Gutierrez SM, Villanueva OK, Gonzalez G, Bobadilla C, Rodriguez IP, Black WC 4th, Flores AE.Susceptibility to chlorpyrifos in pyrethroid-resistant populations of Aedes aegypti (Diptera: Culicidae) from Mexico. J Med Entomol. 2014 May; 51(3):644-9.
Not related to neurodevelopmental effects	Lundqvist A, Bertilsson S, Goedkoop W	2012	Nov	Lundqvist A, Bertilsson S, Goedkoop W.Interactions with DOM and biofilms affect the fate and bioavailability of insecticides to invertebrate grazers. Ecotoxicology. 2012 Nov; 21(8):2398-408.
Not related to neurodevelopmental effects	Maloney KM, Ancca-Juarez J, Salazar R, Borrini-Mayori K, Niemierko M, Yukich JO, Naquira C, Keating JA, Levy MZ	2013	Jun	Maloney KM, Ancca-Juarez J, Salazar R, Borrini-Mayori K, Niemierko M, Yukich JO, Naquira C, Keating JA, Levy MZ.Comparison of insecticidal paint and deltamethrin against Triatoma infestans (Hemiptera: Reduviidae) feeding and mortality in simulated natural conditions. J Vector Ecol. 2013 Jun; 38(1):6-11.
Not related to neurodevelopmental effects	Mbata GN, Shapiro-Ilan D	2013	Oct	Mbata GN, Shapiro-Ilan D.The potential for controlling Pangaeus bilineatus (Heteroptera: Cydnidae) using a combination of entomopathogens and an insecticide. J Econ Entomol. 2013 Oct; 106(5):2072-6.
Not related to neurodevelopmental effects	Mironidis GK, Kapantaidaki D, Bentila M, Morou E, Savopoulou-Soultani M, Vontas J	2013	Aug	Mironidis GK, Kapantaidaki D, Bentila M, Morou E, Savopoulou-Soultani M, Vontas J.Resurgence of the cotton bollworm Helicoverpa armigera in northern Greece associated with insecticide resistance. Insect Sci. 2013 Aug; 20(4):505-12.
Not related to neurodevelopmental effects	Monteiro VB, Lima DB, Gondim MG Jr, Siqueira HA	2012	Aug	Monteiro VB, Lima DB, Gondim MG Jr, Siqueira HA.Residual bioassay to assess the toxicity of Acaricides against Aceria guerreronis (Acari: Eriophyidae) under laboratory conditions. J Econ Entomol. 2012 Aug; 105(4):1419-25.

Not related to neurodevelopmental effects	Mukherjee I, Aman Kumar, Ashok Kumar	2012	Mar	Mukherjee I, Aman Kumar, Ashok Kumar.Persistence behavior of combination mix crop protection agents in/on eggplant fruits. Bull Environ Contam Toxicol. 2012 Mar; 88(3):338-43.
Not related to neurodevelopmental effects	Nair PM, Park SY, Choi J	2013	Nov	Nair PM, Park SY, Choi J.Characterization and expression of cytochrome p450 cDNA (CYP9AT2) in Chironomus riparius fourth instar larvae exposed to multiple xenobiotics. Environ Toxicol Pharmacol. 2013 Nov; 36(3):1133-40.
Not related to neurodevelopmental effects	Nong X, Tan YJ, Wang JH, Xie Y, Fang CL, Chen L, Liu TF, Yang DY, Gu XB, Peng XR, Wang SX, Yang GY	2013	Nov	Nong X, Tan YJ, Wang JH, Xie Y, Fang CL, Chen L, Liu TF, Yang DY, Gu XB, Peng XR, Wang SX, Yang GY.Evaluation acaricidal efficacy of botanical extract from Eupatorium adenophorum against the hard tick Haemaphysalis longicornis (Acari: Ixodidae). Exp Parasitol. 2013 Nov; 135(3):558-63.
Not related to neurodevelopmental effects	Noworyta-GÅ,owacka J, BaÅ„kowski R, Siennicka J, Wiadrowska B, Ludwicki JK	2012		Noworyta-GÅ,owacka J, BaÅ„kowski R, Siennicka J, Wiadrowska B, Ludwicki JK.Influence of chlorpyrifos on the profile of subpopulations of immunoactive cells and their phagocytic activity in an experimental in vivo model. Ann Agric Environ Med. 2012; 19(3):483-6.
Not related to neurodevelopmental effects	Ostwal P, Dabadghao VS, Sharma SK, Dhakane AB	2013	Oct	Ostwal P, Dabadghao VS, Sharma SK, Dhakane AB.Chlorpyrifos toxicity causing delayed myeloneuropathy. Ann Indian Acad Neurol. 2013 Oct; 16(4):736.
Not related to neurodevelopmental effects	Ozkan F, GÃ¼ndÃ¼z SG, BerkÃ¶z M, Hunt AO, YalÄ±n S	2012	Jun	Ozkan F, GÃ¼ndÃ¼z SG, BerkÃ¶z M, Hunt AO, YalÄ±n S.The protective role of ascorbic acid (vitamin C) against chlorpyrifos-induced oxidative stress in Oreochromis niloticus. Fish Physiol Biochem. 2012 Jun; 38(3):635-43.
Not related to neurodevelopmental effects	PÃ©rez J, Monteiro MS, Quintaneiro C, Soares AM, Loureiro S	2013	Nov	PÃ©rez J, Monteiro MS, Quintaneiro C, Soares AM, Loureiro S.Characterization of cholinesterases in Chironomus riparius and the effects of three herbicides on chlorpyrifos toxicity. Aquat Toxicol. 2013 Nov 15; 144-145:296-302.

