Message

From: Beck.Nancy@epa.gov [Beck.Nancy@epa.gov]
Sent: 8/15/2018 9:28:19 PM
To: janet collins [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=usera98e8fe5]
Subject: Re: If tonight doesn't work

8am or 10:30 should work for me. With 8am being more reliable. Thanks

****

Nancy B. Beck, Ph.D., DABT
Deputy Assistant Administrator
Office of Chemical Safety and Pollution Prevention
P: 202-564-1273
M: Ex. 6 Personal Privacy (PP)
beck.nancy@epa.gov

On Aug 15, 2018, at 5:27 PM, Janet Collins <jcollins@croplifeamerica.org> wrote:

> Let's talk tomorrow!
>
> Sent from my iPhone

Message

From: Beck.Nancy@epa.gov [Beck.Nancy@epa.gov]
Sent: 8/15/2018 8:52:01 PM
To: janet collins [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=usera98e8fe5]
Subject: Re: Call update

I'm running behind at my meetings. Can we chat at 6pm instead.

****
Nancy B. Beck, Ph.D., DABT
Deputy Assistant Administrator
Office of Chemical Safety and Pollution Prevention
P: 202-564-1273
M: Ex. 6 Personal Privacy (PP)
beck.nancy@epa.gov

On Aug 15, 2018, at 4:35 PM, Janet Collins <jcollins@croplifeamerica.org> wrote:

> Nancy- Jay Vroom has requested to be part of the discussion at 5::00.
>
> Issues- Prop 65 labeling, and EPA leads or contacts for a couple of issues.
>
> Thank you
>
> Sent from my iPhone

Appointment

From: Janet Collins [jcollins@croplifeamerica.org]
Sent: 8/15/2018 5:44:42 PM
To: Janet Collins [jcollins@croplifeamerica.org]; Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]

Subject: Updates
Location: JEC to phone Nancy

Start: 8/15/2018 9:00:00 PM
End: 8/15/2018 9:30:00 PM
Show Time As: Tentative

Recurrence: (none)

Nancy- if this time doesn't work or you run late, please call my office Ex. 6 Personal Privacy (PP)

Message

From: Francesca Purcell [fpurcell@croplifeamerica.org]
Sent: 2/13/2018 10:03:54 PM
To: Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]
Subject: Sorry that you cannot attend the CropLife America 2018 Winter Board Meeting & Legislative Rally

Dear Nancy,

Thank you for your response. We are sorry that you will not be attending the CropLife America 2018 Winter Board Meeting & Legislative Rally. We hope that you will join us at one of our future events.

Sincerely,
Francesca Purcell
CropLife America
fpurcell@croplifeamerica.org

If you no longer want to receive emails from Francesca Purcell, please Opt-Out.

powered by cvent

Message

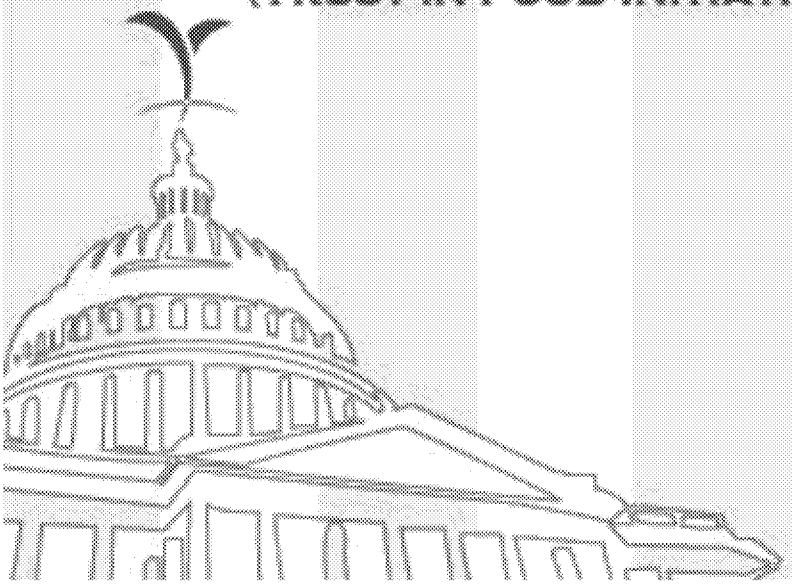
From: Francesca Purcell [fpurcell@croplifeamerica.org]
Sent: 2/13/2018 9:19:56 PM
To: Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]
Subject: Join CropLife America for dinner!

Flag: Flag for follow up

CROPLIFE AMERICA CORDIALLY INVITES YOU TO JOIN OUR BOARD OF DIRECTORS FOR COCKTAILS AND DINNER

MARCH 5, 2018
THE RITZ-CARLTON, PENTAGON CITY
1250 S HAYES ST, ARLINGTON, VA

DIPLOMAT ROOM
COCKTAILS: 6:00 PM
DINNER: 7:00 PM
SPEAKER: CHARLENE FINCK
(TRUST IN FOOD INITIATIVE)



CropLife

Dear Nancy,

You are invited to join us for dinner on Monday evening, Monday, March 5, 2018. Please respond by clicking either **Yes** or **No**. We look forward to your response!

Sincerely,
CropLife America
fpurcell@croplifeamerica.org

If you no longer want to receive emails from CropLife America, please Opt-Out.

powered by cvent

Message

From: Janet Collins [jcollins@croplifeamerica.org]
Sent: 8/15/2018 9:01:29 PM
To: Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]
Subject: Re: Call update

Thank you

Sent from my iPhone

On Aug 15, 2018, at 4:52 PM, Beck, Nancy <Beck.Nancy@epa.gov> wrote:

> I'm running behind at my meetings. Can we chat at 6pm instead.
>
> **********
> Nancy B. Beck, Ph.D., DABT
> Deputy Assistant Administrator
> Office of Chemical Safety and Pollution Prevention
> P: 202-564-1273
> **Ex. 6**
> beck.nancy@epa.gov
>
> On Aug 15, 2018, at 4:35 PM, Janet Collins <jcollins@croplifeamerica.org> wrote:
>
>> Nancy- Jay Vroom has requested to be part of the discussion at 5::00.
>>
>> Issues- Prop 65 labeling, and EPA leads or contacts for a couple of issues.
>>
>> Thank you
>>
>> Sent from my iPhone

Message

From: Janet Collins [jcollins@croplifeamerica.org]
Sent: 2/28/2018 5:44:43 AM
To: Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]
CC: Courtney DeMarco [cdemarco@croplifeamerica.org]
Subject: Re: Follow up discussion

Thank you Nancy

On Feb 27, 2018, at 11:31 PM, Beck, Nancy <Beck.Nancy@epa.gov> wrote:

> Janet,
> I have not yet had the briefing with staff yet on this issue, although I know its on the calendar somewhere.
> Let me find out when it will happen and I can get back to you.
>
> Nancy
>
> _____
> Nancy B. Beck, Ph.D., DABT
> Deputy Assistant Administrator, OCSPP
> P: 202-564-1273
> Ex. 6
> beck.nancy@epa.gov
>
> **From:** Janet Collins [mailto:jcollins@croplifeamerica.org]
> **Sent:** Tuesday, February 27, 2018 2:12 PM
> **To:** Beck, Nancy <Beck.Nancy@epa.gov>
> **Cc:** Courtney DeMarco <cdemarco@croplifeamerica.org>
> **Subject:** Follow up discussion
> **Importance:** High
>
> Nancy- would you have about an hour to catch up on the documents we have sent in the next 10 days to two weeks?
>
> Happy to make my schedule work with what might work for you.
>
> Thank you.
>
> Janet
>
> Ex. 6

Message

From: Janet Collins [jcollins@croplifeamerica.org]
Sent: 8/15/2018 6:19:22 PM
To: Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]
Subject: Re: Tentative: Updates

Then I will wait for your call or your signal that it won't work.

I also can be available after 6:00 if that works for you

Sent from my iPhone

> On Aug 15, 2018, at 1:53 PM, Beck, Nancy <Beck.Nancy@epa.gov> wrote:
>
> I will be coming back from another meeting and I'm not sure it will end on time but will call if I can.
>
> <meeting.ics>

Message

From: Jay Vroom [JVroom@croplifeamerica.org]
Sent: 1/26/2018 8:23:12 PM
To: Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]
Subject: Thank You

Nancy,

Thanks again for taking time out of your evening to come and speak with the CropLife Strategic Oversight Council last night. Your remarks were informative and gave us much food for thought. We appreciate the ability to engage in dialogue with you and many others at EPA. We know your first year at EPA has been intense as you've tackled many ongoing and new issues.

I hope you can engage at our April Regulatory Conference, whether Administrator Pruitt is able to attend and speak there or not. Again, the dates of that event are April 26-27, at Potomac Yards, should you want to put that on your calendar.

Thanks again,

Jay

Jay Vroom
President & CEO
CropLife America
1156 15th Street, NW
Suite 400
Washington, DC 20005
Ex. 6
Main Switchboard (202) 296-1585
Ex. 6
Fax (202) 466-5832
Email vroom@croplifeamerica.org
Executive Assistant Mary Jo Tomalewski (mjtomalewski@croplifeamerica.org, Ex. 6
Web www.croplifeamerica.org

Message

From: Janet Collins [jcollins@croplifeamerica.org]
Sent: 2/27/2018 10:31:52 PM
To: Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]
Subject: Automatic reply: Follow up discussion

I am currently out of the office on business travel but have some access to email. I will respond as quickly as possible. For urgent matters please phone Courtney DeMarco Ex. 6 *We will get back to you as soon as possible.*

Message

From: Janet Collins [jcollins@croplifeamerica.org]
Sent: 2/9/2018 3:54:35 PM
To: Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]
Subject: Automatic reply: updates

I am currently out of the office but have access to email. I will respond as quickly as possible (or you can phone me directly- 703-868-3280). For urgent matters please phone Courtney DeMarco Ex. 6 *We will get back to you as soon as possible.*

Message

From: Mary Jo Tomalewski [mjtomalewski@croplifeamerica.org]
Sent: 3/2/2018 11:36:23 PM
To: Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]
Subject: RE: Invitation to Meet

Thank you, Nancy.

Mary Jo Tomalewski
Executive Assistant to the President & CEO
CropLife America
Ex. 6
Email mjtomalewski@croplifeamerica.org

-----Original Message-----
From: Beck, Nancy [mailto:Beck.Nancy@epa.gov]
Sent: Friday, March 2, 2018 12:24 PM
To: Mary Jo Tomalewski <mjtomalewski@croplifeamerica.org>; Dravis, Samantha <dravis.samantha@epa.gov>; Bennett, Tate <Bennett.Tate@epa.gov>; Greenwalt, Sarah <greenwalt.sarah@epa.gov>
Cc: Willis, Sharnett <Willis.Sharnett@epa.gov>
Subject: RE: Invitation to Meet

I will be attending the Biopesticides meeting on Tuesday and will be unable to attend.

Regards,
Nancy

Nancy B. Beck, Ph.D., DABT
Deputy Assistant Administrator, OCSPP
Ex. 6
beck.nancy@epa.gov

-----Original Message-----
From: Mary Jo Tomalewski [mailto:mjtomalewski@croplifeamerica.org]
Sent: Friday, March 2, 2018 12:17 PM
To: Beck, Nancy <Beck.Nancy@epa.gov>; Dravis, Samantha <dravis.samantha@epa.gov>; Bennett, Tate <Bennett.Tate@epa.gov>; Greenwalt, Sarah <greenwalt.sarah@epa.gov>
Cc: Willis, Sharnett <Willis.Sharnett@epa.gov>
Subject: Invitation to Meet

Good afternoon,

Jay Vroom from CropLife America asked me to reach out to you to invite you to an hour-long meeting that we are having on Tuesday, March 6 at 9 AM, with Henry Darwin in his offices. A group of our Board of directors and other industry leaders are in town for CLA winter board meeting and they want to meet to discuss a number of issues and EPA processes.

If you are available we would be delighted if you would join us.

MJ

Sent from my iPhone~Please excuse any typos!

Message

From: Janet Collins [jcollins@croplifeamerica.org]
Sent: 1/31/2018 10:56:39 AM
To: Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]; Keigwin, Richard [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=151baabb6a2246a3a312f12a706c0a05-Richard P Keigwin Jr]
CC: Jay Vroom [JVroom@croplifeamerica.org]; Mary Jo Tomalewski [mjtomalewski@croplifeamerica.org]; Courtney DeMarco [cdemarco@croplifeamerica.org]
Subject: updates
Attachments: Memo Beck on 2016 OPP Framework .pdf; FINAL CLA Comments OPP Framework Epidemiology.pdf

Dear Nancy and Rick- thank you for the opportunity to discuss various regulatory issues raised by CLA and its members. As a follow up, attached please find a summary of some discussion points as well as a recent CLA comment posted to an EPA docket on pesticide registrations.

We welcome further discussion on these important issues, and others of concern to our members.

My best,

Janet E Collins, Ph.D., R.D.
CropLife America
1156 15th Street, NW; Suite 400
Washington DC 20001

Ex. 6

25 January 2018

Ms. Dana Friedman
Pesticide Re-Evaluation Division
Office of Pesticide Programs
Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, DC 20460-8015

Submitted via Regulations.gov EPA-HQ-OPP-2012-0330

RE: Registration Reviews; Draft Human Health and/or Ecological Risk Assessments for Several Pesticides, December 15, 2017; Fed. Reg 82: 59596; FR Doc No: 2017-27098; Docket ID: EPA-HQ-OPP-2012-0330.

Dear Ms. Friedman:

CropLife America (CLA) appreciates the opportunity to provide comment to the U.S. Environmental Protection Agency (EPA or Agency), Office of Pesticide Programs (OPP) on its Registration Reviews; Draft Human Health and/or Ecological Risk Assessments for Several Pesticides, EPA-HQ-OPP-2012-0330; and on its use of the "Framework for Incorporating Human Epidemiologic and Incident Data in Risk Assessments for Pesticides," (Framework) EPA-HQ-OPP-0316-DRAFT-0075.pdf.[1]

Established in 1933, CLA represents the developers, manufacturers, formulators and distributors of plant science solutions for agriculture and pest management in the United States. CLA's member companies produce, sell and distribute virtually all the vital and necessary crop protection and biotechnology products used by American farmers, ranchers and landowners. CLA is committed to working with EPA, as the primary federal agency responsible for the regulation of pesticides, to encourage practical, science-based regulation of its members' products.

CLA provided suggestions on the "Draft Framework for Incorporating Human Epidemiologic and Incident Data in Risk Assessments for Pesticides" (Draft Framework), as part of its April 8, 2016 comments on the EPA Docket for the FIFRA Scientific Advisory Panel on chlorpyrifos.[2] By reference, those comments are incorporated herein. To summarize the CLA comments on the Draft Framework, we extract the following from the CLA comments of April 8, 2016.

---

[1] US EPA. December 28, 2016. Office of Pesticide Programs' Framework for Incorporating Human Epidemiologic & Incident. Data in Risk Assessments for Pesticides. https://www3.epa.gov/pesticides/EPA-HQ-OPP-2008-0316-DRAFT-0075.pdf

[2] US EPA. FIFRA Scientific Advisory Panel (FIFRA SAP) to consider and review Chlorpyrifos: Analysis of Biomonitoring Data, March 8, 2016; FR Doc No: 2016-05174; Docket ID: EPA-HQOPP-2016-0062-0001.

> *In its Draft Framework EPA refers to use of a modified Bradford Hill[3] criteria approach to assessing strength and appropriate use of epidemiological studies in human health risk assessment. The criteria support sound approaches to evaluating associations in epidemiological data cohorts but are not intended to be used to establish a cause and effect association between exposure and health or environmental impact. It is important to note that even when primary data are available for statistical reassessment, epidemiological studies are not intended to replace toxicological data collected from animal studies intended to establish effects in studies.*

EPA did not incorporate CLA's comments into the Framework. Nevertheless, CLA and its member companies continue to believe that a consistent and scientifically defensible approach to use of all sources of data in regulatory decision making is required. For this reason, CLA does not support the weight of evidence approach taken by EPA in integration of literature, epidemiologic, and other sources of study outcomes in its regulatory decision making.

The Framework contains sound scientific principles, but it is incomplete and does not consider the practitioner perspective. CLA submits a detailed report, "Comments on Office of Pesticide Programs' Framework for incorporating human epidemiologic & incident data in risk assessments for pesticides (Framework)," attached hereto intended to highlight the Framework's limitations and EPA's missteps in its development of it. We welcome the opportunity to work with EPA to correct some of the missteps that have occurred, and importantly that have negatively impacted our members' registration timelines and outcomes.

CLA's comments are intended to address the lack of consistency in approach taken by EPA, relative to the recommendations in the Framework. Risk assessment approaches including various sources of information and data, and inconsistent use of study outcomes within a study when reported across various health outcomes, limit consistency and predictability to assessments intended to follow the Framework.

We continue to welcome the opportunity to work with EPA and other Federal Agencies in pursuit of scientifically balanced approaches to human health risk assessment. Should you have questions or wish to discuss these issues further, please contact me directly [(jcollins@croplifeamerica.org) or (+1) 202-833-4474].

Thank you for your consideration of these comments.

Respectfully,

Janet E. Collins, Ph.D., R.D.
Executive Vice President, Science and Regulatory Affairs

Cc: Mr. Rick Keigwin

---

[3] Hill A. B., 1965. "The environment and disease: Association or causation?" Proc R Soc Med 58, pp 295-380.

Comments on Office of Pesticide Programs' 2016 "Framework for incorporating human epidemiologic & incident data in risk assessments for pesticides" (Framework).

Framework Issue Date: December 28, 2016.

Accessed: https://www3.epa.gov/pesticides/EPA-HQ-OPP-2008-0316-DRAFT-0075.pdf

Key findings.