Not related to neurodevelopmental effects	Park IK	2012		Park IK.Insecticidal activity of isobutylamides derived from Piper nigrum against adult of two mosquito species, Culex pipiens pallens and Aedes aegypti. Nat Prod Res. 2012; 26(22):2129-31.
Not related to neurodevelopmental effects	Prabhaker N, Gispert C, Castle SJ	2012	Aug	Prabhaker N, Gispert C, Castle SJ.Baseline susceptibility of Planococcus ficus (Hemiptera: Pseudococcidae) from California to select insecticides. J Econ Entomol. 2012 Aug; 105(4):1392-400.
Not related to neurodevelopmental effects	Qin G, Jia M, Liu T, Zhang X, Guo Y, Zhu KY, Ma E, Zhang J	2013		Qin G, Jia M, Liu T, Zhang X, Guo Y, Zhu KY, Ma E, Zhang J.Characterization and functional analysis of four glutathione S-transferases from the migratory locust, Locusta migratoria. PLoS One. 2013; 8(3):e58410.
Not related to neurodevelopmental effects	Quesada-Moraga E, Yousef M, Ortiz A, RuÃz-Torres M, Garrido-Jurado I, EstÃ©vez A	2013	Aug	Quesada-Moraga E, Yousef M, Ortiz A, RuÃz-Torres M, Garrido-Jurado I, EstÃ©vez A.Beauveria bassiana (Ascomycota: Hypocreales) wound dressing for the control of Euzophera pinguis (Lepidoptera: Pyralidae). J Econ Entomol. 2013 Aug; 106(4):1602-7.
Not related to neurodevelopmental effects	Ramu S, Seetharaman B	2014		Ramu S, Seetharaman B.Biodegradation of acephate and methamidophos by a soil bacterium Pseudomonas aeruginosa strain Is-6. J Environ Sci Health B. 2014; 49(1):23-34.
Not related to neurodevelopmental effects	RodrÃguez-Vivas RI, Miller RJ, Ojeda-Chi MM, Rosado-Aguilar JA, Trinidad-MartÃnez IC, PÃ©rez de LeÃ³n AA	2014	Feb	RodrÃguez-Vivas RI, Miller RJ, Ojeda-Chi MM, Rosado-Aguilar JA, Trinidad-MartÃnez IC, PÃ©rez de LeÃ³n AA.Acaricide and ivermectin resistance in a field population of Rhipicephalus microplus (Acari: Ixodidae) collected from red deer (Cervus elaphus) in the Mexican tropics. Vet Parasitol. 2014 Feb 24; 200(1-2):179-88.
Not related to neurodevelopmental effects	Rouimi P, Zucchini-Pascal N, Dupont G, Razpotnik A, FouchÃ© E, De Sousa G, Rahmani R	2012	Aug	Rouimi P, Zucchini-Pascal N, Dupont G, Razpotnik A, FouchÃ© E, De Sousa G, Rahmani R.Impacts of low doses of pesticide mixtures on liver cell defence systems. Toxicol In Vitro. 2012 Aug; 26(5):718-26.

Not related to neurodevelopmental effects	Rubach MN, Baird DJ, Boerwinkel MC, Maund SJ, Roessink I, Van den Brink PJ	2012	Oct	Rubach MN, Baird DJ, Boerwinkel MC, Maund SJ, Roessink I, Van den Brink PJ.Species traits as predictors for intrinsic sensitivity of aquatic invertebrates to the insecticide chlorpyrifos. Ecotoxicology. 2012 Oct; 21(7):2088-101.
Not related to neurodevelopmental effects	Sehgal B, Subramanyam B, Arthur FH, Gill BS	2013	Aug	Sehgal B, Subramanyam B, Arthur FH, Gill BS.Variation in susceptibility of field strains of three stored grain insect species to spinosad and chlorpyrifos-methyl plus deltamethrin on hard red winter wheat. J Econ Entomol. 2013 Aug; 106(4):1911-9.
Not related to neurodevelopmental effects	Shetty NJ, Hariprasad TP, Sanil D, Zin T	2013	Nov	Shetty NJ, Hariprasad TP, Sanil D, Zin T.Chromosomal inversions among insecticide-resistant strains of Anopheles stephensi Liston, a malaria mosquito. Parasitol Res. 2013 Nov; 112(11):3851-7.
Not related to neurodevelopmental effects	Singh AK, Parashar A, Singh AK, Singh R	2013	Apr-Jun	Singh AK, Parashar A, Singh AK, Singh R.Pre-natal/juvenile chlorpyrifos exposure associated with immunotoxicity in adulthood in Swiss albino mice. J Immunotoxicol. 2013 Apr-Jun; 10(2):141-9.
Not related to neurodevelopmental effects	Speed HE, Blaiss CA, Kim A, Haws ME, Melvin NR, Jennings M, Eisch AJ, Powell CM	2012	Jan	Speed HE, Blaiss CA, Kim A, Haws ME, Melvin NR, Jennings M, Eisch AJ, Powell CM.Delayed reduction of hippocampal synaptic transmission and spines following exposure to repeated subclinical doses of organophosphorus pesticide in adult mice. Toxicol Sci. 2012 Jan; 125(1):196-208.
Not related to neurodevelopmental effects	Speed HE, Blaiss CA, Kim A, Haws ME, Melvin NR, Jennings M, Eisch AJ, Powell CM	2012	Jan	Speed HE, Blaiss CA, Kim A, Haws ME, Melvin NR, Jennings M, Eisch AJ, Powell CM.Delayed reduction of hippocampal synaptic transmission and spines following exposure to repeated subclinical doses of organophosphorus pesticide in adult mice. Toxicol Sci. 2012 Jan; 125(1):196-208.
Not related to neurodevelopmental effects	Srinivasulu M, Jaffer Mohiddin G, Subramanyam K, Rangaswamy V	2012	Jun	Srinivasulu M, Jaffer Mohiddin G, Subramanyam K, Rangaswamy V.Effect of insecticides alone and in combination with fungicides on nitrification and phosphatase activity in two groundnut (Arachis hypogeae L.) soils. Environ Geochem Health. 2012 Jun; 34(3):365-74.

Not related to neurodevelopmental effects	Srivastava PK, Singh VP, Prasad SM	2012	Sep	Srivastava PK, Singh VP, Prasad SM.Compatibility of ascorbate-glutathione cycle enzymes in cyanobacteria against low and high UV-B exposures, simultaneously exposed to low and high doses of chlorpyrifos. Ecotoxicol Environ Saf. 2012 Sep; 83:79-88.
Not related to neurodevelopmental effects	Stoner KA, Eitzer BD	2013		Stoner KA, Eitzer BD.Using a hazard quotient to evaluate pesticide residues detected in pollen trapped from honey bees (Apis mellifera) in Connecticut. PLoS One. 2013; 8(10):e77550.
Not related to neurodevelopmental effects	Su J, Zhang Z, Wu M, Gao C	2014	Feb	Su J, Zhang Z, Wu M, Gao C.Changes in insecticide resistance of the rice striped stem borer (Lepidoptera: Crambidae). J Econ Entomol. 2014 Feb; 107(1):333-41.
Not related to neurodevelopmental effects	Terry AV Jr, Beck WD, Warner S, Vandenhuerk L, Callahan PM	2012	Jan-Feb	Terry AV Jr, Beck WD, Warner S, Vandenhuerk L, Callahan PM.Chronic impairments in spatial learning and memory in rats previously exposed to chlorpyrfos or diisopropylfluorophosphate. Neurotoxicol Teratol. 2012 Jan-Feb; 34(1):1-8.
Not related to neurodevelopmental effects	Tirello P, Pozzebon A, Duso C	2013	Oct	Tirello P, Pozzebon A, Duso C.The effect of insecticides on the non-target predatory mite Kampimodromus aberrans: laboratory studies. Chemosphere. 2013 Oct; 93(6):1139-44.
Not related to neurodevelopmental effects	Tirello P, Pozzebon A, Duso C	2012	Jan	Tirello P, Pozzebon A, Duso C.Resistance to chlorpyriphos in the predatory mite Kampimodromus aberrans. Exp Appl Acarol. 2012 Jan; 56(1):1-8.
Not related to neurodevelopmental effects	Tiwari S, Killiny N, Mann RS, Wenninger EJ, Stelinski LL	2013	Apr	Tiwari S, Killiny N, Mann RS, Wenninger EJ, Stelinski LL.Abdominal color of the Asian citrus psyllid, Diaphorina citri, is associated with susceptibility to various insecticides. Pest Manag Sci. 2013 Apr; 69(4):535-41.
Not related to neurodevelopmental effects	Uzun FG, Kalender Y	2013	May	Uzun FG, Kalender Y.Chlorpyrifos induced hepatotoxic and hematologic changes in rats: the role of quercetin and catechin. Food Chem Toxicol. 2013 May; 55:549-56.

Not related to neurodevelopmental effects	Ventura C, Nunez M, Miret N, Martinel Lamas D, Randi A, Venturino A, Rivera E, Cocca C	2012	Sep	Ventura C, NÃºÃ±ez M, Miret N, Martinel Lamas D, Randi A, Venturino A, Rivera E, Cocca C.Differential mechanisms of action are involved in chlorpyrifos effects in estrogen-dependent or -independent breast cancer cells exposed to low or high concentrations of the pesticide. Toxicol Lett. 2012 Sep 3; 213(2):184-93.
Not related to neurodevelopmental effects	Wang C, Lu G, Cui J	2012	Jan	Wang C, Lu G, Cui J.Responses of AChE and GST activities to insecticide coexposure in Carassius auratus. Environ Toxicol. 2012 Jan; 27(1):50-7.
Not related to neurodevelopmental effects	Wang JJ, Wei D, Dou W, Hu F, Liu WF, Wang JJ	2013	Apr	Wang JJ, Wei D, Dou W, Hu F, Liu WF, Wang JJ.Toxicities and synergistic effects of several insecticides against the oriental fruit fly (Diptera: Tephritidae). J Econ Entomol. 2013 Apr; 106(2):970-8.
Not related to neurodevelopmental effects	Wang S, Zhang C, Yan Y	2012	Feb	Wang S, Zhang C, Yan Y.Biodegradation of methyl parathion and p-nitrophenol by a newly isolated Agrobacterium sp. strain Yw12. Biodegradation. 2012 Feb; 23(1):107-16.
Not related to neurodevelopmental effects	Wang SY, Zhou XH, Zhang AS, Li LL, Men XY, Zhang SC, Liu YJ, Yu Y	2012	Jul	Wang SY, Zhou XH, Zhang AS, Li LL, Men XY, Zhang SC, Liu YJ, Yu Y.[Resistance mechanisms and cross-resistance of phoxim-resistant Frankliniella occidentalis Pergande population]. Ying Yong Sheng Tai Xue Bao. 2012 Jul; 23(7):1933-9.
Not related to neurodevelopmental effects	Williamson SM, Moffat C, Gomersall MA, Saranzewa N, Connolly CN, Wright GA	2013		Williamson SM, Moffat C, Gomersall MA, Saranzewa N, Connolly CN, Wright GA.Exposure to acetylcholinesterase inhibitors alters the physiology and motor function of honeybees. Front Physiol. 2013; 4:13.
Not related to neurodevelopmental effects	Xia X, Zheng D, Zhong H, Qin B, Gurr GM, Vasseur L, Lin H, Bai J, He W, You M	2013		Xia X, Zheng D, Zhong H, Qin B, Gurr GM, Vasseur L, Lin H, Bai J, He W, You M.DNA sequencing reveals the midgut microbiota of diamondback moth, Plutella xylostella (L.) and a possible relationship with insecticide resistance. PLoS One. 2013; 8(7):e68852.