The 2016 Framework incorporates some aspects of the 2010 SAP recommendations for change to the Draft Framework. Notably, the 2016 Framework document devotes more attention to study quality. However, there remain important limitations. The Framework is "final" and will be updated on as-needed basis.

Missed opportunity. The Office of Pesticide Programs[4] (OPP) provides no guidance to epidemiologists or funding agencies about what is required for risk assessment. As a result, there is little value to sharing this document with other epidemiologists. They will merely say, yes, we know the difference between case control and cohort studies. Similarly, OPP does not offer any recommendations to change the status quo with respect to study limitations, data access or interpretation of epidemiology data.

This is not a framework for integration with toxicology[2]. There is little discussion or even recognition about the state of the science of registered pesticides. The OPP Framework reads as if all epidemiology studies are new discoveries on new pesticides. The Framework makes little effort to incorporate the known with the new. OPP cannot both require GLP studies for pesticide active ingredients and then turn a blind eye to those results when evaluating epidemiology studies.

"Weight of Evidence" is poorly defined. Elements of the Bradford Hill Criteria are listed but the descriptions permit any interpretation.

What about the "missing" or unpublished data? There are multiple places in the Framework that beg for a conversation about access to data. In Section IIIA, OPP mentions missing data. Section IV discusses statistical analyses and null results. If indeed there are missing data or incomplete analyses, OPP should be recommending a discussion with OPP, the registrant(s), and the epidemiology investigators to develop a plan to make the missing data available and/or conduct additional analyses. This is not present in the 2016 Framework.

---

[4] Interchange OPP and Framework in "saying" and "recommending". We use the term OPP for the US EPA OPP. [2] In toxicology, the terms toxicology, *in vivo* and Guideline Studies are used interchangeably. There are differences, but are considered similarly for the purposes of comparing them to human epidemiology data.

Study quality evaluation will not be transparent. Tables of ideal study elements and quality criteria are part of the Framework. However, there is little direction or discussion regarding how these will be used or how individual studies will be scored. The OPP has previously classified epidemiology studies as being of high and medium quality without providing the details of its interpretation on individual elements.

Incident data should be "complementary." OPP recommends that incident data can provide useful, complementary information for real world risk of pesticides. Complementary is the critical point, all data should be evaluated together.

Contents

Opportunities for dialogue and discussion with OPP. 5
Recommendations from the 2010 SAP: 5
 Integration of multidisciplinary data. 5
 What strong epidemiology studies look like. 6
 Exposure 6
Elements of the 2016 Framework. 6
II. Introduction 7
III Systematic review in pesticide risk assessment: Epidemiology 7
IV. Reviewing epidemiology studies for use in pesticide risk assessment 8
V. Human incident surveillance data 11

Opportunities for dialogue and discussion with OPP.

What are their messages to epidemiologists and funders?

How will they OPP integrate epidemiology results with toxicology results?

What is the difference between regulatory science and discovery science? For toxicology? For epidemiology?

What is the solution for publication bias, and lack of published information on null results?

If a publication does not provide full disclosure of analyses, sensitivity, confounding, or dose-response, does OPP have a plan or requirement to either have the authors complete the required analyses or to acquire the raw data?

What is the vision for registrant requirements in the future? Will epidemiology be required?

Recommendations from the 2010 SAP[5]:

The February 2 – 4, 2010 FIFRA Scientific Advisory Panel (SAP) evaluated the Draft Framework on epidemiology and provided extensive comments. Several topics are highlighted.

Integration of multidisciplinary data.

The SAP noted that the 2010 Draft Framework described problem formulation but did not indicate specifically how the toxicological and epidemiologic information would be "considered in an integrated fashion." For example, the SAP suggested that an integrated approach would identify limitations with a recommendation for additional research. Other recommendations:

1. Define "biological plausibility." Several interpretations of plausibility are possible. Emphasis should be placed on dose-response relationships, temporal sequence, strength of the association and consistency of findings across studies.

[5] Minutes published on April 22, 2010 regarding the "Draft framework and case studies on atrazine, human incidents, and the Agricultural Health Study: Incorporation of epidemiology and human incident data into human health risk assessment."

ED_002061_00038399-00005

2. Use the "source to adverse outcome pathway" to identify critical data gaps.
3. The SAP discussed making a logical progression from the key events in animal studies. It is important to consider the relevancy across species from high dosing that is unlikely to occur in humans and to separately consider differences in physiology or biology.

□ What strong epidemiology studies look like.

Robust epidemiology studies are characterized by strong design with well-characterized exposures. However, all studies have weaknesses. "The Agency needs to remain cognizant of those when considering use of data from any single study or an aggregation of studies for a specific pesticide." The 2010 SAP recommended the Agency establish a set of quality criteria. Quality elements include:

1. Validation of exposure assessment
2. Adequate sample size
3. Well defined outcome (disease vs. not diseased)
4. Attention to reduced sources of bias
5. Control for confounding and identification of effect modifying factors
6. Potential for generalizing to other populations.

□ Exposure

1. Exposure must be robustly and quantitatively addressed. Exposure should be paired with identification of key events in a mode of action context.
2. "[E]xposure assessment should be evaluated for accuracy, precision and reliability, and should include validation where feasible… Exposure metrics can represent dose estimates (for example average daily dose or peak dose), duration of exposure, or a combination of these in a cumulative exposure metric."

Elements of the 2016 Framework.

OPP characterizes the 2016 Framework as a description of overall conceptual scientific consideration when evaluating epidemiology studies. It is not intended to be binding or serve as a reviewer's guide. OPP states on page three, that "since the number of pesticides for which quality epidemiology data either exist or are being developed remains relatively low in the near term, experimental laboratory data will likely continue to be the primary source of data for use in quantitative risk assessment for most pesticides."

Interpretation: The page three statement implies that for pesticides with some epidemiology data, experimental data may be supplanted with epidemiology data. It also suggests that epidemiology results will be used more frequently in the future.

This Framework is a "final" document. OPP says that will be updated from time-to-time and on an as needed basis.

II. Introduction

OPP lists uses of human data.

1. Provide insight in effects caused by actual chemical exposures
2. Guide additional analyses (doses, endpoint selection)
3. Identify potentially susceptible population
4. Identify new health effects
5. Confirm the existing toxicological observations

OPP discusses the NRC 2007 Tox21 report, in that a strong WOE draws from the "best available information" from multiple data sources. The Framework presents existing guidance documents and continues to draw from the Bradford Hill criteria (guideline elements). OPP recognizes that epidemiology studies tend to report on widely used pesticides, which also have a significant body of data from toxicology, exposure, pharmacokinetics, MOA and AOP, etc. On page five, OPP states that "it is noteworthy that the availability of a fully elucidated MOA/AOP is not [a] requirement for using epidemiology studies in human health risk assessment."

Interpretation: The Framework recommends using multiple data sources. We might interpret this as OPP will use toxicology when reviewing epidemiology. It is also possible (and likely) that OPP is using this as mandate that it must use epidemiology, to the exclusion of toxicology.

III Systematic review in pesticide risk assessment: Epidemiology

A. Problem Formulation

Here OPP describes how it plans to define the scope of an analysis. OPP continues to point out that a review may be focused on exposure pathways and certain health outcomes. It notes (page nine), "If missing data are critical to the assessment, options are discussed as to how best to obtain that information."

Interpretation: This section lists many questions that would be considered. At first look, it seems like a good practice. However, it is unclear if these are standard questions posed for all pesticides and risk assessments or if they are raised after reviewing an epidemiology publication. The comment about missing data is an opportunity for enhancement to the Framework. Indeed, the Framework does not list how to obtain missing data. If indeed there are missing data, there should be a discussion among OPP, registrant(s), and the epidemiology investigators to develop a plan to make the missing data available.

B. Data Collection

This section details how OPP will search and report on published and unpublished sources. On page 10 of the Framework, it states, "In the case of epidemiology, most studies are expected to be found in the open scientific literature. Although in some cases supplemental analyses or information may be available, dialogue with the researchers may provide additional, important information not published in the original paper in understanding and interpreting epidemiology studies."

Interpretation. It is a good practice to assure the reader that OPP will not selectively pick certain studies. However, if indeed OPP is willing to use epidemiology data *in lieu* of toxicology, waiting

for epidemiology studies to be conducted, and reported seems unduly random. If epidemiology data are important, the process to conduct and report should be more systematic.

C. Data Evaluation

OPP promises to use study quality in its review. More on this later in IV.C.

D. Data integration: Weight of Evidence (WOE)

OPP promises to use a WOE analysis, and that conclusions will be made on the preponderance of information rather than relying on any one study. Specific aspects are listed:

- Key events. The events can come from the MOA/AOP, PK or any human or animal study. In other words, there is no guideline.
- Dose-response. A well-characterized exposure-response relationship strongly suggests cause and effect in epidemiology studies.
- Temporal association. This is another argument in favor of casualty.
- Consistency. A pattern across several independent studies supports causality. OPP also states that discordant results cannot be used to rule out a causal connection.
- Strength of association. A large and precise risk increases confidence in a true association. OPP also states that a small effect can also be important.
- Specificity of the association. Specificity may or may not be a strong argument for causation.
- Coherence. When animal and human data show a similar toxic profile, there is high confidence in the human health risk assessment. If they are dissimilar it is important to consider other factors.
- Biological plausibility. A proposed mechanism is an important source of support for causality. Lack of mechanistic data is not a reason to reject causality.

Interpretation: These elements are the Bradford Hill Criteria. For many elements, OPP first makes a strong point and then gives an excuse to dismiss it. In other words, the list is correct but the interpretation is weak. These elements were designed the be used collectively to dispassionately evaluate a body of literature. When there is evidence of a causal effect, the reviewer does not have to offer excuses for each element.

The Hill criteria have been criticized because reviewers can pick and choose certain elements. Further they do not consider bias and confounding. However, reproducibility in independently conducted studies should correct for errors in any individual study. In its Framework, OPP is providing no guidance at all for WOE.

E. Overall conclusion, recommendation for risk assessment, statement of areas of confidence and uncertainty.

Epidemiology studies have the potential to inform multiple components of the risk assessment.

IV. Reviewing epidemiology studies for use in pesticide risk assessment

A. Introduction

This section gives the merits of epidemiology. It recognizes that exposures (to pesticides) have changed (declined) over time. This is important when reviewing a body of literature.

Epidemiology data study real-world chemical exposures in humans and provide information on hazard/risk characterization. The results can be used to guide future research. It also can inform uncertainties associated with specific extrapolation.

Interpretation. No argument. Of course, devil lies in the details.

B. Types of epidemiology studies

This section reads like an epidemiology textbook. OPP describes the type of study and when each is used.

C. Evaluating epidemiology studies for use in pesticide risk assessment

The Framework also has a table on page 24 with five parameters for quality consideration ranked as high/moderate/low. On page 22, OPP uses a list of eight aspects that are considered important (listed below). These include:

1. Clear articulation of the hypothesis, even if the study is hypothesis-generating in nature;
2. Adequate assessment of exposure for the relevant critical windows of the health effects, the range of exposure of interest for the risk assessment target population, and the availability of a dose/exposure-response trend from the study, among other qualities of exposure assessment;
3. Reasonably valid and reliable outcome ascertainment (the correct identification of those with and without the health effect in the study population);
4. Appropriate inclusion and exclusion criteria that result in a sample population representative of the target population, and absent systematic bias;
5. Adequate measurement and analysis of potentially confounding variables, including measurement or discussion of the role of multiple pesticide exposure, or mixtures exposure in the risk estimates observed;
6. Overall characterization of potential systematic biases in the study including errors in the selection of participation and in the collection of information; this can include performing sensitivity analysis to determine the potential influence of systematic error on the risk estimates presented;
7. Evaluation of the statistical power of the study to observe health effects with appropriate discussion and/or presentation of power estimates; and,
8. Use of appropriate statistical modeling techniques, given the study design and the nature of the outcomes under study. "

Interpretation: As we saw with the glyphosate and organophosphate (OP) reviews, these aspects/considerations are reasonable but OPP *application* of them is important. The terms of "good," "adequate," and "appropriate" are highly subjective. Further, there is no discussion in the Framework about interpretation of study quality if one or more parameter scores has a low score. Previously, OPP had not revealed how it scored each parameter but only gave how the overall study was ranked. Transparency in these elements is an important "ask" for future assessments.

9. Exposure assessment

The Framework goes into detail about direct and indirect approaches. It incorporates the biomarker evaluation instrument (BEES-C) from Lakind et al. (2014).[6]

Interpretation. The validity of an exposure assessment is more important than defining if it is direct or indirect. OPP omitted the consideration of Exposure Variability and Misclassification from the BEES-C instrument, which addresses the number of samples (spot vs. many). OPP concludes that exposure assessment methods should be able to provide exposure estimates that are reliable and valid. However, the Framework does not incorporate a recommendation to this effect.

10. Confounding factors

This is appropriately defined and discussed. The Framework mentions that "it is not sufficient to simply raise the possibility of confounding; one should make a persuasive argument explaining why a risk factor is likely to be a confounder, what its impact might be, and how important that impact might be to the interpretation of findings."

Interpretation: This conclusion appears to be a warning to industry and other critics.

11. Statistical analysis

This is appropriately defined and discussed.

Interpretation: This is an area in which investigators may not disclose all analyses in the publication(s) or may not conduct the analyses in question. There is no discussion or guidance how to improve the status quo.

12. Potential bias in observational research

OPP recognizes that no study is devoid of bias. Most authors (and journals request) some discussion of study biases and limitations. Quantitation of the amount and direction of bias is less common. Computational tools are increasingly available to evaluate potential biases.

Interpretation: As above, there is no discussion or guidance how to improve the status quo. These are issues related to access to raw data and open discussions with investigators.

13. Interpretation of null studies

OPP states that lack of associations will be evaluated carefully. The bulk of this section states the opposite. In fact, OPP states, "the absence of evidence should not be interpreted as the evidence of absence" (page 35). The Framework mentions the effects of publication bias where the published literature disproportionately excludes null findings.

Interpretation. This section fails to account for what is already known about the risk profile of a registered pesticide. Contrary to OPP's view, the lack of human evidence, in the context of vast guideline studies, could be interpreted as evidence of absence. Furthermore, when relying upon

[6] J. Lakind et al. (2014). Biomonitoring, Environmental Epidemiology, and Short-lived Chemicals (BEES-C).

published epidemiology data, OPP must account for the lack of disclosure of negative data. There is no discussion or guidance how to improve the status quo.

14. External validity (generalizability)

OPP mentions that results from one human study must be evaluated relative to another population.

Interpretation. This section is weak. OPP makes no attempt to discuss biology or human physiology. Nor does OPP apply knowledge of chemical properties of pesticides to infer exposure in different population.

V. Human incident surveillance data

OPP lists the resources for national poison control.

Interpretation. On page 38, OPP makes an important point that medical reports should be reliable, reasonable and consistent with current knowledge. However, for the incident/poison control data, OPP does not discuss the merits of using incident data that has not been classified as "definite" (i.e. eliminating probable and possible data). It is well known that many incident reports do not confirm exposure to a specific pesticide. On page 43 of the Framework, OPP states that incident data can provide useful, complementary information in evaluating the real-world risks of pesticides. The complementary nature of incident data is the key aspect. Incident data should only be considered in the context of other known data.

CropLife ★AMERICA★

MEMO

Prepared by:	Janet E. Collins, Ph.D., R.D.
CC:	Jay Vroom
Date:	January 30, 2018
To:	Dr. Nancy Beck, Mr. Rick Keigwin

Comments on Office of Pesticide Programs' Framework for incorporating human epidemiologic & incident data in risk assessments for pesticides. Issue date: December 28, 2016. Found here.

Questions from CLA members for open discussion/dialogue

- What is the appropriate way for EPA to integrate epidemiology results with toxicology results?
- What is the appropriate way to address the lack of published information on null results for epidemiology studies (i.e. selective reporting for results for specific pesticides)?
- If a publication does not provide sufficient disclosure of analysis, sensitivity, confounding, or dose-response, does OPP have a plan or requirement to either have the authors complete the required analyses or to acquire the raw data?

General comments

The US Environmental Protection Agency (EPA or Agency) Office of Pesticide Programs' (OPP) Framework for Incorporating Human Epidemiologic & Incident Data in Risk Assessments for Pesticides [EPA-HQ-OPP-2008-0316) (2016 Framework)], states it is "not intended to be a reviewer's guide or manual or Standard Operating Procedure for assessing or using epidemiology data." Rather, OPP characterizes the 2016 Framework as a description of overall conceptual scientific consideration when evaluating epidemiology studies and highlights the importance of human data for risk assessment.

The pesticide industry agrees that a systematic and transparent review process is necessary when evaluating epidemiology data. The 2016 Framework does incorporate some aspects of the 2010 SAP recommendations, such as developing an assessment of study quality. However, there remain important limitations in the 2016 Framework that require clarification and in turn, reduce its effectiveness. While this request is not exhaustive, as we continue to have concerns about the Framework and its implementation, three important areas are discussed more fully below and include:

1. Integration of epidemiology and EPA required guideline studies (the Weight of Evidence assessment)
2. Quality assessment (how will "appropriate and sufficient be defined?)
3. Transparency of epidemiology data (how will unpublished results be obtained?)

It is our opinion that incorporating these suggestions into the 2016 Framework will enhance the potential to provide for a process that instills greater confidence in OPP and Agency integration of epidemiology into the human risk assessment process.

1. Integration of epidemiology and EPA-required guideline studies

In its 2016 Framework, OPP has provided little guidance for weight of evidence (WOE). The OPP promises to use a WOE analysis, and that conclusions will be made on the preponderance of information rather than relying on any one study. The 2016 Framework presents existing guidance documents and continues to draw from the Bradford Hill criteria (guideline elements). Specific aspects from the Bradford Hill elements are listed. For many of the Hill elements, OPP first makes a strong point and then gives an excuse to dismiss it. In other words, the list is correct but the interpretation is weak and seemingly arbitrary. These elements were designed to be used collectively, to dispassionately evaluate a body of literature. When there is evidence of a causal effect, the reviewer does not have to offer excuses for each element.

The 2016 Framework recommends using multiple data sources and information from different disciplines. One might interpret this as OPP will use toxicology when reviewing epidemiology. However, it appears that in practice, OPP is using epidemiology to the exclusion of toxicology.

There is little discussion or even recognition in the 2016 Framework about the robust state of the science of registered pesticides. The 2016 Framework reads as if all epidemiology studies are new discoveries on new pesticides. The document makes little effort to incorporate the known with the new. OPP cannot both require Good Laboratory Practice-compliant studies and then turn a blind eye to their results when evaluating epidemiology studies, which often are conducted for a completely different purpose. The EFSA Panel on Plant Protection Products and their residues, in its assessment of the methodological limitations of pesticide epidemiology studies (EFSA, 2017), discussed incorporation of pesticide epidemiology studies into the regulatory risk assessment process [EFSA Journal 2017; 15(10):5007]. While recognizing the potential important role that human health outcomes can play, the EFSA PPR (2017) cautioned against use of a single, not replicated, epidemiological study in the absence of other studies on the same substance, a practice the EPA did not follow in the assessment of the organophosphates.

OPP discusses the NRC 2007 Tox21 report, that a strong WOE draws from the "best available information" from multiple data sources. OPP recognizes that epidemiology studies tend to report on widely used pesticides, which also have a significant body of data from toxicology, exposure, pharmacokinetics, mode of action (MOA) and adverse outcome pathway (AOP), etc. On page five of the Framework, OPP states that "it is noteworthy that the availability of a fully elucidated MOA/AOP is not [a] requirement for using epidemiology studies in human health risk assessment."

When the epidemiology data and the guideline toxicology data are inconsistent and contradictory, either the WOE must be extremely robust with a detailed scientific rationale as to how and why the epidemiology, or toxicology data carry the greater weight. Neither the discounting of robust epidemiology data indicating an adverse association not observed in the guideline studies, nor the discounting of robust toxicology data for weak epidemiology data

suggesting an association not observed in the guideline studies or without a plausible MOA, is acceptable without a strong WOE justification to support the decision.

It is not clear how data with a defined MOA will be integrated with information lacking a plausible MOA.

2. Quality assessment

The integration of toxicology and epidemiology relies upon using the "best available information." Discussion of ideal study elements and quality criteria are part of the 2016 Framework. The Framework has a table on page 24 with five parameters for quality considerations ranked as high/moderate/low. However, there is little direction or discussion regarding how these will be used or how individual studies will be ranked. The rubric for study quality considerations (Table 2) uses vague terms such as "good", "moderately good" and "low-quality." Further, there is no discussion in the 2016 Framework about interpretation of study quality if one or more parameter scores low.

In recent evaluations of glyphosate and the organophosphates, OPP classified epidemiology studies as being high, medium and low quality without providing details of its interpretation on individual elements. Notably, the quality evaluation was inconsistently applied in OPP's 2016 Updated Literature Review on Neurodevelopment Effects for the Organophosphates, in which several studies were excluded from the WOE based on a single quality element, while others were not (excluded using the same criteria). Transparency in these elements is an important requirement for future assessments.

Following the table on quality considerations, the 2016 Framework goes into detail about direct and indirect approaches for exposure assessment in epidemiology studies. However, the reliability and validity of an exposure assessment is more important than defining if it is direct or indirect. The 2016 Framework also displays a portion of the biomarker evaluation instrument from Lakind et al., 2014, "Biomonitoring, Environmental Epidemiology, and Short-lived Chemicals (BEES-C)." OPP omitted consideration of Exposure Variability and Misclassification from the BEES-C instrument, which addresses the number of samples (spot vs. many) and reliability testing for this parameter. OPP concludes that exposure assessment methods should be able to provide exposure estimates that are reliable and valid. However, the 2016 Framework does not incorporate any quality recommendation to this effect.

The importance of reliable and valid exposure data was emphasized in the scientific opinion of the EFSA PPR panel. The PPR panel specifically recommended that improvement in the accuracy of exposure measurement is increasingly important.

While OPP recognizes the 2016 Framework is not designed to be a rigid guideline, the document lacks direction for peer reviewers and investigators. Alternatively, an expanded quality rubric that includes additional elements (from Table 2), such as sensitivity analyses to quantify the direction of bias and dose response analyses, would provide a minimum expectation for epidemiology data for risk assessment.

In summary, the 2016 Framework provides commentary on quality elements and considerations. However, the organization of the document and presentation of a quality rubric makes the actual approach unclear as to OPP's quality interpretation of epidemiology studies.

3. Transparency of epidemiology results

Access to full analytical results (i.e. unpublished) from epidemiology studies is a critical difference from the EPA guideline data currently available for risk assessment. There are multiple places in the 2016 Framework that necessitate a conversation about access to data. In Section IIIA, OPP mentions missing data. Section IV discusses statistical analyses and null results. If indeed there are missing data or incomplete analyses, OPP should be recommending a discussion among OPP, registrant(s), and the epidemiology investigators to develop a plan to make the missing data available and/or conduct additional analyses. This is not present in the 2016 Framework. A *post-hoc* review of published studies does not meet the same standard as regulatory data for HHRA.

In the Problem Formulation section, OPP describes how it plans to define the scope of an analysis. OPP continues to point out that a review may be focused on exposure pathways and certain health outcomes. It notes on page 9, "If missing data are critical to the assessment, options are discussed as to how best to obtain that information." At first look, it seems like a good practice. However, it is unclear if these are standard questions posed for all pesticides and risk assessments or if they are raised after reviewing an epidemiology publication. Indeed, the 2016 Framework does not list how to obtain missing data (or if the study results will be used if the data are not available).

The Data Collection section describes how OPP will search and report on published and unpublished sources. On page 10 the 2016 Framework states, "In the case of epidemiology, most studies are expected to be found in the open scientific literature. Although in some cases supplemental analyses or information may be available, dialogue with the researchers may provide additional, important information not published in the original paper in understanding and interpreting epidemiology studies." A comprehensive and systematic review demonstrates best practices. However, there is a notable lack of synchronization of the process of regulatory review of specific pesticides and the conduct and reporting of epidemiology results. In other words, the process to conduct and report epidemiology findings prior to the OPP search should be more systematic.

The section on "Interpretation of null studies" fails to account for what is already known about the risk profile of a registered pesticide. OPP states that lack of associations will be evaluated carefully. The bulk of this section states the opposite. In fact, the 2016 Framework states, "the absence of evidence should not be interpreted as the evidence of absence" (page 35).

The 2016 Framework mentions the effects of publication bias that the published literature disproportionately excludes null findings. Contrary to OPP's view, the lack of human evidence, in the context of vast guideline studies, could be interpreted as evidence of absence. The null data could be interpreted as evidence below the No Observed Effect Level. Furthermore, when relying upon published epidemiology data, the OPP must account for the lack of disclosure of negative data. There is no discussion or guidance how to improve the *status quo*.

In summary, it is well known that null (non-adverse) findings of epidemiology studies are not fully disclosed in the published literature. For both hazard identification and risk assessment, the ability to know and evaluate these results must be developed.

Message

From: Janet Collins [jcollins@croplifeamerica.org]
Sent: 3/2/2018 12:37:57 AM
To: Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]
CC: Courtney DeMarco [cdemarco@croplifeamerica.org]; Keller, Kaitlin [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=d7a6b15adfd745c6ada1c121dec27ac4-Keller, Kai]
Subject: RE: Follow up discussion

Thanks Nancy- we look forward to the discussion.

Janet

Ex. 6

From: Beck, Nancy [mailto:Beck.Nancy@epa.gov]
Sent: Thursday, March 1, 2018 6:55 PM
To: Janet Collins <jcollins@croplifeamerica.org>
Cc: Courtney DeMarco <cdemarco@croplifeamerica.org>; Keller, Kaitlin <keller.kaitlin@epa.gov>
Subject: RE: Follow up discussion

Janet,
On our end it would be best to shoot for the 2nd week of April.
Kaitlin can assist with finding a window when all the right folks are available.

Thanks,
Nancy

Nancy B. Beck, Ph.D., DABT
Deputy Assistant Administrator, OCSPP
P: 202-564-1273
M: 202-731-9910
beck.nancy@epa.gov

From: Janet Collins [mailto:jcollins@croplifeamerica.org]
Sent: Tuesday, February 27, 2018 2:12 PM
To: Beck, Nancy <Beck.Nancy@epa.gov>
Cc: Courtney DeMarco <cdemarco@croplifeamerica.org>
Subject: Follow up discussion
Importance: High

Nancy- would you have about an hour to catch up on the documents we have sent in the next 10 days to two weeks?

Happy to make my schedule work with what might work for you.

Thank you.

Janet

Ex. 6

Message

From: Janet Collins [jcollins@croplifeamerica.org]
Sent: 2/9/2018 3:55:21 PM
To: Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]; Keigwin, Richard [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=151baabb6a2246a3a312f12a706c0a05-Richard P Keigwin Jr]
CC: Jay Vroom [JVroom@croplifeamerica.org]; Mary Jo Tomalewski [mjtomalewski@croplifeamerica.org]; Courtney DeMarco [cdemarco@croplifeamerica.org]; Keller, Kaitlin [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=d7a6b15adfd745c6ada1c121dec27ac4-Keller, Kai]
Subject: RE: updates

Thanks very much Nancy. We look forward to the discussion.

Janet

Ex. 6

From: Beck, Nancy [mailto:Beck.Nancy@epa.gov]
Sent: Friday, February 9, 2018 10:55 AM
To: Janet Collins <jcollins@croplifeamerica.org>; Keigwin, Richard <Keigwin.Richard@epa.gov>
Cc: Jay Vroom <JVroom@croplifeamerica.org>; Mary Jo Tomalewski <mjtomalewski@croplifeamerica.org>; Courtney DeMarco <cdemarco@croplifeamerica.org>; Keller, Kaitlin <keller.kaitlin@epa.gov>
Subject: RE: updates

Janet,
Many thanks for the input.
We are going to have some internal discussions on the topic and then will circle back with your team.

Regards,
Nancy

Nancy B. Beck, Ph.D., DABT
Deputy Assistant Administrator, OCSPP
Ex. 6
beck.nancy@epa.gov

From: Janet Collins [mailto:jcollins@croplifeamerica.org]
Sent: Wednesday, January 31, 2018 5:57 AM
To: Beck, Nancy <Beck.Nancy@epa.gov>; Keigwin, Richard <Keigwin.Richard@epa.gov>
Cc: Jay Vroom <JVroom@croplifeamerica.org>; Mary Jo Tomalewski <mjtomalewski@croplifeamerica.org>; Courtney DeMarco <cdemarco@croplifeamerica.org>
Subject: updates

Dear Nancy and Rick- thank you for the opportunity to discuss various regulatory issues raised by CLA and its members. As a follow up, attached please find a summary of some discussion points as well as a recent CLA comment posted to an EPA docket on pesticide registrations.

We welcome further discussion on these important issues, and others of concern to our members.

My best,

Janet E Collins, Ph.D., R.D.
CropLife America
1156 15th Street, NW; Suite 400
Washington DC 20001

Ex. 6

Message

From: Norman, Caffey [caffey.norman@squirepb.com]
Sent: 3/5/2018 8:44:06 PM
To: Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]
Subject: Presentation at SPB General Counsel Roundtable
Attachments: 2018 Roundtable for General Counsel in the Chemical and Performance Materials Industry; USA Rountable Attendance through 2017.pdf

Dear Nancy,

Thank you for considering participation in a panel discussion at the SPB General Counsel Roundtable April 5-6 here in Washington. Attached is a save-the-date which will be supplemented with materials tailored to you and David Banks, along with a list of attendees in previous years so you can have a sense of the audience.

Please let me know if your schedule will accommodate (late morning on the 6th is preferred but not critical time)

Best regards,

SQUIRE PATTON BOGGS

W. Caffey Norman
Partner
Squire Patton Boggs (US) LLP
2550 M Street, NW
Washington, DC 20037
T +1 202 457 5270
F +1 202 457 6315
M +1 202 460 9495

---
47 Offices in 20 Countries

This message is confidential and may be legally privileged or otherwise protected from disclosure. If you are not the intended recipient, please telephone or email the sender and delete this message and any attachment from your system; you must not copy or disclose the contents of this message or any attachment to any other person.

Squire Patton Boggs (US) LLP is part of the international legal practice Squire Patton Boggs, which operates worldwide through a number of separate legal entities. Please visit www.squirepattonboggs.com for more information.

#US

Message

From: Buller, Carolyn J. [carolyn.buller@squirepb.com]
Sent: 11/28/2017 2:57:26 PM
To: Bernstein, Carol [carol_bernstein@cabotcmp.com]; Berube, Brian A. [brian_berube@cabot-corp.com]; Blanchard, Eric [eablanchard@olin.com]; Bost II, Glenn E. [gbost@ppg.com]; Brown, Pauletta J. [pauletta.brown@lucite.com]; Chemnitz, Gregory R. [Gregory.Chemnitz@materion.com]; Claiborne, Hobie [hobie.claiborne@carpenter.com]; Day, Suzanne F. [suzanne.day@lubrizol.com]; Duesenberg, Mark H. [mark.duesenberg@ferro.com]; Ederington, Ben [bederington@westlake.com]; Edison, Sheri [shedison@bemis.com]; Ellis, Scott [sellis@tatachemicals.com]; Feeney, Kevin [kevin.feeney@airliquide.com]; Feuss, Linda U. [linda.feuss@bayer.com]; Ganz, Peter J. [pganz@ashland.com]; Garceau, Mary [mary.garceau@sherwin.com]; Golden, David A. [dgolden@eastman.com]; Gupta, Ravila [ravila.gupta@am.umicore.com]; Harrelson, Kimberly [kim.harrelson@lonza.com]; Hernandez, Francisco [francisco.hernandez@mexichem.com]; Hill, Timothy J. [hillt@cpchem.com]; Horton, Andrean [andrean.horton@us.aschulman.com]; Isaacson, Mark [mark.isaacson@mosaicco.com]; Johns, Douglas [douglas.johns@hexion.com]; Johnson, Celia [cjohnson@graymont.com]; Kaplan, Jeffrey A. [jeffrey.kaplan@lyb.com]; Keenan, Timothy J. [tim.keenan@hbfuller.com]; Kunkle, Lisa K. [lisa.kunkle@polyone.com]; Lattimore, Rachel G. [rlattimore@croplifeamerica.org]; LeMay, James C. [jim.lemay@omnova.com]; Lepore, Matthew [matt.lepore@basf.com]; Major, Sean [majors@airproducts.com]; Masterson, Thomas [thomas.masterson@siigroup.com]; Matthews, Elizabeth [elizabeth.matthews@total.com]; McCaskill, Randy A. [ramccaskill@tkinet.com]; McGrath, Marlene M. [mmmcgrath@mmm.com]; Mitchell, William C. [bill.mitchell@novachem.com]; Moran, John D. [john.moran@momentive.com]; Muglia, Richard [richard.muglia@tronox.com]; Mulligan, Gregory J. [gregory.mulligan@evonik.com]; Narwold, Karen G. [karen_narwold@albemarle.com]; Nightingale, Alice [anightingale@fpcusa.com]; Nivica Jr., Gjon N. [gjon.nivica.jr@celanese.com]; Podwika, Joseph A. [japodwika@potashcorp.com]; Powell, Gary E. [gary.powell@emeryoleo.com]; Price, Kevin [kprice@methanex.com]; Rachford, Laurie A. [laurie.a.rachford@exxonmobil.com]; Radcliffe, Susan [susan.radcliffe@kemira.com]; Rzepecki, David S. [david.rzepecki@covestro.com]; Saunders, Charles T. [charles.saunders@ineos.com]; Saviano, Anthony T. [anthony.saviano@solvay.com]; Seifert, James [jim.seifert@ecolab.com]; Shastri, Naveena [naveena.shastri@sabic.com]; Shelton, David C. [david.c.shelton@chemours.com]; Simmons, Jay [Jay.Simmons@Kraton.com]; Snively, David F. [david.f.snively@monsanto.com]; Stolle, Russ [russ.stolle@huntsman.com]; Stryker, David M. [david_stryker@huntsman.com]; Sussman, Jason [jason.sussman@lubrizol.com]; Traub, Robert T. [traubr@quakerchem.com]; Tuckett III, Ernest A. [ernest.tuckett@akzonobel.com]; Utecht, Andrea E. [andrea_utecht@fmc.com]; Valente, Michael W [michael.valente@versummaterials.com]; Van Horn, James R. [jim.vanhorn@sunchemical.com]; West, Rudy [rudy.west@newmarket.com]; Williams, David E. [david.e.williams@innospecinc.com]
CC: Ling, Simeon [simeon.ling@squirepb.com]; Smith, Shirley A. [shirley.smith@squirepb.com]
Subject: 2018 Roundtable for General Counsel in the Chemical and Performance Materials Industry

Hi everyone. The 12th Annual Roundtable will be held April 5-6, 2018. More details to follow. Best. C

2018 Roundtable for General Counsel in the Chemical and Performance Materials Industry

Save the Date

April 5-6, 2018

Please save April 5-6, 2018 for our 12th Annual Roundtable for General Counsel in the Chemical and Performance Materials Industry, to be held in Washington DC.

Guided by a steering committee of industry general counsel (noted to the right), the roundtable will bring together leading practitioners and officials in industry and government for this invitation-only event.

There is no cost for attending and CLE credits will be available. Updates regarding the venue and the agenda will follow soon.