Not related to neurodevelopmental effects	Xiang Y, Wang N, Song J, Cai D, Wu Z	2013	Jun	Xiang Y, Wang N, Song J, Cai D, Wu Z.Micro-nanopores fabricated by high-energy electron beam irradiation: suitable structure for controlling pesticide loss. J Agric Food Chem. 2013 Jun 5; 61(22):5215-9.
Not related to neurodevelopmental effects	Xie H, Li Q, Wang M, Zhao L	2013	Jun	Xie H, Li Q, Wang M, Zhao L.Production of a recombinant laccase from Pichia pastoris and biodegradation of chlorpyrifos in a laccase/vanillin system. J Microbiol Biotechnol. 2013 Jun 28; 23(6):864-71.
Not related to neurodevelopmental effects	Xie J, Zhao Y, Zhang H, Liu Z, Lu Z	2014	Jan	Xie J, Zhao Y, Zhang H, Liu Z, Lu Z.Improving methyl parathion hydrolase to enhance its chlorpyrifos-hydrolysing efficiency. Lett Appl Microbiol. 2014 Jan; 58(1):53-9.
Not related to neurodevelopmental effects	Yan C, Jiao L, Zhao J, Yang H, Peng S	2012	Jul	Yan C, Jiao L, Zhao J, Yang H, Peng S.Repeated exposures to chlorpyrifos lead to spatial memory retrieval impairment and motor activity alteration. Neurotoxicol Teratol. 2012 Jul; 34(4):442-9.
Not related to neurodevelopmental effects	Yang X, Li X, Zhang Y	2013	Dec	Yang X, Li X, Zhang Y.Molecular cloning and expression of CYP9A61: a chlorpyrifos-ethyl and lambda-cyhalothrin-inducible cytochrome P450 cDNA from Cydia pomonella. Int J Mol Sci. 2013 Dec 13; 14(12):24211-29.
Not related to neurodevelopmental effects	Zein MA, McElmurry SP, Kashian DR, Savolainen PT, Pitts DK	2014	Jan	Zein MA, McElmurry SP, Kashian DR, Savolainen PT, Pitts DK.Optical bioassay for measuring sublethal toxicity of insecticides in Daphnia pulex. Environ Toxicol Chem. 2014 Jan; 33(1):144-51.
Not related to neurodevelopmental effects	Zhang J, Li D, Ge P, Yang M, Guo Y, Zhu KY, Ma E, Zhang J	2013	Oct	Zhang J, Li D, Ge P, Yang M, Guo Y, Zhu KY, Ma E, Zhang J.RNA interference revealed the roles of two carboxylesterase genes in insecticide detoxification in Locusta migratoria. Chemosphere. 2013 Oct; 93(6):1207-15.
Not related to neurodevelopmental effects	Zhang NN, Liu CF, Yang F, Dong SL, Han ZJ	2012		Zhang NN, Liu CF, Yang F, Dong SL, Han ZJ.Resistance mechanisms to chlorpyrifos and F392W mutation frequencies in the acetylcholine esterase ace1 allele of field populations of the tobacco whitefly, Bemisia tabaci in China. J Insect Sci. 2012; 12:41.

Not related to neurodevelopmental effects	Zhang SK, Ren XB, Wang YC, Su J	2014	Apr	Zhang SK, Ren XB, Wang YC, Su J.Resistance in Cnaphalocrocis medinalis (Lepidoptera: Pyralidae) to new chemistry insecticides. J Econ Entomol. 2014 Apr; 107(2):815-20.
Not related to neurodevelopmental effects	Zhang X, Wallace AD, Du P, Kibbe WA, Jafari N, Xie H, Lin S, Baccarelli A, Soares MB, Hou L	2012	Aug	Zhang X, Wallace AD, Du P, Kibbe WA, Jafari N, Xie H, Lin S, Baccarelli A, Soares MB, Hou L.DNA methylation alterations in response to pesticide exposure in vitro. Environ Mol Mutagen. 2012 Aug; 53(7):542-9.
Not related to neurodevelopmental effects	Zhao L, Teng S, Liu Y	2012	Apr	Zhao L, Teng S, Liu Y.Characterization of a versatile rhizospheric organism from cucumber identified as Ochrobactrum haematophilum. J Basic Microbiol. 2012 Apr; 52(2):232-44.
Not related to neurodevelopmental effects	Zheng Y, Zhao JW, He YX, Huang J, Weng QY	2012	Jan	Zheng Y, Zhao JW, He YX, Huang J, Weng QY.[Development of insecticide resistance and its effect factors in field population of Bemisia tabaci in Fujian Province, East China]. Ying Yong Sheng Tai Xue Bao. 2012 Jan; 23(1):271-7.
Not related to neurodevelopmental effects	Zhou WW, Li XW, Quan YH, Cheng J, Zhang CX, Gurr G, Zhu ZR	2012	Sep	Zhou WW, Li XW, Quan YH, Cheng J, Zhang CX, Gurr G, Zhu ZR.Identification and expression profiles of nine glutathione S-transferase genes from the important rice phloem sap-sucker and virus vector Laodelphax striatellus (FallÃ©n) (Hemiptera: Delphacidae). Pest Manag Sci. 2012 Sep; 68(9):1296-305.
Not related to neurodevelopmental effects	Zhou WW, Liang QM, Xu Y, Gurr GM, Bao YY, Zhou XP, Zhang CX, Cheng J, Zhu ZR	2013		Zhou WW, Liang QM, Xu Y, Gurr GM, Bao YY, Zhou XP, Zhang CX, Cheng J, Zhu ZR.Genomic insights into the glutathione S-transferase gene family of two rice planthoppers, Nilaparvata lugens (StÃ¥l) and Sogatella furcifera (HorvÃ¡th) (Hemiptera: Delphacidae). PLoS One. 2013; 8(2):e56604.
Not related to neurodevelopmental effects/Mixtures	Ojha A, Yaduvanshi SK, Pant SC, Lomash V, Srivastava N	2013	Oct	Ojha A, Yaduvanshi SK, Pant SC, Lomash V, Srivastava N.Evaluation of DNA damage and cytotoxicity induced by three commonly used organophosphate pesticides individually and in mixture, in rat tissues. Environ Toxicol. 2013 Oct; 28(10):543-52.