Contact for Questions
Shirley A. Smith
+1 216 479 8064

Hope to see you there.

Carolyn J. Buller
Partner and Chair Global Chemicals Practice
Squire Patton Boggs
T +1 212 872 9850
E carolyn.buller@squirepb.com

Steering Committee

Eric A. Blanchard
Vice President, General Counsel and Secretary
Olin Corporation

Peter J. Ganz
Senior Vice President, General Counsel and Secretary
Ashland Global Holdings Inc.

Douglas A. Johns
Executive Vice President, General Counsel and Secretary
Hexion Inc.

Karen G. Narwold
Executive Vice President and Chief Administrative Officer
Albemarle Corporation

Naveena Shastri
Vice President and General Counsel
SABIC

David C. Shelton
Senior Vice President, General Counsel and Corporate Secretary
The Chemours Company

David M. Stryker
Executive Vice President, General Counsel, Chief Compliance Officer and Secretary
Huntsman

Jason R. Sussman
General Counsel
The Lubrizol Corporation

© 2018 | Squire Patton Boggs | All Rights Reserved

We respect your right to privacy – view our policy

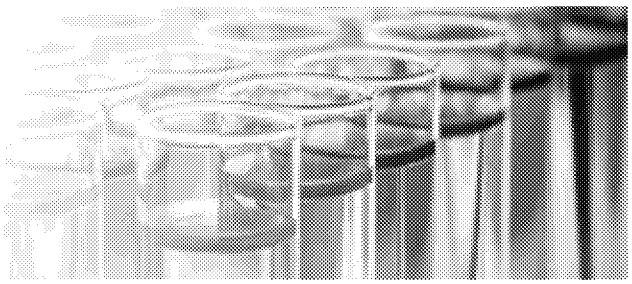
Unsubscribe if you wish to no longer receive these mailings.

Please inform us upon registration if you require any specific adjustments or accommodations to make our event fully accessible to you.

This email was sent by Squire Patton Boggs | 4900 Key Tower, 127 Public Square, Cleveland, OH 44114, USA

Squire Patton Boggs refers to an international legal practice which operates worldwide through a number of separate legal entities. Please visit squirepattonboggs.com for more information.

SQUIRE PATTON BOGGS



NORTH AMERICA
ROUNDTABLE FOR GENERAL COUNSEL IN THE CHEMICAL AND PERFORMANCE MATERIALS INDUSTRY

HISTORICAL ATTENDANCE

Company	Name and Title
Air Liquide USA LLC	Kevin M. Feeney Senior Vice President and Chief Legal Officer - Americas
Air Products and Chemicals, Inc.	John D. Stanley Senior Vice President, General Counsel and Chief Administrative Officer
AkzoNobel	Rogier Roelen, Director, Legal IP and Compliance Steven J. Miller, Senior Vice President and General Counsel, Legal and IP Ernest A. Tuckett III General Counsel Americas
Albemarle Corporation	Karen G. Narwold Executive Vice President, Chief Administrative Officer and General Counsel
A. Schulman, Inc.	Andrean R. Horton Executive Vice President, Chief Legal Officer and Secretary
Ashland Global Holdings Inc.	Peter J. Ganz Senior Vice President, General Counsel and Secretary
BASF Corporation	Matthew Lepore Senior Vice President and General Counsel
Cabot Corporation	Brian A. Berube Vice President and General Counsel
Celanese Corporation	Gjon N. Nivica Jr. Senior Vice President, General Counsel and Corporate Secretary
The Chemours Company	David C. Shelton Senior Vice President – General Counsel & Corporate Secretary

Company	Name and Title
Chevron Phillips Chemical Company	Timothy J. Hill Senior Vice President, Legal and Public Affairs, General Counsel and Secretary
Covestro LLC	Scott G. Brown Vice President, General Counsel, Secretary and Compliance Officer
Cristal USA Inc.	James G. Koutras Director-Senior Corporate Counsel and Secretary
CropLife America	Rachel G. Lattimore Senior Vice President and General Counsel
DuPont	Sharon A. Leyhow Associate General Counsel
Emery Oleochemicals	Gary Powell General Counsel, North America
Evonik Corporation	Gregory J. Mulligan Vice President, General Counsel and Secretary
ExxonMobil Chemical Company	Laurie A. Rachford General Counsel
Ferro Corporation	Mark H. Duesenberg Vice President, General Counsel and Secretary
FMC Corporation	Andrea E. Utecht Executive Vice President, General Counsel and Secretary
Hexion Inc.	Douglas A. Johns Executive Vice President and General Counsel
Huntsman Corporation	David M. Stryker Executive Vice President and General Counsel
INEOS	Charles T. Saunders General Counsel
Kemira Chemicals, Inc.	Susan B. Radcliffe General Counsel – Americas
Kraton Performance Polymers, Inc.	Steven W. Duffy Vice President, General Counsel and Secretary
The Lubrizol Corporation	Suzanne F. Day Senior Vice President and Chief Legal and Ethics Officer Jason R. Sussman General Counsel
Lucite International, Inc.	Pauletta J. Brown General Counsel

Company	Name and Title
LyondellBasell Industries	Jeffrey A. Kaplan Executive Vice President and Chief Legal Officer
Methanex Corporation	Kevin Price General Counsel and Corporate Secretary
Mexichem	Francisco Hernandez Corporate Vice President and General Counsel
Momentive Performance Materials	John D. Moran Senior Vice President and General Counsel
The Mosaic Company	Richard L. Mack Executive Vice President, General Counsel and Secretary
Olin Corporation	Eric A. Blanchard Vice President, General Counsel and Secretary
OMNOVA Solutions Inc.	James C. LeMay Senior Vice President-Corporate Development and General Counsel
PPG Industries, Inc.	Glenn E. Bost II Senior Vice President and General Counsel
Quaker Chemical Corporation	Robert Traub Vice President, General Counsel and Corporate Secretary
SABIC	Naveena Shastri Vice President and General Counsel
SI Group, Inc.	Thomas Masterson Vice President and General Counsel
Total Petrochemicals and Refining USA, Inc.	Elizabeth Matthews Vice President, General Counsel and Corporate Secretary
Umicore USA Inc.	Ravila Gupta Legal Counsel, North America
Univar Inc.	Amy E. Weaver Executive Vice President and General Counsel
Westlake Chemical Corporation	L. Benjamin Ederington Vice President, General Counsel and Secretary

Message

From: Bolen, Derrick [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=1FFC58B0468C4DECA51A8BAD735B7D95-BOLEN, DERR]
Sent: 1/16/2018 2:15:00 PM
To: Jay Vroom [JVroom@croplifeamerica.org]
CC: Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]
Subject: RE: Meeting Invitation and Happy New Year!

Jay-

A working dinner the night of January 25 will work with Nancy's schedule.

Thank you,
Derrick Bolen

From: Beck, Nancy
Sent: Wednesday, January 10, 2018 8:28 PM
To: Jay Vroom <JVroom@croplifeamerica.org>
Cc: Keigwin, Richard <Keigwin.Richard@epa.gov>; Keller, Kaitlin <keller.kaitlin@epa.gov>; Bolen, Derrick <bolen.derrick@epa.gov>
Subject: RE: Meeting Invitation and Happy New Year!

Hi Jay,
Happy New Year to you as well.
Thanks for the invitation. Let us check the calendars and we will get back to you shortly.

Regards,
Nancy

Nancy B. Beck, Ph.D., DABT
Deputy Assistant Administrator, OCSPP
P: 202-564-1273
M: 202-731-9910
beck.nancy@epa.gov

From: Jay Vroom [mailto:JVroom@croplifeamerica.org]
Sent: Wednesday, January 3, 2018 11:42 AM
To: Beck, Nancy <Beck.Nancy@epa.gov>
Cc: Keigwin, Richard <Keigwin.Richard@epa.gov>
Subject: Meeting Invitation and Happy New Year!

Hi Nancy, and happy new year.

I had a good chat with Rick yesterday covering a wide range of topics, including the NMFS "BIOP surprise" of last Friday!!

Rick tells me you're on a ship in the Pacific for a few more days—enjoy the down time.

Our CLA Strategic Oversight Council is meeting January 25-26 here in DC—would like to invite you to meet with the group. Best time would be a working dinner the night of January 25—at the Madison Hotel in downtown DC. Would that work? If not could you stop by to join our meeting the afternoon of the 25th or morning (through lunch) on the 26th?

Thanks,

Jay

Jay Vroom
President & CEO
CropLife America
1156 15th Street, NW
Suite 400
Washington, DC 20005
Ex. 6
Main Number: 202.296.1585
Ex. 6
Email: vroom@croplifeamerica.org
Executive Assistant: Mary Jo Tomalewski Ex. 6 mjtomalewski@croplifeamerica.org)

Message

From: Ray McAllister [RMcAllister@croplifeamerica.org]
Sent: 7/19/2018 1:49:01 PM
To: Bodine, Susan [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=8c2cc6086fcc44c3be6b5d32b262d983-Bodine, Sus]
CC: Starfield, Lawrence [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=8a89d6cd217d4254a5879abecb3f314e-Starfield, Lawrence]; Morris, Jeff [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=55c34872e6ea40cab78be910aec63321-Morris, Jeff]; Wise, Louise [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=cf7be035da4b45a3a7d45c84c9f4b4a3-LWise]; Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]; Keigwin, Richard [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=151baabb6a2246a3a312f12a706c0a05-Richard P Keigwin Jr]; Messina, Edward [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=95521fbf4e34496a879e364faf7e5aa8-Messina, Edward]; Letendre, Daisy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=b691cccca6264ae09df7054c7f1019cb-Letendre, D]; Sharpe, Kristinn [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=f440784f845946cca8b5102d93e8e1d0-Vazquez, Kristinn]; janet collins [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=usera98e8fe5]; Jay Vroom [JVroom@croplifeamerica.org]; Allison Jones (allisonjones@naicc.org) [allisonjones@naicc.org]
Subject: Importance of the GLP Audit and Inspection Program

Ms. Bodine:

On behalf of Crop Life America (CLA) and the National Association of Independent Crop Consultants (NAICC), we want to follow up the CLA visit with you on May 10 with more detail on the importance of the Good Laboratory Practice (GLP) Audit and Inspection program to the crop protection industry. We would welcome the opportunity to continue this conversation. I am taking the liberty of copying other EPA leaders with a stake in this program.

- We are concerned about a loss of vision within the management at the Environmental Protection Agency (EPA) regarding what the GLP program should do and be and accomplish.
- The GLP inspection and audit program is being starved of resources and personnel. In 1994, when the program was under the Office of Prevention, Pesticides, and Toxic Substances (OPPTS), there were 19 inspectors, 6 support staff, and a contractor supporting the GLP program. Currently in the Office of Enforcement and Compliance Assurance (OECA) there are 4 inspectors and no support staff.
- A reasonable frequency of audit and inspection of the individual labs and facilities is necessary to assure EPA of the quality and integrity of the data supporting pesticide product registrations, as required by law, regulation, and international agreement.
- There are some 1400 laboratories, facilities, and field sites in the US participating in GLP research on pesticides. With current staffing of the audit and inspection program, keeping up with that number of facilities seems like an impossible task.
- By comparison, the burden of other GLP audit and inspection programs is more balanced, for example: US-FDA (300 labs, 75 inspectors); Canada (40 labs, 23 inspectors); UK (100 labs, 8 inspectors); Germany (160 labs, 53 inspectors). Many of these inspectors in other programs are part time.
- If inspections are not conducted with sufficient frequency, registrants may feel obligated to take their research to foreign contract research organizations (CROs), leading to loss of business for US laboratories.
- The US is obligated as a member of the Organization for Economic Cooperation and Development (OECD) to comply with requirements of formal OECD Decisions regarding GLP and audits and inspections. This has a direct bearing on the ability of US industry to operate internationally. Among other things, these requirements cover:
    - The nature and frequency of audits and inspections;

    - Providing statements of such audits and inspections to foreign governments in a timely manner.
- Historically, US has had a preeminent role in the development and management of the GLP and Mutual Acceptance of Data (MAD) programs under OECD. In recent years, EPA participation in the OECD GLP Committee and other international forums has been curtailed, resulting in loss of leadership, where the US should be in the forefront. The US should maintain active engagement in moulding and shaping the future direction of MAD.
- Because the EPA does not issue compliance certificates to GLP facilities, the inspection closure letters from EPA are vital in the registration submission process to many other countries, to assure studies have been conducted in a GLP-compliant facility. Lack of the closure letter creates a significant barrier to acceptance of US studies by other countries.
- Registrants experience delays in registrations when they have to obtain a closure letter from the laboratory to send to the monitoring authority in the foreign government. The current practice is to obtain the closure letter in advance to include with the study report in the registration application, and not wait for the monitoring authority to make a request.
- New CROs have a hard time breaking into the business, because of lack of inspections and lack of the ability to be inspected.
- The industry – both registrants and CROs – have a great deal of confidence in and respect for Francis Liem who has led the audit and inspection effort for many years. The Agency must maintain this level of experience and expertise.
- Interaction of audit and inspection staff with industry has been curtailed. We depend on frequent interaction with them in meetings and conferences to keep up to date on the latest developments in GLP.
- The prospect of additional funding authorized by the Pesticide Registration Improvement Act (PRIA) to bolster the GLP program is heartening. It is the clear intent of PRIA legislation that this additional funding supplement, and not replace, current funding from appropriations. It is essential that the new funds set aside for this purpose be spent exclusively on the GLP program.
- In 2016 there was serious consideration of moving the audit and inspection program to the Office of Chemical Safety and Pollution Prevention (OCSPP). We felt then and still feel now that this would be a very positive step for the program.
    - The GLP program began in OPPTS {now known as OCSPP), and was located there until the mid 1990s.
    - The principle purpose of EPA's GLP program is to support the registration decisions made by the Office of Pesticide Programs (OPP) within OCSPP.
    - With such an organizational change, the GLP program could be more responsive to the audit and inspection needs of OPP for specific studies and facilities.
    - Administration of funds from product maintenance fees under PRIA for the GLP program would be simpler and more straightforward in OCSPP, which administers all other PRIA funds.
    - The GLP program does not audit or inspect research performed by OPP, so the organizational connection would not represent a conflict of interest.
    - OCSPP can maintain the appropriate organizational structure to assure independence of the GLP program.
- A robust GLP program in full compliance with the OECD MAD requirements demonstrates to all stakeholders the integrity of industry-supported and generated data that underpin pesticide registrations in the US and around the world. The EPA has a significant responsibility to vigorously defend its Pesticide Programs, and the GLP program should contribute in that regard.

Ray S. McAllister, Ph.D.
Senior Director, Regulatory Policy
CropLife America
202-872-3874 (office)
Ex. 6
ray@croplife.us

Allison Jones

Executive Vice President
National Alliance of Independent Crop Consultants (NAICC)
901.861.0511
AllisonJones@NAICC.org
www.NAICC.org

CC:

Larry Starfield, Principal Deputy Assistant Administrator, OECA
Jeff Morris, Director, OPPT; chief US Head of Delegation to OECD on Chemicals
Nancy Beck, Acting Assistant Administrator, OSCPP
Louise Wise, Deputy Assistant Administrator, OSCPP
Rick Keigwin, Director, OPP
Ed Messina, Acting Deputy Director, OPP
Daisy Letendre, Smart Sectors Program
Kristinn Sharp, Smart Sectors Program

Message

From: Jay Vroom [JVroom@croplifeamerica.org]
Sent: 9/18/2017 6:35:05 PM
To: Keigwin, Richard [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=151baabb6a2246a3a312f12a706c0a05-Richard P Keigwin Jr]; Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]
Subject: FW: Special Invite: 9/19 Food Evolution & SMART Farm Educational Seminar
Attachments: ATT00001.htm; Food Evolution SMART Farm reception.pdf; ATT00002.htm

Dear colleagues at EPA—

Maybe you've heard about the new film "Food Evolution"—and if you have some curiosity I wanted you to know that a screening of the film will be tomorrow late afternoon/evening here in DC. The event is free to the public. The attached flyer explains the details and how to sign up for free.

Feel free share it with your coworkers!

Thanks

Jay

Jay Vroom
President & CEO
CropLife America
Ex. 6
Executive Assistant: Mary Jo Tomalewski (202.872.3849, mjtomalewski@croplifeamerica.org)

From: Katie Foster [mailto:Kfoster@usfraonline.org]
Sent: Monday, September 18, 2017 1:40 PM
To: Jay Vroom <JVroom@croplifeamerica.org>
Cc: Randy Krotz <rkrotz@usfraonline.org>
Subject: Special Invite: 9/19 Food Evolution & SMART Farm Educational Seminar

Hi Jay,

Below is the invitation to tomorrow's Food Evolution & SMART Farm Educational Seminar at The U.S. Capitol. I have also attached the invite for your reference.

Thank you again for your help is sharing this exclusive event with your Directors and staff. Let us know if there is anything else I can provide.

Best,

Katie

5:00 p.m. SMART Farm Reception
6:00 p.m. Food Evolution Documentary
7:30 p.m. Panel Discussion

Food Evolution & SMART Farm Educational Seminar

U.S. Farmers & Ranchers Alliance (USFRA) invites you to an important discussion with farmers, ranchers, agribusiness leaders and the entertainment industry about today's polarized debate marked by fear, distrust and confusion: the controversy surrounding GMOs and our food.

Hear from an esteemed panel of experts about the tools and technologies used in agriculture, which will help inform important decisions on The Hill impacting rural America, including the 2018 Farm Bill. The panelists will discuss ways that agriculture can continually improve in the areas of animal welfare, crop technology and sustainability and ideate ways to inform consumers about how we grow and raise food.

Also, join USFRA for a SMART Farm reception including 360-degree videos showcasing a modern pig farm. In addition, the program includes a showing of the Food Evolution documentary.