Not related to neurodevelopmental effects/Mixtures	Rajpoot DS, Prakash A, Mandil R, Rahal A, Garg SK	2013		Rajpoot DS, Prakash A, Mandil R, Rahal A, Garg SK.Differential modulation of xenobiotic-metabolizing enzymes in rats following single and concurrent exposure to chlorpyrifos, arsenic, and ascorbic acid. J Toxicol Environ Health A. 2013; 76(24):1354-65.
Not relevant	Slotkin TA, Card J, Infante A, Seidler FJ	2013	May-Jun	Slotkin TA, Card J, Infante A, Seidler FJ.BDE99 (2,2',4,4',5-pentabromodiphenyl ether) suppresses differentiation into neurotransmitter phenotypes in PC12 cells. Neurotoxicol Teratol. 2013 May-Jun; 37:13-7.
Not Relevant /Exposure	Khairy MA, Kolb M, Mostafa AR, El-Fiky A, Bahadir M	2012	Mar	Khairy MA, Kolb M, Mostafa AR, El-Fiky A, Bahadir M.Risk posed by chlorinated organic compounds in Abu Qir Bay, East Alexandria, Egypt. Environ Sci Pollut Res Int. 2012 Mar; 19(3):794-811.
Not relevant/Acute	Duysen EG, Cashman JR, Schopfer LM, Nachon F, Masson P, Lockridge O	2012	Feb	Duysen EG, Cashman JR, Schopfer LM, Nachon F, Masson P, Lockridge O.Differential sensitivity of plasma carboxylesterase-null mice to parathion, chlorpyrifos and chlorpyrifos oxon, but not to diazinon, dichlorvos, diisopropylfluorophosphate, cresyl saligenin phosphate, cyclosarin thiocholine, tabun thiocholine, and carbofuran. Chem Biol Interact. 2012 Feb 5; 195(3):189-98.
Not Relevant/Acute Mechanisms	Li T, Zhao H, Hung GC, Han J, Tsai S, Li B, Zhang J, Puri RK, Lo SC	2012	Dec	Li T, Zhao H, Hung GC, Han J, Tsai S, Li B, Zhang J, Puri RK, Lo SC.Differentially expressed genes and pathways induced by organophosphates in human neuroblastoma cells. Exp Biol Med (Maywood). 2012 Dec; 237(12):1413-23.
Not Relevant/Acute Mechanisms	SchÃ¤fer M, Koppe F, Stenger B, Brochhausen C, Schmidt A, Steinritz D, Thiermann H, Kirkpatrick CJ, Pohl C	2013	Dec	Schafer M, Koppe F, Stenger B, Brochhausen C, Schmidt A, Steinritz D, Thiermann H, Kirkpatrick CJ, Pohl C.Influence of organophosphate poisoning on human dendritic cells. Chem Biol Interact. 2013 Dec 5; 206(3):472-8.
Not relevant/Acute/Lower Taxa	Buono S, Manzo S, Maria G, Sansone G	2012	Apr	Buono S, Manzo S, Maria G, Sansone G.Toxic effects of pentachlorophenol, azinphos-methyl and chlorpyrifos on the development of Paracentrotus lividus embryos. Ecotoxicology. 2012 Apr; 21(3):688-97.

Not relevant/In vitro study, chlorpyrifos used as positive control with no new information on effects in this cell line	Slotkin TA, Card J, Infante A, Seidler FJ	2013	May-Jun	Slotkin TA, Card J, Infante A, Seidler FJ.Prenatal dexamethasone augments the sex-selective developmental neurotoxicity of chlorpyrifos: implications for vulnerability after pharmacotherapy for preterm labor. Neurotoxicol Teratol. 2013 May-Jun; 37:1-12.
Not relevant/Mixtures	Chen L, Qu G, Sun X, Zhang S, Wang L, Sang N, Du Y, Liu J, Liu S	2013		Chen L, Qu G, Sun X, Zhang S, Wang L, Sang N, Du Y, Liu J, Liu S.Characterization of the interaction between cadmium and chlorpyrifos with integrative techniques in incurring synergistic hepatoxicity. PLoS One. 2013; 8(3):e59553.
Not Relevant: Ecological Effects	Alexander AC, Luis AT, Culp JM, Baird DJ, Cessna AJ	2013	Sep	Alexander AC, Luis AT, Culp JM, Baird DJ, Cessna AJ.Can nutrients mask community responses to insecticide mixtures? Ecotoxicology. 2013 Sep; 22(7):1085-100.
Not Relevant: Ecological Effects	Amaral MJ, Sanchez-Hernandez JC, Bicho RC, Carretero MA, Valente R, Faustino AM, Soares AM, Mann RM	2012	Oct	Amaral MJ, Sanchez-Hernandez JC, Bicho RC, Carretero MA, Valente R, Faustino AM, Soares AM, Mann RM.Biomarkers of exposure and effect in a lacertid lizard (Podarcis bocagei Seoane) exposed to chlorpyrifos. Environ Toxicol Chem. 2012 Oct; 31(10):2345-53.
Not Relevant: Ecological Effects	Ashauer R, Hintermeister A, O'Connor I, Elumelu M, Hollender J, Escher BI	2012	Mar	Ashauer R, Hintermeister A, O'Connor I, Elumelu M, Hollender J, Escher BI.Significance of xenobiotic metabolism for bioaccumulation kinetics of organic chemicals in Gammarus pulex. Environ Sci Technol. 2012 Mar 20; 46(6):3498-508.
Not Relevant: Ecological Effects	Assis CR, Linhares AG, Oliveira VM, FranÃ§a RC, Carvalho EV, Bezerra RS, de Carvalho LB Jr	2012	Dec	Assis CR, Linhares AG, Oliveira VM, FranÃ§a RC, Carvalho EV, Bezerra RS, de Carvalho LB Jr.Comparative effect of pesticides on brain acetylcholinesterase in tropical fish. Sci Total Environ. 2012 Dec 15; 441:141-50.
Not Relevant: Ecological Effects	Baylay AJ, Spurgeon DJ, Svendsen C, Griffin JL, Swain SC, Sturzenbaum SR, Jones OA	2012	Jul	Baylay AJ, Spurgeon DJ, Svendsen C, Griffin JL, Swain SC, Sturzenbaum SR, Jones OA.A metabolomics based test of independent action and concentration addition using the earthworm Lumbricus rubellus. Ecotoxicology. 2012 Jul; 21(5):1436-47.

Not Relevant: Ecological Effects	BottÃ© ES, Jerry DR, Codi King S, Smith-Keune C, Negri AP	2012		BottÃ© ES, Jerry DR, Codi King S, Smith-Keune C, Negri AP.Effects of chlorpyrifos on cholinesterase activity and stress markers in the tropical reef fish Acanthochromis polyacanthus. Mar Pollut Bull. 2012; 65(4-9):384-93.
Not Relevant: Ecological Effects	Cacciatore LC, Guerrero NV, CochÃ³n AC	2013	Mar	Cacciatore LC, Guerrero NV, CochÃ³n AC.Cholinesterase and carboxylesterase inhibition in Planorbarius corneus exposed to binary mixtures of azinphos-methyl and chlorpyrifos. Aquat Toxicol. 2013 Mar 15; 128-129:124-34.
Not Relevant: Ecological Effects	Cacciatore LC, Kristoff G, Verrengia Guerrero NR, CochÃ³n AC	2012	Jul	Cacciatore LC, Kristoff G, Verrengia Guerrero NR, CochÃ³n AC.Binary mixtures of azinphos-methyl oxon and chlorpyrifos oxon produce in vitro synergistic cholinesterase inhibition in Planorbarius corneus. Chemosphere. 2012 Jul; 88(4):450-8.
Not Relevant: Ecological Effects	Campillo JA, Albentosa M, ValdÃ©s NJ, Moreno-GonzÃ¡lez R, LeÃ³n VM	2013	Oct	Campillo JA, Albentosa M, ValdÃ©s NJ, Moreno-GonzÃ¡lez R, LeÃ³n VM.Impact assessment of agricultural inputs into a Mediterranean coastal lagoon (Mar Menor, SE Spain) on transplanted clams (Ruditapes decussatus) by biochemical and physiological responses. Aquat Toxicol. 2013 Oct 15; 142-143:365-79.
Not Relevant: Ecological Effects	Carvalho RA, Omoto C, Field LM, Williamson MS, Bass C	2013		Carvalho RA, Omoto C, Field LM, Williamson MS, Bass C.Investigating the molecular mechanisms of organophosphate and pyrethroid resistance in the fall armyworm Spodoptera frugiperda. PLoS One. 2013; 8(4):e62268.
Not Relevant: Ecological Effects	Chen S, Liu C, Peng C, Liu H, Hu M, Zhong G	2012		Chen S, Liu C, Peng C, Liu H, Hu M, Zhong G.Biodegradation of chlorpyrifos and its hydrolysis product 3,5,6-trichloro-2-pyridinol by a new fungal strain Cladosporium cladosporioides Hu-01. PLoS One. 2012; 7(10):e47205.
Not Relevant: Ecological Effects	Chen X, Song M, Qi S, Wang C	2013	Feb	Chen X, Song M, Qi S, Wang C.Safety evaluation of eleven insecticides to Trichogramma nubilale (Hymenoptera: Trichogrammatidae). J Econ Entomol. 2013 Feb; 106(1):136-41.