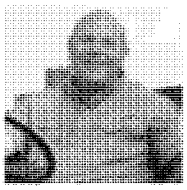
Click the invitation to RSVP or to learn more.
Space is limited.

• U.S. Farmers & Ranchers Alliance •

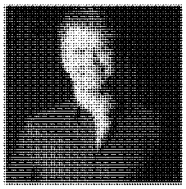
Food Evolution & SMART Farm Educational Seminar

Tuesday, September 19, 2017

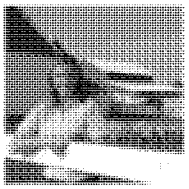
5:00 pm *SMART Farm Reception*
6:00 pm *Food Evolution Documentary*
7:30 pm *Panel Discussion*



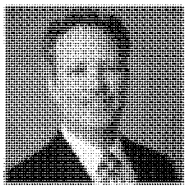
Zippy Duvall
President
American Farm Bureau Federation



Scott Hamilton Kennedy
Food Evolution Director, Producer, Writer



Emily Buck, Ph.D.,
Ohio State University Professor & Crop / Sheep Farmer



Chris Galen
Sr. VP, Communications
National Milk Producers Federation

U.S. Farmers & Ranchers Alliance (USFRA) invites The Hill, USDA, and EPA staff for an important discussion with farmers, ranchers, agribusiness leaders and the entertainment industry about today's polarized debate marked by fear, distrust and confusion, the controversy surrounding GMOs and our food.

Hear from an esteemed panel of experts about the tools and technologies used in agriculture, which will help inform important decisions on The Hill impacting rural America, including the 2018 Farm Bill. The panelists will discuss ways that agriculture can continually improve in the areas of animal welfare, crop technology and sustainability and ideate ways to inform consumers about how we grow and raise food.

Additionally, join USFRA for a SMART Farm reception including 360-degree videos showcasing a modern pig farm. In addition, the program includes a showing of the Food Evolution documentary.

LOCATION:

United States Capitol
Reception: Congressional Atrium
Documentary & Discussion: Congressional Auditorium

Please RSVP: http://www.fooddialogues.com/food-evolution/

If you have any questions, please contact Jennifer Johnson at jjohnson@usfraonline.org or call her at 636-449-5049.

We look forward to bringing everyone together.

Sincerely,

Brad Greenway
USFRA Chairman

Randy P. Krotz
USFRA CEO

U.S. Farmers and Ranchers Alliance,
16020 Swingley Ridge Rd, Ste. 300, Chesterfield, MO 63017

SafeUnsubscribe™ kfoster@usfraonline.org

About our service provider

Sent by rkrotz@usfraonline.org in collaboration with

Constant Contact

Try it free today

<!--[endif]-->**Katie Foster** - Director, Executive Affairs

U.S. Farmers & Ranchers Alliance

kfoster@usfraonline.org O: 636-449-5037 Ex. 6

16020 Swingley Ridge Road, Suite 300, Chesterfield, MO 63017

Learn more about USFRA at www.FoodDialogues.com

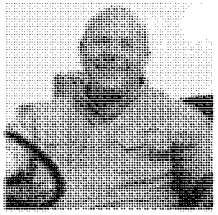
The information contained in this communication, and in any accompanying documents, may constitute confidential or proprietary information belonging to U.S. Farmers & Ranchers Alliance. If you are not the intended recipient of this message, then you may not disclose, print, copy, or disseminate this information, nor take any action in reliance on this information. If you have received this communication in error, please reply and notify the sender (only) and then delete the message.

U.S. Farmers & Ranchers Alliance

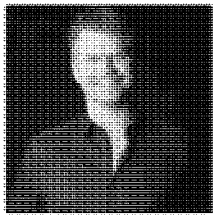
Food Evolution & SMART Farm Educational Seminar

Tuesday, September 19, 2017

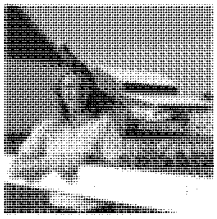
5:00 pm	*SMART Farm Reception*
6:00 pm	*Food Evolution Documentary*
7:30 pm	*Panel Discussion*



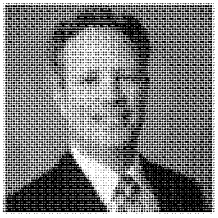
Zippy Duvall
President
American Farm Bureau Federation



Scott Hamilton Kennedy
Food Evolution Director, Producer, Writer



Emily Buck, Ph.D.,
Ohio State University
Professor & Crop / Sheep Farmer



Chris Galen
Sr. VP, Communications
National Milk Producers Federation

U.S. Farmers & Ranchers Alliance (USFRA) invites The Hill, USDA, and EPA staff for an important discussion with farmers, ranchers, agribusiness leaders and the entertainment industry about today's polarized debate marked by fear, distrust and confusion: the controversy surrounding GMOs and our food.

Hear from an esteemed panel of experts about the tools and technologies used in agriculture, which will help inform important decisions on The Hill impacting rural America, including the 2018 Farm Bill. The panelists will discuss ways that agriculture can continually improve in the areas of animal welfare, crop technology and sustainability and ideate ways to inform consumers about how we grow and raise food.

Additionally, join USFRA for a SMART Farm reception including 360-degree videos showcasing a modern pig farm. In addition, the program includes a showing of the Food Evolution documentary.

LOCATION:

United States Capitol
Reception: Congressional Atrium
Documentary & Discussion: Congressional Auditorium

Please RSVP: http://www.fooddialogues.com/food-evolution/

Message

From: Jay Vroom [JVroom@croplifeamerica.org]
Sent: 9/8/2017 11:01:59 PM
To: Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]
Subject: RE: Invitation to CLA Annual Meeting -- September 22-27, 2017

Thanks for considering our annual meeting this year, Nancy—we'll try to plan a bit more ahead with invites to future CLA events. Have a great weekend.

Jay

Jay Vroom
President & CEO
CropLife America

Ex. 6

Executive Assistant: Mary Jo Tomalewski (202.872.3849, mjtomalewski@croplifeamerica.org)

From: Beck, Nancy [mailto:Beck.Nancy@epa.gov]
Sent: Tuesday, September 5, 2017 5:57 PM
To: Jay Vroom <JVroom@croplifeamerica.org>
Subject: RE: Invitation to CLA Annual Meeting -- September 22-27, 2017

Jay,
Thanks for the offer, but I will need to decline this one. Between a trip to NY for the Jewish Holidays and also potential hearing dates for Mike Dourson, its likely best if I plan to stay local that week. Of course my putting this in writing may very well jinx any hope for an actual hearing for the EPA nominees—but you never know.

Please think of me for future meetings.
Regards,
Nancy

Nancy B. Beck, Ph.D., DABT
Deputy Assistant Administrator, OCSPP

Ex. 6

beck.nancy@epa.gov

From: Jay Vroom [mailto:JVroom@croplifeamerica.org]
Sent: Tuesday, August 29, 2017 11:54 AM
To: Beck, Nancy <Beck.Nancy@epa.gov>
Cc: Milhouse, Gloria <Milhouse.Gloria@epa.gov>; Marshall, Venus <Marshall.Venus@epa.gov>; Mary Jo Tomalewski <mjtomalewski@croplifeamerica.org>
Subject: Invitation to CLA Annual Meeting -- September 22-27, 2017

Dear Nancy,

Thanks for the time on the phone last Friday, and for scheduling the time to meet with us on September 8. We are preparing our agenda and team for that meeting, and I'll follow up with more details later.

All the topics we discussed last Friday are vitally important. I'm working on all those currently and we will definitely have a chance to recap all of those and more when we meet on September 8.

In connection with my mention of our CropLife America Government Policy Weekend and Annual Meeting in southern California at the end of September, this letter is to formally invite you to consider attending some or all of our meeting, being held at the Ritz-Carlton Laguna Niguel, in Dana Point, CA. We would make every effort to insure a speaking platform for you, to address and meet with key stakeholders. If your calendar is open and you can justify the trip, we would appreciate your participation.

Attached please find the current program for your review. The Government Policy Weekend begins with a welcome reception Friday night, September 22, and runs through Sunday morning, September 24. Our committees meet on Sunday morning, and then our Board meets at lunch and through the afternoon on Sunday. That evening, the annual meeting program kicks off with a welcome reception. We hold General Sessions on Monday and Tuesday mornings, giving our membership plenty of time in the afternoons to hold networking and business meetings of their own.

The Annual Meeting ends with a gala reception and dinner on Tuesday night, September 26.

I know your schedule is busy, so I wanted to put this in from of you as a possible opportunity for you to have access to the entirety of our membership at this critical time.

Please let me know if you need more information or have any questions. I hope you can join us!

Jay

Jay Vroom
President & CEO
CropLife America
1156 15th Street, NW
Suite 400
Washington, DC 20005

Ex. 6

Fax (202) 466-5832
Email vroom@croplifeamerica.org
Executive Assistant Mary Jo Tomalewski (mjtomalewski@croplifeamerica.org, 202.872.3849 o Ex. 6
Web www.croplifeamerica.org

Message

From: Beau Greenwood [BGreenwood@croplifeamerica.org]
Sent: 10/2/2017 9:52:43 PM
To: Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]
Subject: RE: CLA

Ex. 6

From: Beck, Nancy [mailto:Beck.Nancy@epa.gov]
Sent: Monday, October 2, 2017 5:44 PM
To: Beau Greenwood <BGreenwood@croplifeamerica.org>
Subject: RE: CLA

Beau,
What is your phone number?

Nancy B. Beck, Ph.D., DABT
Deputy Assistant Administrator, OCSPP
Ex. 6
beck.nancy@epa.gov

From: Beau Greenwood [mailto:BGreenwood@croplifeamerica.org]
Sent: Monday, October 2, 2017 4:47 PM
To: Beck, Nancy <Beck.Nancy@epa.gov>
Subject: Re: CLA

Okay. Thanks.

On Oct 2, 2017, at 4:28 PM, Beck, Nancy <Beck.Nancy@epa.gov> wrote:

After 5pm I hope.

Nancy B. Beck, Ph.D., DABT
Deputy Assistant Administrator, OCSPP
Ex. 6
beck.nancy@epa.gov

From: Beau Greenwood [mailto:BGreenwood@croplifeamerica.org]
Sent: Monday, October 2, 2017 4:15 PM
To: Beck, Nancy <Beck.Nancy@epa.gov>
Subject: CLA

Hi Nancy. Are you available to talk?

Beau.

Message

From: Dale Moore [dalem@fb.org]
Sent: 8/31/2017 12:27:03 PM
To: Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]; Jay Vroom [JVroom@croplifeamerica.org]
Subject: Ex. 6 d

Nancy,

Ex. 6

Dale

From: Beck, Nancy [mailto:Beck.Nancy@epa.gov]
Sent: Wednesday, August 30, 2017 9:01 PM
To: Jay Vroom
Cc: Dale Moore
Ex. 6

Thank you Jay.

Nancy B. Beck, Ph.D., DABT
Deputy Assistant Administrator, OCSPP
Ex. 6
Beck.Nancy@epa.gov

On Aug 30, 2017, at 6:18 PM, Jay Vroom <JVroom@croplifeamerica.org> wrote:

Nancy—

Per our conversation last Friday about potential candidates willing and capable and eager to come to EPA to assist with FIFRA and other major agricultural issues—I share the Bio and

Ex. 6

Let me know what other questions in can help address.

Best,

Jay

Jay Vroom
President & CEO
CropLife America
1156 15th Street, NW
Suite 400
Washington, DC 20005
Ex. 6
Vroom@croplifeamerica.org
www.croplifeamerica.org

Ex. 6

Message

From: Bennett, Tate [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=1FA92542F7CA4D01973B18B2F11B9141-BENNETT, EL]
Sent: 10/2/2017 3:13:41 PM
To: Ethan Mathews [emathews@croplifeamerica.org]
CC: Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]; Lyons, Troy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=15e4881c95044ab49c6c35a0f5eef67e-Lyons, Troy]
Subject: RE: PRIA question info

Thanks, we will be in touch with the committee on the best way to handle these questions. Our position is the same as it was the other week.

From: Ethan Mathews [mailto:emathews@croplifeamerica.org]
Sent: Monday, October 2, 2017 10:50 AM
To: Bennett, Tate <Bennett.Tate@epa.gov>
Subject: PRIA question info

Tate –

Below is information on PRIA which we expect to be asked of Dr. Dourson at the hearing on Wednesday 10/4. If you have any questions or need additional info please do not hesitate to reach out.

Ethan

PRIA/OPP Funding Question
The Pesticide Registration Improvement Act (PRIA) was first enacted in 2003 and established a fee schedule for pesticide registration requests. It lists specific decision time periods for EPA to make a regulatory decision on pesticide registration and tolerance actions submitted to the Agency. The goal of PRIA was to create a more predictable and effective evaluation system for affected pesticide decisions and couple the collection of individual fees with specific decision review periods. It also promoted shorter decision review periods for reduced-risk applications.

It has been tremendously successful, providing hundreds of millions of dollars in funding to EPA and providing product developers with clarity on timelines for agency actions and facilitating investment in research and development of new products. Importantly, it also has provided $1 million annually in worker protection and pesticide safety training, funded by industry fees.

PRIA has been reauthorized twice since it was first enacted – in 2007 and 2012 – each time by unanimous consent. It has been supported by large and small manufacturers of agricultural and non-agricultural products, antimicrobial products, biotech companies, and biopesticides, as well as labor and environmental advocates. The current law expired on September 30, 2017. HR 1029, the Pesticide Registration Enhancement Act, which would reauthorize these authorities passed the House on March 20, 2017 and was reported by the Senate Agriculture Committee on June 29, 2017.

What would the impact be to worker protection programs if PRIA is not reauthorized?
Answer:

- The $1 million annually that goes to program funding for worker protection safety and training – largely in cooperation with State Departments of Agriculture and Cooperative Extension Service -- would

cease. Therefore those programs would either have to be funded with other EPA funds (difficult in a time of shrinking budgets), funded by our state partners, or terminated.

What would the impact be to EPA if PRIA is not reauthorized?

Answer:

- The loss of maintenance and registration fees would result in the elimination of 200 full-time-equivalent positions in EPA's Office of Pesticide Programs.
- The authority to collect product maintenance fees expires on 9/30/2017, resulting in an annual loss of resources of $27.8 million. However, EPA's obligation to conduct registration review continues. Without additional resources, it will be impossible for EPA to comply with the 2022 review deadline.
- New registration applications submitted after 9/30/2017 have no completion deadlines. Companies will face tremendous uncertainty about whether to make new R&D investment in new products.

Ethan Mathews
Director of Government Affairs
CropLife America
emathews@croplifeamerica.org

Ex. 6

Message

From: Mary Jo Tomalewski [mjtomalewski@croplifeamerica.org]
Sent: 8/25/2017 2:36:21 PM
To: Milhouse, Gloria [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=0a424462e03c4a82ba83121d59d8b34d-Gmilhous]; Marshall, Venus [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=dbd81a18f6ad447f90b8abbcb90fe9db-Venus Ashton]
CC: Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]
Subject: Meeting with Jay Vroom

Gloria and Venus,

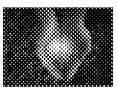
Jay and Nancy spoke this morning, and they agreed to meet on Friday, September 8, since she isn't able to meet with our SOC on September 6 or 7. Nancy said she's available in the morning after 10:00a, so please confirm with me that we can schedule a meeting from 10:00a – 11:00a (preferably 11:30a) in your offices, on Friday, September 8.

Thank you!
MJ

Mary Jo Tomalewski
Executive Assistant to the President & CEO
CropLife America
1156 15th Street, NW
Suite 400
Washington, DC 20005

Ex. 6

Fax (202) 466-5832
Email mjtomalewski@croplifeamerica.org
Web www.croplifeamerica.org



How can I serve you today?

Future Meetings
2017 Spring Regulator Conference – April 6-7, Arlington, VA
2017 Annual Meeting – September 22-27, Dana Point, CA
2018 Winter Board of Directors Meeting – March 5-7, Washington, DC
2018 Annual Meeting – September 21-26, The Ritz-Carlton Amelia Island

Message

From: Jay Vroom [JVroom@croplifeamerica.org]
Sent: 8/30/2017 10:17:19 PM
To: Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]
CC: Dale Moore [dalem@fb.org]

Ex. 6

Nancy—

Per our conversation last Friday about potential candidates willing and capable and eager to come to EPA to assist with FIFRA

Ex. 6

Let me know what other questions in can help address.

Best,

Jay

Jay Vroom
President & CEO
CropLife America
1156 15th Street, NW
Suite 400
Washington, DC 20005
Ex. 6
Vroom@croplifeamerica.org
www.croplifeamerica.org

Message

From: Jay Vroom [JVroom@croplifeamerica.org]
Sent: 8/24/2017 7:25:33 PM
To: Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]
CC: Mary Jo Tomalewski [mjtomalewski@croplifeamerica.org]
Subject: Re: Still like to connect with you by phone

Great-- will do my best to connect in one or both time windows! Thanks!

Sent from my iPhone

> On Aug 24, 2017, at 8:51 PM, Beck, Nancy <Beck.Nancy@epa.gov> wrote:
>
> Jay,
> I hope your trip was fantastic. I should have some availability between 9:30-10 and 11-12 tomorrow.
> One of those windows should work.
> Safe Travels,
> Nancy
>
> _____
> Nancy B. Beck, Ph.D., DABT
> Deputy Assistant Administrator, OCSPP
>
> Ex. 6
> beck.nancy@epa.gov
>
> -----Original Message-----
> From: Jay Vroom [mailto:JVroom@croplifeamerica.org]
> Sent: Thursday, August 24, 2017 2:36 PM
> To: Beck, Nancy <Beck.Nancy@epa.gov>
> Cc: Mary Jo Tomalewski <mjtomalewski@croplifeamerica.org>
> Subject: Still like to connect with you by phone
>
> Nancy
>
> Greetings from South Africa! I'm enjoying my last night here -- start the journey home tomorrow.
>
> You may have noticed a few missed calls from me on Monday August 7-- the day I flew out to Africa and you arrived in California for the specialty crops tour-- sorry we did not connect that day-- I would still like a chance to catch up on a number of issues by phone.
>
> What day/time would be best for you?
>
> 1. Monday or Tuesday or Wednesday next week I'll be in Florida for meetings-- could maybe find a couple time windows?
>
> 2. Tomorrow I have a 3 hour layover in Johannesburg from about 9 am your time until about noon-- would there be a few minutes in there I could call you?
>
> Thanks-
>
> Jay
>
> Sent from my iPhone

Message

From: Mary Jo Tomalewski [mjtomalewski@croplifeamerica.org]
Sent: 8/24/2017 6:52:22 PM
To: Milhouse, Gloria [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=0a424462e03c4a82ba83121d59d8b34d-Gmilhous]; Marshall, Venus [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=dbd81a18f6ad447f90b8abbcb90fe9db-Venus Ashton]
CC: Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]
Subject: RE: Still like to connect with you by phone

Importance: High

Gloria and Venus,

If tomorrow morning doesn't work for a call with Nancy, Jay would also be available next week:

- Tuesday, August 29, before 8:00a or after noon
- Wednesday, August 30, before 8:00a; or between 8:30a and 10:30a

He's supposed to attend the RISE Board meeting on Monday between noon and 5:00p, but he could step out if that's the only time that works for Nancy.

MJ

Mary Jo Tomalewski
Executive Assistant to the President & CEO
CropLife America
Ex. 6
Email mjtomalewski@croplifeamerica.org

How can I serve you today?

Future Meetings
2017 Spring Regulator Conference – April 6-7, Arlington, VA
2017 Annual Meeting – September 22-27, Dana Point, CA
2018 Winter Board of Directors Meeting – March 5-7, Washington, DC
2018 Annual Meeting – September 21-26, The Ritz-Carlton Amelia Island

-----Original Message-----
From: Jay Vroom
Sent: Thursday, August 24, 2017 2:36 PM
To: Nancy Beck (PhD, DABT) <beck.nancy@epa.gov>
Cc: Mary Jo Tomalewski <mjtomalewski@croplifeamerica.org>
Subject: Still like to connect with you by phone

Nancy

Greetings from South Africa! I'm enjoying my last night here -- start the journey home tomorrow.

You may have noticed a few missed calls from me on Monday August 7-- the day I flew out to Africa and you arrived in California for the specialty crops tour-- sorry we did not connect that day-- I would still like a chance to catch up on a number of issues by phone.

What day/time would be best for you?

1. Monday or Tuesday or Wednesday next week I'll be in Florida for meetings-- could maybe find a couple time windows?

2. Tomorrow I have a 3 hour layover in Johannesburg from about 9 am your time until about noon-- would there be a few minutes in there I could call you?

Thanks-

Jay

Sent from my iPhone

Message

From: Jay Vroom [JVroom@croplifeamerica.org]
Sent: 8/29/2017 3:53:52 PM
To: Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]
CC: Milhouse, Gloria [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=0a424462e03c4a82ba83121d59d8b34d-Gmilhous]; Marshall, Venus [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=dbd81a18f6ad447f90b8abbcb90fe9db-Venus Ashton]; Mary Jo Tomalewski [mjtomalewski@croplifeamerica.org]
Subject: Invitation to CLA Annual Meeting -- September 22-27, 2017
Attachments: CLA GPW AM 2017 Agenda 8.23.pdf

Dear Nancy,

Thanks for the time on the phone last Friday, and for scheduling the time to meet with us on September 8. We are preparing our agenda and team for that meeting, and I'll follow up with more details later.

All the topics we discussed last Friday are vitally important. I'm working on all those currently and we will definitely have a chance to recap all of those and more when we meet on September 8.

In connection with my mention of our CropLife America Government Policy Weekend and Annual Meeting in southern California at the end of September, this letter is to formally invite you to consider attending some or all of our meeting, being held at the Ritz-Carlton Laguna Niguel, in Dana Point, CA. We would make every effort to insure a speaking platform for you, to address and meet with key stakeholders. If your calendar is open and you can justify the trip, we would appreciate your participation.

Attached please find the current program for your review. The Government Policy Weekend begins with a welcome reception Friday night, September 22, and runs through Sunday morning, September 24. Our committees meet on Sunday morning, and then our Board meets at lunch and through the afternoon on Sunday. That evening, the annual meeting program kicks off with a welcome reception. We hold General Sessions on Monday and Tuesday mornings, giving our membership plenty of time in the afternoons to hold networking and business meetings of their own.

The Annual Meeting ends with a gala reception and dinner on Tuesday night, September 26.

I know your schedule is busy, so I wanted to put this in from of you as a possible opportunity for you to have access to the entirety of our membership at this critical time.

Please let me know if you need more information or have any questions. I hope you can join us!

Jay

Jay Vroom
President & CEO
CropLife America
1156 15th Street, NW
Suite 400
Washington, DC 20005

Ex. 6

Fax (202) 466-5832
Email vroom@croplifeamerica.org

Executive Assistant Mary Jo Tomalewski (mjtomalewski@croplifeamerica.org
Web www.croplifeamerica.org

Ex. 6

CropLife America
Government Policy Weekend 2017

Government Policy Weekend Agenda

The dress code for our Government Policy weekend is business casual. All evening events are business casual. Our events will be an indoor/outdoor experience so please dress for the cool West Coast climate and bring sunglasses.

The meeting space for our event will be on level two unless otherwise noted and guests may find the pool and fitness center access on this level. Main arrivals, restaurants and valet are located on level three.

Friday, September 22, 2017

3:30 pm – 5:00 pm	Registration	*Ritz-Carlton Foyer*
5:00 pm – 7:00 pm	Welcome Reception *Dress is business casual*	*Monarch Bay Sunset Terrace*

Saturday, September 23, 2017

7:00 am – 8:00 am	Breakfast	*Terrace Salon Balcony*
8:00 am – 9:50 am	Federal Affairs Panel	*Terrace Salon*
10:10 am – 12:00 pm	State & Local Affairs Panel	*Terrace Salon*
6:00 pm – 7:00 pm	Government Policy & Board Reception *Dress is business casual*	*Terrace Salon Balcony (TEN)*
7:00 pm – 9:00 pm	Government Policy & Board Dinner	*Terrace Salon (TEN)*

Sunday, September 24, 2017

7:00 am – 8:00 am	Breakfast	*Terrace Salon Balcony*
8:00 am – 10:00 am	ESA Panel	*Terrace Salon*

Annual Meeting Agenda

The dress code for our Annual Meeting session during the day is business casual. All evening events are business casual except Tuesday. Tuesday evening's gala is taking us "Back to the Future" – so come attired for a festive 50's event. Other evening events will be indoor/outdoor (weather permitting) so please dress for the windy and cooler climates of Laguna Niguel. General Sessions, committee meetings and other receptions are all business casual. Hats, sunscreen and sunglasses are encouraged for outdoor activities, golf and tours.

The meeting space for our event will be focused on level two unless otherwise noted – guests may find the pool, fitness center and the self-service business center on this level. Main arrivals, restaurants and valet at located on level three. Affiliate meetings will be on level two as well as in suites and guest rooms on levels one, two and three, accessible via the guest room elevators or stairs.

Sunday, September 24, 2017

8:00 am – 9:00 am	Compensation Committee	*Salon I*
9:00 am – 11:00 am	Executive Committee	*Salon II*
11:00 am – 11:30 am	Investment Committee	*Salon I*
11:45 am – 1:00 pm	Board Luncheon & Distributor Forum	*Salon IV*
1:00 pm – 5:00 pm	Board of Directors Meeting *Dress is business casual*	*Salon III*
5:00 pm – 7:00 pm	Welcome Reception *Dress is business casual*	*Dana Lawn*

Monday, September 25, 2017

7:00 am – 8:00 am	Breakfast	*Monarch Bay Courtyard*
8:00 am – 10:00 am	General Session *Dress is business casual*	*Salon IV - VII*
11:00 am – 3:30 pm	Whale Watching Off-site Adventure	*Meeting at the Eco-Adventure Center*
11:00 am – 5:00 pm	CLPAC Golf Tournament, Monarch Bay Club *Shotgun start at 12 pm* *Dress is golf attire*	*Shuttles departing from Main Entrance*
5:30 pm – 7:00 pm	Networking Reception *Dress is business casual*	*Monarch Pool*
6:00 pm – 6:30 pm	New & Individual Member Reception	*Monarch Pool*

CropLife America
Annual Meeting 2017

Tuesday, September 26, 2017

7:00 am – 8:00 am	Breakfast	*Monarch Bay Courtyard*
8:00 am – 10:00 am	General Session *Dress is business casual*	*Salon IV - VII*
10:45 am – 4:00 pm	Communications Committee	*Plaza Room*
11:00 am – 2:30 pm	Gardens & Greens Cooking Tour	*Meeting at the Eco-Adventure Center*
11:45 am – 1:00 pm	FFA & AFA Giving Council Luncheon	*Monarch Bay Courtyard*
6:00 pm – 7:00 pm	Gala Reception & Silent Auction	*Ritz-Carlton Ballroom foyer*
7:00 pm – 9:00 pm	Gala Dinner *Festive 50's Back to the Future attire encouraged*	*Ritz-Carlton Ballroom*

Message

From: Jay Vroom [JVroom@croplifeamerica.org]
Sent: 7/10/2017 7:30:48 PM
To: Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]; Greenwalt, Sarah [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=6c13775b8f424e90802669b87b135024-Greenwalt,]
CC: Brittany Benton [BBenton@croplifeamerica.org]
Subject: Invitation to Meet with CropLife America Strategic Oversight Council (SOC)

Dear Nancy and Sarah,

On behalf of CLA's Strategic Oversight Council, I am inviting you to come and meet with SOC at its meeting here in DC in July, specifically around the topic of ESA. The meeting will be held at CLA's offices, at 1156 15th Street, NW, Suite 400.

The meeting starts at 10:00a on Tuesday, July 25, goes through lunch, and ends at 5:00p. We will have a brief cocktail reception at 5:30p, followed by dinner, close by our offices. The meeting will resume on Wednesday morning at 8:00a with breakfast, through lunch and ending at 2:00p.

We would be more than happy to work with your schedule if you are available those dates.

I look forward to hearing from you.

Jay

Jay Vroom
President & CEO
CropLife America
1156 15th Street, NW
Suite 400
Washington, DC 20005
Ex. 6
Fax (202) 466-5832
Email vroom@croplifeamerica.org
Executive Assistant Mary Jo Tomalewski (mjtomalewski@croplifeamerica.org, Ex. 6
Web www.croplifeamerica.org

Message

From: Jay Vroom [JVroom@croplifeamerica.org]
Sent: 8/4/2017 11:24:57 AM
To: Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]
Subject: Re: Just left you a voicemail in your office phone

Nancy

Thanks! The board meeting I am in is to conclude at 3:30 pm today-- I'll call you then!!

Jay

Sent from my iPhone

> On Aug 3, 2017, at 6:12 PM, Beck, Nancy <Beck.Nancy@epa.gov> wrote:
>
> Hi Jay,
> Happy to chat. I've got meetings til about 3pm tomorrow but should be available afterwards.
> Next week I will be doing the Specialty Crop Tours in California all week. I'm told the schedule is pretty packed so I'm not sure what amount of free time I will have. However, you can always try my cell (number below)
> If tomorrow doesn't work, I'll be back in the office on the 14th.
>
> Regards,
> Nancy
>
> _____
> Nancy B. Beck, Ph.D., DABT
> Deputy Assistant Administrator, OCSPP
>
> Ex. 6
>
> beck.nancy@epa.gov
>
> -----Original Message-----
> From: Jay Vroom [mailto:JVroom@croplifeamerica.org]
> Sent: Thursday, August 3, 2017 4:18 PM
> To: Beck, Nancy <Beck.Nancy@epa.gov>
> Subject: Just left you a voicemail in your office phone
>
> Hi Nancy,
>
> Hoping we might have a chat by phone through tomorrow or maybe even in person early next Monday. Let me know if you might have time for a call tomorrow or Monday?
>
> Jay
>
> Jay Vroom
> CropLife America
>
> Ex. 6
>

Message

From: Mary Jo Tomalewski [mjtomalewski@croplifeamerica.org]
Sent: 7/12/2017 5:25:15 PM
To: Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]
CC: Milhouse, Gloria [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=0a424462e03c4a82ba83121d59d8b34d-Gmilhous]; Marshall, Venus [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=dbd81a18f6ad447f90b8abbcb90fe9db-Venus Ashton]
Subject: Timely Meeting Request re Ellis v Housenger

Good afternoon, Nancy,

Jay Vroom has asked me to reach out to you to schedule a meeting soonest (ideally before Tuesday, July 18) regarding *Ellis v Housenger*. On a conference call yesterday, the Government advised that the political meeting between the registrants/CLA and EPA should occur before the July 18 ADR submission if possible and certainly before the July 25 in-person meeting with Judge Corley.

The Court has ordered mediation before the next phase of litigation. There is a settlement meeting with the arbitrator set for July 25th that we are preparing for, to talk through why a settlement of this particular case is in the best interests of EPA and the registrants, and to obtain your support for pursuing a settlement with the plaintiffs.

We understand that you are the key EPA person/decision-maker, and that is why I am reaching out to you today. In keeping with the hopes of scheduling this meeting sooner rather than later, I can offer the following dates and times:

- Thursday, July 13
- Friday, July 14, between 10:00a and noon
- Monday, July 17

The Government suggested it may be helpful to include Rick Keigwin, Marietta Echeverria, Yu-Ting Guilaran, and someone from the Office of the General Counsel, and we'll leave that to your good offices.

If you are unable to identify a time that could work based on the above suggestions, please let me know and I'll come back with other dates before July 25.

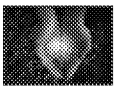
This meeting would include Jay Vroom and our general counsel, Rachel Lattimore, as well as registrant representatives – we estimate 9-12 participants, including Jay and Rachel. In that case, it seems a minimum of 45 minutes would be best, if not an 60 or 90 minutes.

I look forward to hearing from you.
MJ

Mary Jo Tomalewski
Executive Assistant to the President & CEO
CropLife America
1156 15th Street, NW
Suite 400
Washington, DC 20005

Ex. 6

Fax (202) 466-5832
Email mjtomalewski@croplifeamerica.org
Web www.croplifeamerica.org



How can I serve you today?

Future Meetings

2017 Spring Regulator Conference – April 6-7, Arlington, VA
2017 Annual Meeting – September 22-27, Dana Point, CA
2018 Winter Board of Directors Meeting – March 5-7, Washington, DC
2018 Annual Meeting – September 21-26, The Ritz-Carlton Amelia Island

Message

From: Janet Collins [jcollins@croplifeamerica.org]
Sent: 6/5/2017 8:34:03 PM
To: Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]
Subject: RE: Quick question

Flag: Flag for follow up

Perfect…yes.

Ex. 6

Janet

Ex. 6

From: Beck, Nancy [mailto:Beck.Nancy@epa.gov]
Sent: Monday, June 5, 2017 3:06 PM
To: Janet Collins <jcollins@croplifeamerica.org>
Subject: Re: Quick question

5:30?

_____
Nancy B. Beck, Ph.D., DABT
Deputy Assistant Administrator, OCSPP

Ex. 6

Beck.Nancy@epa.gov

On Jun 5, 2017, at 2:53 PM, Janet Collins <jcollins@croplifeamerica.org> wrote:

> Nancy- can you please give me a call for a brief chat about potential for a discussion with our strategic oversight council next week!
>
> Thanks.
>
> Janet E Collins, Ph.D., R.D.
> Executive Vice President, Science and Regulatory Affairs
> CropLife America
> 1156 15th Street, NW; Suite 400
> Washington DC 20001
>
> Ex. 6

Message

From: Janet Collins [jcollins@croplifeamerica.org]
Sent: 6/9/2017 9:27:54 PM
To: Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]
CC: Marshall, Venus [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=dbd81a18f6ad447f90b8abbcb90fe9db-Venus Ashton]
Subject: Re: Meeting w/Crop Life America

Understood. We will make it work.

On Jun 9, 2017, at 4:57 PM, Beck, Nancy <Beck.Nancy@epa.gov> wrote:

Janet,
I have a conflict at 3pm which is why it was set for 2pm. I hope this still works.

Thanks!

Nancy B. Beck, Ph.D., DABT
Deputy Assistant Administrator, OCSPP
Ex. 6
beck.nancy@epa.gov

-----Original Appointment-----
From: Janet Collins [mailto:jcollins@croplifeamerica.org]
Sent: Friday, June 9, 2017 4:17 PM
To: Beck, Nancy
Cc: Marshall, Venus
Subject: Accepted: Meeting w/Crop Life America
When: Wednesday, June 14, 2017 2:00 PM-2:30 PM (UTC-05:00) Eastern Time (US & Canada).
Where: RM 3156 EPA East

Our request had been for a 3:00 meeting which is preferable to our group.
Is it possible to make this 3:00 (I sent a note to Venus Marshall and left a message as well)? If not, we will keep the 2:00.
Many thanks.
Janet

Message

From: Jay Vroom [JVroom@croplifeamerica.org]
Sent: 6/17/2017 11:44:44 AM
To: Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]; janet collins [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=usera98e8fe5]; Bennett, Tate [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=1fa92542f7ca4d01973b18b2f11b9141-Bennett, El]
CC: Mary Jo Tomalewski [mjtomalewski@croplifeamerica.org]; Beau Greenwood [BGreenwood@croplifeamerica.org]; Cindy Baker-Smith (csmith@gowanco.com) [csmith@gowanco.com]; Hunt Shipman [hshipman@cgagroup.com]
Subject: Re: Follow up materials

Nancy and Tate (added on this reply)—

I want to add my thanks for your time and useful conversation. And add two other points:

- PRIA (PREA) Reauthorization— this continued to see additional twists even after our meeting this week and we should stay in close communication as it moves forward. I mentioned the benefit of a good "score" by CBO for the current legislation as an added benefit to the package as introduced. I'll ask, by cc of this to Beau and Hunt Shipman, that our team get you more details about the current CBO score and exchange some further thoughts about any risks from significant change to the language as introduced vis a vis the favorable score.
- ESA—you mentioned planned interagency meetings this coming week on the issue—please keep us in the loop and we will do the same for you.