Not Relevant: Ecological Effects	Ciliberti A, Martin S, Ferrandez E, Belluco S, Rannou B, Dussart C, Berny P, de Buffrenil V	2013	May	Ciliberti A, Martin S, Ferrandez E, Belluco S, Rannou B, Dussart C, Berny P, de Buffrenil V.Experimental exposure of juvenile savannah monitors (Varanus exanthematicus) to an environmentally relevant mixture of three contaminants: effects and accumulation in tissues. Environ Sci Pollut Res Int. 2013 May; 20(5):3107-14.
Not Relevant: Ecological Effects	Dasgupta R, Chakravorty PP, Kaviraj A	2012		Dasgupta R, Chakravorty PP, Kaviraj A.Effects of carbaryl, chlorpyrifos and endosulfan on growth, reproduction and respiration of tropical epigeic earthworm, Perionyx excavatus (Perrier). J Environ Sci Health B. 2012; 47(2):99-103.
Not Relevant: Ecological Effects	Davidson C, Stanley K, Simonich SM	2012	Aug	Davidson C, Stanley K, Simonich SM.Contaminant residues and declines of the Cascades frog (Rana cascadae) in the California Cascades, USA. Environ Toxicol Chem. 2012 Aug; 31(8):1895-902.
Not Relevant: Ecological Effects	de Solla SR, Palonen KE, Martin PA	2014	Jan	de Solla SR, Palonen KE, Martin PA.Toxicity of pesticides associated with potato production, including soil fumigants, to snapping turtle eggs (Chelydra serpentina). Environ Toxicol Chem. 2014 Jan; 33(1):102-6.
Not Relevant: Ecological Effects	Demetrio PM, Bulus Rossini GD, Bonetto CA, Ronco AE	2012	Jan	Demetrio PM, Bulus Rossini GD, Bonetto CA, Ronco AE.Effects of pesticide formulations and active ingredients on the coelenterate Hydra attenuata (Pallas, 1766). Bull Environ Contam Toxicol. 2012 Jan; 88(1):15-9.
Not Relevant: Ecological Effects	DemirdaÄŸ R, Yerlikaya E, Aksakal E, KÃ¼frevioÄŸlu OI, Ekinci D	2012	Sep	DemirdaÄŸ R, Yerlikaya E, Aksakal E, KÃ¼frevioÄŸlu OI, Ekinci D.Influence of pesticides on the pH regulatory enzyme, carbonic anhydrase, from European Seabass liver and bovine erythrocytes. Environ Toxicol Pharmacol. 2012 Sep; 34(2):218-22.
Not Relevant: Ecological Effects	Diepens NJ, Pfennig S, Van den Brink PJ, Gunnarsson JS, Ruepert C, Castillo LE	2014	Jan	Diepens NJ, Pfennig S, Van den Brink PJ, Gunnarsson JS, Ruepert C, Castillo LE.Effect of pesticides used in banana and pineapple plantations on aquatic ecosystems in Costa Rica. J Environ Biol. 2014 Jan; 35(1):73-84.

Not Relevant: Ecological Effects	Dinh Van K, Janssens L, Debecker S, Stoks R	2014	Jul	Dinh Van K, Janssens L, Debecker S, Stoks R.Warming increases chlorpyrifos effects on predator but not anti-predator behaviours. Aquat Toxicol. 2014 Jul; 152:215-21.
Not Relevant: Ecological Effects	Dzul-Caamal R, DomÃnguez-LÃ³pez ML, GarcÃa-Latorre E, Vega-LÃ³pez A	2012	Oct	Dzul-Caamal R, DomÃnguez-LÃ³pez ML, GarcÃa-Latorre E, Vega-LÃ³pez A.Implications of cytochrome 450 isoenzymes, aryl-esterase and oxonase activity in the inhibition of the acetylcholinesterase of Chirostoma jordani treated with phosphorothionate pesticides. Ecotoxicol Environ Saf. 2012 Oct; 84:199-206.
Not Relevant: Ecological Effects	El-Amrani S, Pena-Abaurrea M, Sanz-Landaluze J, Ramos L, Guinea J, CÃ¡mara C	2012	May	El-Amrani S, Pena-Abaurrea M, Sanz-Landaluze J, Ramos L, Guinea J, CÃ¡mara C.Bioconcentration of pesticides in zebrafish eleutheroembryos (Danio rerio). Sci Total Environ. 2012 May 15; 425:184-90.
Not Relevant: Ecological Effects	Enis Yonar M, Yonar SM, Ural MÅž, Silici S, DÃ¼ÅŸÃ¼kcan M	2012	Aug	Enis Yonar M, Yonar SM, Ural MÅž, Silici S, DÃ¼ÅŸÃ¼kcan M.Protective role of propolis in chlorpyrifos-induced changes in the haematological parameters and the oxidative/antioxidative status of Cyprinus carpio carpio. Food Chem Toxicol. 2012 Aug; 50(8):2703-8.
Not Relevant: Ecological Effects	Fu Y, Li M, Liu C, Qu JP, Zhu WJ, Xing HJ, Xu SW, Li S	2013	Aug	Fu Y, Li M, Liu C, Qu JP, Zhu WJ, Xing HJ, Xu SW, Li S.Effect of atrazine and chlorpyrifos exposure on cytochrome P450 contents and enzyme activities in common carp gills. Ecotoxicol Environ Saf. 2013 Aug; 94:28-36.
Not Relevant: Ecological Effects	George T, Beevi SN, Xavier G, Kumar NP, George J	2013	Jun	George T, Beevi SN, Xavier G, Kumar NP, George J.Dissipation kinetics and assessment of processing factor for chlorpyrifos and lambda-cyhalothrin in cardamom. Environ Monit Assess. 2013 Jun; 185(6):5277-84.
Not Relevant: Ecological Effects	Guo W, Yan X, Zhao C, Han R	2013	Jun	Guo W, Yan X, Zhao C, Han R.Efficacy of entomopathogenic Steinernema and Heterorhabditis nematodes against white grubs (Coleoptera: Scarabaeidae) in peanut fields. J Econ Entomol. 2013 Jun; 106(3):1112-7.