Hope you're having a great weekend.

Jay

Jay Vroom
President & CEO
CropLife America
1156 15th Street, NW
Suite 400
Washington, DC 20005
Ex. 6
Vroom@croplifeamerica.org
www.croplifeamerica.org

From: Nancy Beck <beck.nancy@epa.gov>
Date: Friday, June 16, 2017 at 1:46 PM
To: Janet Collins <jcollins@croplifeamerica.org>
Cc: Jay Vroom <jvroom@croplifeamerica.org>, Mary Tomalewski <mjtomalewski@croplifeamerica.org>, Beau Greenwood <bgreenwood@croplifeamerica.org>, Cindy Smith <csmith@gowanco.com>
Subject: RE: Follow up materials

Thank you Janet.
It was good to meet everyone and I appreciate you following up with all this information.

Regards,

Nancy

Nancy B. Beck, Ph.D., DABT
Deputy Assistant Administrator, OCSPP
Ex. 6
beck.nancy@epa.gov

From: Janet Collins [mailto:jcollins@croplifeamerica.org]
Sent: Thursday, June 15, 2017 8:16 PM
To: Beck, Nancy <Beck.Nancy@epa.gov>
Cc: Jay Vroom <JVroom@croplifeamerica.org>; Mary Jo Tomalewski <mjtomalewski@croplifeamerica.org>; Beau Greenwood <BGreenwood@croplifeamerica.org>; Cindy Baker-Smith (csmith@gowanco.com) <csmith@gowanco.com>
Subject: Follow up materials

Nancy- thanks very much for taking an appointment yesterday with CropLife America (CLA) CEO and President, Jay Vroom; CLA member, Cindy Baker Smith from Gowan Company; Executive Vice President, Government Relations, Beau Greenwood; and me to discuss concerns our members have regarding EPA/HED use of epidemiological data and a literature review supporting the EPA position, in spite of the fact that the Administrator has questioned the use of epidemiologic study outcomes in human risk assessment. We are concerned that the continual posting of such documents on open dockets, as supporting documents in those dockets, creates a record as to where EPA is acting and regulating with respect to its approach to integration of data sources and weight of evidence in human risk assessment.

Attached please find documents that provide some perspective as to the approach EPA is taking, and CLA objections to such approach:

- <!--[if !supportLists]--><!--[endif]-->CLA 2010 petition to EPA, requesting guidance from EPA on use of epi studies prior to any regulatory use of such studies in human risk assessment;
- <!--[if !supportLists]--><!--[endif]-->EPA response letter, denying the petition, but stating that EPA would put out guidance on the topic for notice and comment which has not occurred;
- <!--[if !supportLists]--><!--[endif]-->CLA 2016 petition requesting EPA not use such epidemiologic studies until EPA developed criteria for use and design of the studies- November 2016, no response to date;
- <!--[if !supportLists]--><!--[endif]-->2015 EPA literature review to support EPA/HED use of epi studies in organophosphate [OP (and by association, other OPs)] human risk assessment;
- <!--[if !supportLists]--><!--[endif]-->2016 EPA literature review updated from 2015, posted to dockets in late May, 2017; and,
- <!--[if !supportLists]--><!--[endif]-->EPA's 2016 Framework (updated from 2010) for integration of epidemiological studies- posted on the EPA website on December 28 2016, with no notice or comment.

After your review of these documents, should you wish to discuss them or ask specific questions, please let me know and we will arrange a time to meet as quickly as convenient for you.

Once again, thanks for the time you spent with us on this important issue.

My best,

Janet E Collins, Ph.D., R.D.
Executive Vice President, Science and Regulatory Affairs
CropLife America
1156 15th Street, NW; Suite 400
Washington DC 20001

Ex. 6

Ex. 6

Message

From: Janet Collins [jcollins@croplifeamerica.org]
Sent: 6/14/2017 5:50:25 PM
To: Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]
CC: Mary Jo Tomalewski [mjtomalewski@croplifeamerica.org]
Subject: Re: EPA contact?

Thank you - it did not come through on my phone. Nearly there now.

> On Jun 14, 2017, at 1:49 PM, Beck, Nancy <Beck.Nancy@epa.gov> wrote:
>
> This should have been in the calendar invite:
>
> William Jefferson Clinton East Building
> 1201 Constitution Avenue NW
> Third Floor, Room 3156
>
> **Please have security contact 202-564-2902 or 202-564-2910 for an escort into the building.
>
>
> _____
> Nancy B. Beck, Ph.D., DABT
> Deputy Assistant Administrator, OCSPP
>
> Ex. 6
>
> beck.nancy@epa.gov
>
> -----Original Message-----
> From: Janet Collins [mailto:jcollins@croplifeamerica.org]
> Sent: Wednesday, June 14, 2017 1:47 PM
> To: Beck, Nancy <Beck.Nancy@epa.gov>
> Cc: Mary Jo Tomalewski <mjtomalewski@croplifeamerica.org>
> Subject: Re: EPA contact?
>
> Which location are your offices?
>
>> On Jun 14, 2017, at 12:23 PM, Beck, Nancy <Beck.Nancy@epa.gov> wrote:
>>
>> Either Venus Marshall or Gloria Milhouse.
>> Please have security contact 202-564-2902 or 202-564-2910 for an escort into the building.
>>
>> _____
>> Nancy B. Beck, Ph.D., DABT
>> Deputy Assistant Administrator, OCSPP
>>
>> Ex. 6
>> beck.nancy@epa.gov
>>
>> -----Original Message-----
>> From: Janet Collins [mailto:jcollins@croplifeamerica.org]
>> Sent: Wednesday, June 14, 2017 12:18 PM
>> To: Beck, Nancy <Beck.Nancy@epa.gov>
>> Cc: Mary Jo Tomalewski <mjtomalewski@croplifeamerica.org>
>> Subject: EPA contact?
>>
>> Who should we have them call at the desk when we arrive?
>>
>> Thanks- looking forward to meeting with you.

Message

From: Janet Collins [jcollins@croplifeamerica.org]
Sent: 6/14/2017 5:48:49 PM
To: Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]
CC: Marshall, Venus [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=dbd81a18f6ad447f90b8abbcb90fe9db-Venus Ashton]; Mary Jo Tomalewski [mjtomalewski@croplifeamerica.org]
Subject: Re: Meeting w/Crop Life America

Thanks- I see the address here.

On Jun 9, 2017, at 4:57 PM, Beck, Nancy <Beck.Nancy@epa.gov> wrote:

Janet,
I have a conflict at 3pm which is why it was set for 2pm. I hope this still works.

Thanks!

Nancy B. Beck, Ph.D., DABT
Deputy Assistant Administrator, OCSPP

Ex. 6

beck.nancy@epa.gov

-----Original Appointment-----
From: Janet Collins [mailto:jcollins@croplifeamerica.org]
Sent: Friday, June 9, 2017 4:17 PM
To: Beck, Nancy
Cc: Marshall, Venus
Subject: Accepted: Meeting w/Crop Life America
When: Wednesday, June 14, 2017 2:00 PM-2:30 PM (UTC-05:00) Eastern Time (US & Canada).
Where: RM 3156 EPA East

Our request had been for a 3:00 meeting which is preferable to our group.
Is it possible to make this 3:00 (I sent a note to Venus Marshall and left a message as well)? If not, we will keep the 2:00.
Many thanks.
Janet

Message

From: Mary Jo Tomalewski [mjtomalewski@croplifeamerica.org]
Sent: 6/14/2017 5:47:54 PM
To: janet collins [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=usera98e8fe5]; Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]
Subject: RE: EPA contact?

Nancy sent a calendar appointment to everyone, with this information:
William Jefferson Clinton East Building
1201Constitution Avenue NW
Third Floor, Room 3156

Mary Jo Tomalewski
Executive Assistant to the President & CEO
CropLife America
Ex. 6
Email mjtomalewski@croplifeamerica.org

How can I serve you today?

Future Meetings
2017 Spring Regulator Conference - April 6-7, Arlington, VA
2017 Annual Meeting - September 22-27, Dana Point, CA
2018 Winter Board of Directors Meeting - March 5-7, Washington, DC
2018 Annual Meeting - September 21-26, The Ritz-Carlton Amelia Island

-----Original Message-----
From: Janet Collins
Sent: Wednesday, June 14, 2017 1:47 PM
To: Beck, Nancy <Beck.Nancy@epa.gov>
Cc: Mary Jo Tomalewski <mjtomalewski@croplifeamerica.org>
Subject: Re: EPA contact?

Which location are your offices?

> On Jun 14, 2017, at 12:23 PM, Beck, Nancy <Beck.Nancy@epa.gov> wrote:
>
> Either Venus Marshall or Gloria Milhouse.
> Please have security contact 202-564-2902 or 202-564-2910 for an escort into the building.
>
> _____
> Nancy B. Beck, Ph.D., DABT
> Deputy Assistant Administrator, OCSPP
>
> Ex. 6
> beck.nancy@epa.gov
>
> -----Original Message-----
> From: Janet Collins [mailto:jcollins@croplifeamerica.org]
> Sent: Wednesday, June 14, 2017 12:18 PM
> To: Beck, Nancy <Beck.Nancy@epa.gov>
> Cc: Mary Jo Tomalewski <mjtomalewski@croplifeamerica.org>
> Subject: EPA contact?
>
> Who should we have them call at the desk when we arrive?
>
> Thanks- looking forward to meeting with you.

Message

From: Janet Collins [jcollins@croplifeamerica.org]
Sent: 6/8/2017 4:35:48 PM
To: Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]
CC: Brittany Benton [BBenton@croplifeamerica.org]; Mary Jo Tomalewski [mjtomalewski@croplifeamerica.org]
Subject: RE: meeting July 14

Nancy- could we bring a small group over at 3:00? Where would we meet if that works?

Janet

Ex. 6

From: Beck, Nancy [mailto:Beck.Nancy@epa.gov]
Sent: Wednesday, June 7, 2017 4:27 PM
To: Janet Collins <jcollins@croplifeamerica.org>
Subject: meeting July 14

Janet,
I could do a 30 minute meeting the afternoon of the 14th.

Keep me posted.
Nancy

****

Nancy B. Beck, Ph.D., DABT
Deputy Assistant Administrator
Office of Chemical Safety and Pollution Prevention

Ex. 6

beck.nancy@epa.gov

Message

From: Janet Collins [jcollins@croplifeamerica.org]
Sent: 6/16/2017 12:15:43 AM
To: Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]
CC: Jay Vroom [JVroom@croplifeamerica.org]; Mary Jo Tomalewski [mjtomalewski@croplifeamerica.org]; Beau Greenwood [BGreenwood@croplifeamerica.org]; Cindy Baker-Smith (csmith@gowanco.com) [csmith@gowanco.com]
Subject: Follow up materials
Attachments: f CLA Petition for rulemaking_epidemiological criteria 122810.pdf; EPA response to epi petition.pdf; FINAL CLA Petition Regulatory Decision Making 11 29 16.pdf; EPA Literature Review Neuro Dev of OPs Sept 15 2015.pdf; 2016 Literature ReviewEPA-HQ-OPP-2008-0316-0073.pdf; 2016 Framework EPA-HQ-OPP-2008-0316-0072.pdf

Flag: Flag for follow up

Nancy- thanks very much for taking an appointment yesterday with CropLife America (CLA) CEO and President, Jay Vroom; CLA member, Cindy Baker Smith from Gowan Company; Executive Vice President, Government Relations, Beau Greenwood; and me to discuss concerns our members have regarding EPA/HED use of epidemiological data and a literature review supporting the EPA position, in spite of the fact that the Administrator has questioned the use of epidemiologic study outcomes in human risk assessment. We are concerned that the continual posting of such documents on open dockets, as supporting documents in those dockets, creates a record as to where EPA is acting and regulating with respect to its approach to integration of data sources and weight of evidence in human risk assessment.

Attached please find documents that provide some perspective as to the approach EPA is taking, and CLA objections to such approach:

- CLA 2010 petition to EPA, requesting guidance from EPA on use of epi studies prior to any regulatory use of such studies in human risk assessment;
- EPA response letter, denying the petition, but stating that EPA would put out guidance on the topic for notice and comment which has not occurred;
- CLA 2016 petition requesting EPA not use such epidemiologic studies until EPA developed criteria for use and design of the studies- November 2016, no response to date;
- 2015 EPA literature review to support EPA/HED use of epi studies in organophosphate [OP (and by association, other OPs)] human risk assessment;
- 2016 EPA literature review updated from 2015, posted to dockets in late May, 2017; and,
- EPA's 2016 Framework (updated from 2010) for integration of epidemiological studies- posted on the EPA website on December 28 2016, with no notice or comment.

After your review of these documents, should you wish to discuss them or ask specific questions, please let me know and we will arrange a time to meet as quickly as convenient for you.

Once again, thanks for the time you spent with us on this important issue.

My best,

Janet E Collins, Ph.D., R.D.
Executive Vice President, Science and Regulatory Affairs
CropLife America
1156 15th Street, NW; Suite 400
Washington DC 20001

Ex. 6

Office of Pesticide Programs' Framework for Incorporating Human Epidemiologic & Incident Data in Risk Assessments for Pesticides

December 28, 2016

Office of Pesticide Programs
US Environmental Protection Agency

ED_002061_00044424-00001

Table of Contents

I. Purpose & Scope **3**

II. Introduction **4**

III. Systematic Review in Pesticide Risk Assessment: Epidemiology **8**

A. Problem Formulation *9*

B. Data Collection *10*

C. Data Evaluation *11*

D. Data Integration: Weight of Evidence (WOE) *12*

E. Overall conclusions, recommendations for risk assessment, statement of areas of confidence and uncertainty *15*

IV. Reviewing Epidemiology Studies for Use in Pesticide Risk Assessment **16**

A. Introduction *16*

B. Types of Epidemiology Studies *18*

C. Evaluating epidemiology studies for use in pesticide risk assessment *21*

1. Exposure Assessment 25
2. Confounding Factors 30
3. Statistical Analysis 33
4. Potential Bias in Observational Research 34
5. Interpretation of Null studies 35
6. External Validity (Generalizability) 36

V. Human Incident Surveillance Data **37**

VI. Summary & Conclusions **43**

VII. References **44**

ED_002061_00044424-00002

I. PURPOSE & SCOPE

The Environmental Protection Agency's (EPA) Office of Pesticide Programs (OPP) is a licensing program regulating pesticides in the U.S under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug, and Cosmetic Act (FFDCA). As part of this program, OPP evaluates a substantial body of toxicology and exposure data to assess the effects of pesticides on human health and the environment. In evaluating human health, EPA looks first for information directly evaluating the potential for effects to people, including epidemiological data. Historically, however, few epidemiology studies have been available to inform the potential toxicity of pesticide chemicals. As such, OPP has in the past primarily relied on toxicology studies in laboratory animals to assess the hazard potential and to estimate human health risk. With the publication of numerous papers from the Agricultural Health Study[1] and from the National Institute of Environmental Health Sciences (NIEHS)/EPA Children's Centers[2], among others, the availability of epidemiology studies conducted on U.S.-relevant exposures to pesticides is increasing. Nevertheless, since the number of pesticides for which quality epidemiology data either exist or are being developed remains relatively low in the near term, experimental laboratory data will likely continue to be the primary source of data for use in quantitative risk assessment for most pesticides.