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Not Relevant: Ecological Effects	Huynh HP, Nugegoda D	2012	Jan	Huynh HP, Nugegoda D.Effects of chlorpyrifos exposure on growth and food utilization in Australian catfish, Tandanus tandanus. Bull Environ Contam Toxicol. 2012 Jan; 88(1):25-9.
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Not Relevant: Ecological Effects	Key PB, Simonik E, Kish N, Chung KW, Fulton MH	2013		Key PB, Simonik E, Kish N, Chung KW, Fulton MH.Differences in response of two model estuarine crustaceans after lethal and sublethal exposures to chlorpyrifos. J Environ Sci Health B. 2013; 48(11):967-73.
Not Relevant: Ecological Effects	Khalil F, Kang IJ, Undap S, Tasmin R, Qiu X, Shimasaki Y, Oshima Y	2013	Jun	Khalil F, Kang IJ, Undap S, Tasmin R, Qiu X, Shimasaki Y, Oshima Y.Alterations in social behavior of Japanese medaka (Oryzias latipes) in response to sublethal chlorpyrifos exposure. Chemosphere. 2013 Jun; 92(1):125-30.
Not Relevant: Ecological Effects	LÃ³pez-RoldÃ¡n R, Jubany I, MartÃ V, GonzÃ¡lez S, Cortina JL	2013	Dec	LÃ³pez-RoldÃ¡n R, Jubany I, MartÃ V, GonzÃ¡lez S, Cortina JL.Ecological screening indicators of stress and risk for the Llobregat river water. J Hazard Mater. 2013 Dec 15; 263 Pt 1:239-47.
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Not Relevant: Ecological Effects	Li X, Liu L, Zhang Y, Fang Q, Li Y, Li Y	2013	Sep	Li X, Liu L, Zhang Y, Fang Q, Li Y, Li Y.Toxic effects of chlorpyrifos on lysozyme activities, the contents of complement C3 and IgM, and IgM and complement C3 expressions in common carp (Cyprinus carpio L.). Chemosphere. 2013 Sep; 93(2):428-33.
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Not Relevant: Ecological Effects	Narra MR, Rajendera K, Rao JV, Begum G	2013	Jul-Aug	Narra MR, Rajendera K, Rao JV, Begum G.Evaluation of the biochemical stress response to chlorpyrifos in tissues of the edible crab Barytelphusa guerini: withdrawal of exposure improves the nutritional value. Z Naturforsch C. 2013 Jul-Aug; 68(7-8):318-26.
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Not Relevant: Ecological Effects	PÃ©rez J, Domingues I, Monteiro M, Soares AM, Loureiro S	2013	Jul	Perez J, Domingues I, Monteiro M, Soares AM, Loureiro S.Synergistic effects caused by atrazine and terbuthylazine on chlorpyrifos toxicity to early-life stages of the zebrafish Danio rerio. Environ Sci Pollut Res Int. 2013 Jul; 20(7):4671-80.
Not Relevant: Ecological Effects	Pal S, Kokushi E, Koyama J, Uno S, Ghosh AR	2012		Pal S, Kokushi E, Koyama J, Uno S, Ghosh AR.Histopathological alterations in gill, liver and kidney of common carp exposed to chlorpyrifos. J Environ Sci Health B. 2012; 47(3):180-95.
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Not Relevant: Ecological Effects	Richendrfer H, Pelkowski SD, Colwill RM, CrÃ©ton R	2012	Jul	Richendrfer H, Pelkowski SD, Colwill RM, CrÃ©ton R.Developmental sub-chronic exposure to chlorpyrifos reduces anxiety-related behavior in zebrafish larvae. Neurotoxicol Teratol. 2012 Jul; 34(4):458-65.
Not Relevant: Ecological Effects	Rivadeneira PR, Agrelo M, Otero S, Kristoff G	2013	Apr	Rivadeneira PR, Agrelo M, Otero S, Kristoff G.Different effects of subchronic exposure to low concentrations of the organophosphate insecticide chlorpyrifos in a freshwater gastropod. Ecotoxicol Environ Saf. 2013 Apr; 90:82-8.

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Not Relevant: Ecological Effects	Santos MJ, Ferreira MF, Cachada A, Duarte AC, Sousa JP	2012	Nov	Santos MJ, Ferreira MF, Cachada A, Duarte AC, Sousa JP.Pesticide application to agricultural fields: effects on the reproduction and avoidance behaviour of Folsomia candida and Eisenia andrei. Ecotoxicology. 2012 Nov; 21(8):2113-22.
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Not Relevant: Ecological Effects	Vera-Candioti J, Soloneski S, Larramendy ML	2013	Dec	Vera-Candioti J, Soloneski S, Larramendy ML.Single-cell gel electrophoresis assay in the ten spotted live-bearer fish, Cnesterodon decemmaculatus (Jenyns, 1842), as bioassay for agrochemical-induced genotoxicity. Ecotoxicol Environ Saf. 2013 Dec; 98:368-73.
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Not Relevant: Ecological Effects	Weston DP, Asbell AM, Lesmeister SA, Teh SJ, Lydy MJ	2014	Apr	Weston DP, Asbell AM, Lesmeister SA, Teh SJ, Lydy MJ.Urban and agricultural pesticide inputs to a critical habitat for the threatened delta smelt (Hypomesus transpacificus). Environ Toxicol Chem. 2014 Apr; 33(4):920-9.
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Not Relevant: Ecological Effects	Yoo DH, Shin EH, Lee DK, Ahn YJ, Chang KS, Kim HK, Kim SY, Park C	2013		Yoo DH, Shin EH, Lee DK, Ahn YJ, Chang KS, Kim HK, Kim SY, Park C.Insecticide susceptibility of field-collected populations of Culex tritaeniorhynchus in the Republic of Korea. J Insect Sci. 2013; 13:2.

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Pharmacokinetic	Crane AL, Klein K, Olson JR	2012	Dec	Crane AL, Klein K, Olson JR.Bioactivation of chlorpyrifos by CYP2B6 variants. Xenobiotica. 2012 Dec; 42(12):1255-62.
Pharmacokinetic	Crane AL, Klein K, Zanger UM, Olson JR	2012	Mar	Crane AL, Klein K, Zanger UM, Olson JR.Effect of CYP2B6*6 and CYP2C19*2 genotype on chlorpyrifos metabolism. Toxicology. 2012 Mar 11; 293(1-3):115-22.
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Pharmacokinetic	Leoni C, Balduzzi M, Buratti FM, Testai E	2012	Nov	Leoni C, Balduzzi M, Buratti FM, Testai E.The contribution of human small intestine to chlorpyrifos biotransformation. Toxicol Lett. 2012 Nov 23; 215(1):42-8.
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