OPP's goal is to use such information -- when available -- in a scientifically robust and transparent way. To accomplish this, OPP has developed a general epidemiologic framework, as described in this document, that outlines the scientific considerations that OPP will weigh in evaluating how such studies and scientific information can be more fully integrated into risk assessments of pesticide chemicals. The current document is neither a binding regulation nor is it intended to be or serve as a reviewer's guide or manual or as a Standard Operating Procedure for assessing or using epidemiology data. Nor is it intended to be a full treatise on more modern or advanced epidemiological methods or to adequately convey the nuances and complexity that is important for interpreting these types of studies. As such, it does not discuss (or does not discuss in any detail) such important epidemiological topics as causal inference and causal diagrams (Rothman et al., 2012a; Glymor and Greenland, 2012); more recent approaches to confounder identification, assessment, and control; meta-analysis and heterogeneity and its assessment/evaluation (Borenstein et al., 2009; Greenland and O'Rourke, 2012); or sensitivity/quantitative bias analysis for epidemiologic data (Lash et al., 2009; Lash et al,. 2014; Ioannidis, 2008; Greenland and Lash, 2012; Jurek et al., 2007). All these topics, concepts, and issues can and do apply to epidemiology studies concerning pesticides, but are not covered in this OPP framework document. Instead, this document provides overall conceptual considerations concerning the evaluation and use of epidemiology studies on pesticides in

---

[1] https://aghealth.nih.gov/

[2] https://www.epa.gov/research-grants/niehsepa-childrens-environmental-health-and-disease-prevention-research-centers

ED_002061_00044424-00003

the context of human health risk assessments to support OPP's FIFRA and FFDCA activities. An earlier version of this document was reviewed favorably by the FIFRA Scientific Advisory Panel (SAP) in February, 2010 (USEPA, 2010; FIFRA SAP, 2010). This document incorporates improvements recommended by the SAP, public comments, and the experience gained since 2010 conducting assessments on several pesticides for which epidemiological data were available, and should be considered a document that will be updated from time-to-time as we progress and on as-needed basis

II. INTRODUCTION

Two reports by the National Research Council (NRC) of the National Academy of Science (NAS), "Toxicity Testing in the 21st Century: A Vision and A Strategy (2007)" and "Science and Decisions (2009)," together provide new directions in toxicology and risk assessment. These two NRC reports advocate far reaching changes in how toxicity testing is performed, how such data are interpreted, and ultimately how regulatory decisions are made. Specifically, the 2007 report on 21st century toxicity testing advocates a shift away from the current focus of using apical toxicity endpoints to using toxicity pathways[3] to inform toxicity testing, risk assessment, and ultimately decision making. This approach is based on the rapidly evolving scientific understanding of how genes, proteins, and small molecules interact to form molecular pathways that maintain cell function in human cells. The goal for the new toxicity testing paradigm is to determine how exposure to environmental agents can perturb these pathways, thereby causing a cascade of subsequent key events leading to adverse health effects. Human information like that found in epidemiology studies, human incident databases, and biomonitoring studies, along with experimental toxicological information are expected to play a significant role in this new approach. Specifically, these types of human information provide insight into the effects caused by actual chemical exposures in humans and thus can contribute to problem formulation and hazard/risk characterization. In addition, epidemiologic and human incident data can guide additional analyses or data generations (e.g., dose and endpoint selection for use in *in vitro* and targeted *in vivo* experimental studies), identify potentially susceptible populations, identify new health effects, or confirm the existing toxicological observations.

This new vision of toxicity testing and risk assessment will involve data from multiple levels of biological organization ranging from the molecular level up to population-based surveillance with a goal of considering chemical effects from their source to the ultimate health outcome and effects on populations. Such data will come from *in vitro* and *in vivo* experimental studies along with *in silico* and modeled data. OPP's framework for incorporating epidemiology and incident data is conceptually consistent with the 2007 NRC report on 21st century toxicity testing in that both emphasize the use of the best available information from multiple data sources are compiled in a weight of the evidence (WOE) analysis.

---

[3] Toxicity pathways are cellular response pathways that, when sufficiently perturbed, are expected to result in adverse health effects.

ED_002061_00044424-00004

As a general principle, occupational and environmental epidemiology studies are conducted only on widely used pesticides; these pesticides also tend to have to be well-studied in the scientific literature. Thus, OPP expects in many cases where epidemiologic data are available, a significant body of literature data on toxicology, exposure, pharmacokinetics (PK), and mode of action/adverse outcome pathway information (MOA/AOP) may also be available. Human incident data are available on a broader range of chemicals, some of which have robust databases and others which do not. In those situations, where there are significant human incident cases and little is known about the MOA/AOP or PK of a particular pesticide, the WOE analysis can be used to identify areas of new research.

OPP's approach in this framework for incorporating epidemiology and human incident data is not a new or novel approach. Instead, this approach is a reasonable, logical extension of existing tools and methods. This document relies on existing guidance documents and frameworks (Table 1) as the starting point for reviewing and evaluating epidemiology and human incident data for use in pesticide risk assessment. This framework on using epidemiology and incident data in human health risk assessment is consistent with the recommendations of the NRC in its 2009 report on *Science and Decisions*, and with the agency's recent Human Health Risk Assessment Framework (USEPA, 2014a) with respect to emphasizing the use of problem formulation as a tool for scoping, planning, and reviewing available, particularly in the context of risk management needs.

Similarly, OPP's framework is consistent with updates to the World Health Organization/International Programme on Chemical Safety MOA/human relevance framework, which highlights the importance of problem formulation and the need to integrate information at different levels of biological organization (Meek et al., 2014). The MOA/HR framework begins with identifying the series of key events that are along the causal path, that are established on weight of evidence, using principles like those described by Bradford Hill, taking into account factors such as dose-response and temporal concordance, biological plausibility, coherence and consistency (Hill, 1965). Using this analytic approach, epidemiologic findings can be evaluated in the context of other human information (including human incident findings) and experimental studies and for identifying areas of uncertainty and future research. However, it is noteworthy that the availability of a fully elucidated MOA/AOP is a not requirement for using epidemiology studies in human health risk assessment. As the agency continues to move forward in implementing the transformative approach in the 2007 and 2009 NRC reports and as OPP gains experience in integration of epidemiology and human incident information, OPP will re-evaluate and update this framework as appropriate.

ED_002061_00044424-00005

Figure 1. Schematic of the adverse outcome pathway. Adapted from Ankley *et al.* (2010).

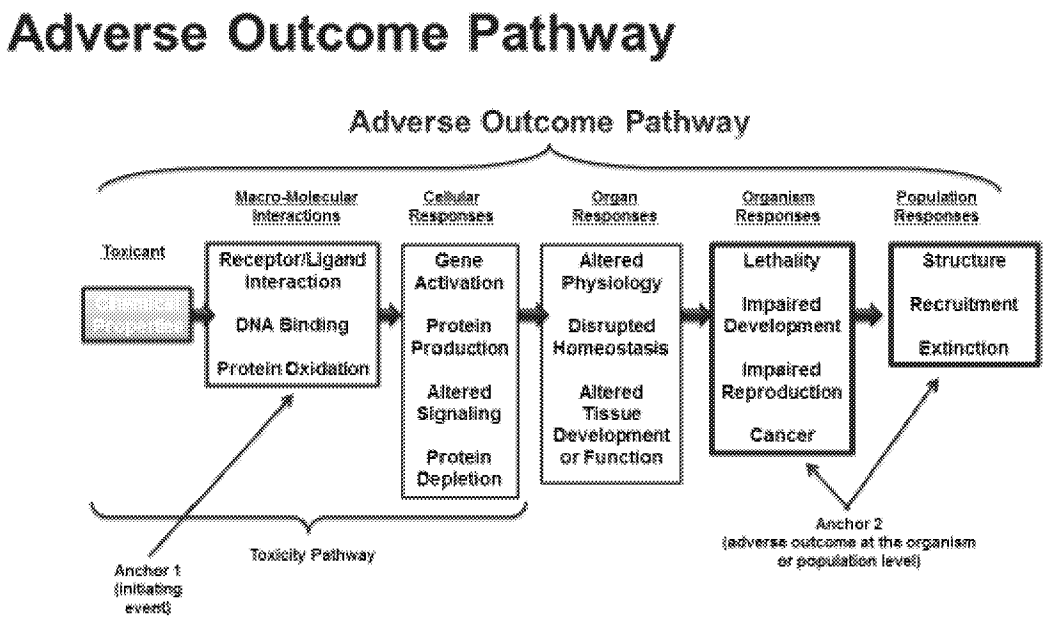


Table 1. Key guidance documents and frameworks used by OPP

NAS	1983: Risk Assessment in the Federal Government: Managing the Process
	1994: Science and Judgment
	2007: Toxicity Testing in the 21st Century
	2009: Science and Decisions: Advancing Risk Assessment
	2011: NAS report on Formaldehyde
	2014: Review of EPA's Integrated Risk Information System (IRIS) Process
WHO/IPCS	2001-2007: Mode of Action/Human Relevance Framework
	2005: Chemical Specific Adjustment Factors (CSAF)
	2014: New developments in the evolution and application of the WHO/IPCS framework on mode of action/species concordance analysis.

ED_002061_00044424-00006

EPA	1991-2005: Risk Assessment Forum Guidances for Risk Assessment (e.g., guidelines for carcinogen, reproductive, developmental, neurotoxicity, ecological, and exposure assessment, guidance for benchmark dose modeling, review of reference dose and reference concentration processes)[4]
	2000: Science Policy Handbook on Risk Characterization
	2006b. Approaches for the Application of Physiologically Based Pharmacokinetic (PBPK) Models and Supporting Data in Risk Assessment
	2014a. Framework for Human Health Risk Assessment to Inform Decision Making.
	2014b. Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation
OPP	2001: Aggregate risk assessment
	2001 and 2002: Cumulative risk assessment
OECD	2013: Organisation for Economic Co-operation and Development Guidance Document On Developing And Assessing Adverse Outcome Pathways

Although there are other sources of human information, the focus of this framework is on interpreting and using ***epidemiology*** and ***human incident data*** in human risk assessment; other sources of human information are not addressed in this document in any depth. Specifically, this document does not extensively discuss research with pesticides involving intentional exposure of human subjects[5] or on studies done to measure dermal or inhalation exposures in agricultural workers as they perform their activities[6,7] .

[4] https://www.epa.gov/osa/products-and-publications-relating-risk-assessment-produced-office-science-advisor

[5] Both the conduct of such research and OPP's reliance on data from such research are governed by EPA's Rule for the Protection of Human Subjects of Research (40 CFR Part 26.) Among other things, these rules forbid research involving intentional exposure of pregnant or nursing women or of children, require prior review of proposals for new research by EPA-OPP and by the Human Studies Review Board (HSRB), and require further review by EPA-OPP and the HSRB of reports of completed research.

[6] In the last several years, OPP has extensively evaluated existing observational studies with agricultural workers in efforts to improve the data and approaches used in worker exposure assessment; those evaluations can be found elsewhere (http://www.epa.gov/scipoly/sap/meetings/2007/010907_mtg.htm)

[7] For additional information on how such worker exposure studies are conducted and used by OPP, see PPP-48 "Pesticides and human Health Risk Assessment: Policies, Processes, and Procedures "available at https://www.extension.purdue.edu/extmedia/PPP/PPP-48.pdf.

ED_002061_00044424-00007

III. SYSTEMATIC REVIEW IN PESTICIDE RISK ASSESSMENT: EPIDEMIOLOGY

In recent years, the NRC 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 (NRC 2011, 2014). The NRC defines systematic review as "a scientific investigation that focuses on a specific question and uses explicit, pre-specified scientific methods to identify, select, assess, and summarize the findings of similar but separate studies" (NRC, 2014). Consistent with NRC's recommendations, the Office of Chemical Safety and Pollution Prevention (OCSPP) employs fit-for-purpose systematic reviews that rely on standard methods for collecting, evaluating and integrating the scientific data supporting our decisions.

According to the NRC, systematic reviews "have several common elements: transparent and explicitly documented methods, consistent and critical evaluation of all relevant literature, application of a standardized approach for grading the strength of evidence, and clear and consistent summative language (NRC, 2014)." In recent years, several groups (Rooney et al., 2014; Woodruff and Sutton, 2014; Hartung, 2010) have published systematic review approaches for use in environmental health sciences. The OCSPP approach to systematic review is consistent with the principles articulated in the Cochrane Handbook for Systematic Reviews of Interventions for evidence-based medicine and with the principles of the Grading of Recommendations Assessment, Development and Evaluation (GRADE). GRADE guidelines used by systematic review approaches for environmental health sciences developed by the National Institute of Environmental Health Sciences (NIEHS) Office of Health Assessment and Translation (OHAT) (Rooney et al., 2014) and University of California, San Diego (Woodruff and Sutton, 2014). According to the *Cochrane Handbook*, the key characteristics of a systematic review are:

- a clearly stated set of objectives with pre-defined eligibility criteria for studies;
- an explicit, reproducible methodology;
- a systematic search that attempts to identify all studies that would meet the eligibility criteria;
- an assessment of the validity of the findings from the identified studies;
- a systematic presentation and synthesis of the characteristics and findings of the included studies.

Each approach mentioned above share common themes and workflow starting with a statement of scientific context (e.g., problem formulation or protocol) followed by literature review with explicit search strategy methods, analysis of study quality (often called risk of bias), evaluation of the quality of the totality of the evidence (e.g., integration) and ultimately leading to a conclusion(s). Each approach recommends transparent and pre-determined criteria for inclusion/exclusion of scientific literature, evaluation of study quality, and reporting of study quality (e.g., high, medium, low). Each approach recommends a pre-stated tool for data integration that provides the foundation for the conclusion(s).

ED_002061_00044424-00008

So far, no single nomenclature has been agreed upon by the risk assessment community for systematic review and OCSPP expects terminology to evolve over time as more broad experience is gained. OCSPP considers its systematic review process and workflow as starting with problem formulation followed by data collection, data evaluation, data integration, and summary findings with critical data gaps identified. Scientific analysis is often iterative in nature as new knowledge is obtained.

A. Problem Formulation

In the NRC report *Science and Decisions-Advancing Risk Assessment,* the National Academy of Sciences (NAS) recommended to EPA that risk assessments and associated scientific analyses be developed to be useful to policy makers; in order to attain this goal, the NRC recommended that the agency more broadly use problem formulation in developing its risk assessments. In response to the NRC, the agency published the Human Health Risk Assessment Framework (USEPA, 2014) which highlights the importance of problem formulation. Problem formulation entails an initial dialogue between scientists and risk managers and provides the regulatory context for the scientific analysis and helps define the scope of an analysis. Problem formulation draws from regulatory, decision-making and policy context of the assessment, informs the technical approach to the assessment and systematically identifies the major factors to be considered. As such, the complexity and scope of each systematic review will vary among the different risk assessment contexts. In other words, an OCSPP systematic review is conducted as "fit-for-purpose" (NRC, 2009) based on the pre-determined scope and purpose determined from problem formulation.

The problem formulation involves consideration of the available information along with key gaps in data or scientific information. OPP uses problem formulation as a tool to identify exposure pathways and potential health outcomes along with the appropriate methods, data sources, and approaches for the scientific analysis. If missing data are critical to the assessment, options are discussed as to how best to obtain that information (e.g., required testing, research). The peer review process is identified and the timeline for completing the assessment is defined.

Systematic review provides a transparent tool for organizing available information and identifying gaps in information for the regulatory purpose for the analysis. As such, in problem formulation, the regulatory context of a scientific analysis is described which in turn defines the scope of and purpose for collection and evaluation of scientific literature. Some considerations in problem formulation may be related to population or life-stage, exposure pathways (e.g., route, duration, frequency), and/or health outcomes of interest identified from *in vitro* or *in vivo* laboratory studies along with epidemiology or human incident studies along with resources available and regulatory timeframe. In the context of considering epidemiology and human incident information, an initial evaluation of the study quality, study design, and uncertainties are considered.

ED_002061_00044424-00009

Key scientific issues related to hazard assessment considered in problem formulation include: What are the effects associated with exposure? What are the MOA/AOPs associated with these effects? What are the temporal aspects of the effects? Are there susceptible populations and if so, who are they and what factors contribute to susceptibility? Are there differences in PK or pharmacodynamics (PD) between laboratory animals and humans? Exposure information is also evaluated in problem formulation. Key scientific issues related to exposure assessment considered in problem formulation include: How is the pesticide used? What are all of the relevant use sites of exposure? To what chemical substances will people be exposed? What are the routes, durations, and frequencies of exposures? Who may be exposed? Does the exposure pose different risks to different groups (e.g., due age or activity patterns?) In the specific case of epidemiology data, this review considers a variety of factors including, but not limited to, research hypothesis, study design (i.e., sample size, sufficient controls, quality of measurements, etc.), exposure dose/concentration, statistical analysis, and conclusions.

B. Data Collection

The data collection phase of systematic review is the collection of available information from various published and unpublished sources, such as the open scientific literature and submitted studies for pesticide registration. OPP reviews data collected under the Organisation for Economic Cooperation and Development (OECD) test guidelines, OCSPP harmonized test guidelines, and other pesticide (OPP guidelines). These guideline studies are collected primarily from in-house databases of submitted studies and are found through searches of such internal databases.

In the case of epidemiology, most studies are expected to be found in the open scientific literature. Although in some cases supplemental analyses or information may be available, dialogue with the researchers may provide additional, important information not published in the original paper in understanding and interpreting epidemiology studies. The sources of human incident information are summarized in Section IV.

Open literature search strategies use specified criteria to retrieve health effects information from the open scientific literature and unpublished sources. After identifying and selecting the most appropriate sources/databases and determining the most resource effective strategy utilizing classification codes, medical subject headings, and/or keywords, a search is conducted of the literature. Depending on the complexity of the scientific evaluation, support from a reference librarian may or may not be needed. The goal of a human health literature search is to perform a reliable and reproducible literature search by providing proper documentation of the literature search process. The following steps are conducted to retrieve relevant studies:

- The purpose of the scientific analysis and inclusion criteria are established.
- Combinations of terms/key words and/or MeSH (Medical Subject Heading) terms and their Boolean combinations (AND; OR; NOT) are used and documented.

Advanced Search and Field Search by author, title, keywords or subject heading may also be performed as needed. Knowledge of database structure, and using a separate search strategy for a specific database is helpful in retrieving relevant studies. In addition to an initial comprehensive search, periodic searches may be conducted to update the literature list.

- The search strategy is documented, including the date(s) of the search(es)to ensure that all the searches of all the databases are reproducible.
- Reference lists of retrieved articles are examined[2] for additional background and to look for articles that were not discovered in the initial search.
- After combining the retrieved articles from different databases and removing duplicates, the available titles and abstracts are screened. For some of the articles where relevance could not be determined from the title and the abstract, the article is retrieved for further review.
- Following the initial screening, articles that were not relevant (exclusion criteria) – such as opinion articles, studies not in English, and those consisting only of abstracts are excluded. Additional exclusion criteria can be identified on a case by case basis. All exclusion criteria are documented. The rest of the articles, even those that found no adverse health effects, are included for review and evaluation.

C. Data Evaluation

In the data evaluation phase, data quality is reviewed and conclusions are made about the utility of such data. Study quality reflects the overall confidence that reports findings are correct (Balshem et al., 2011). As such, study quality can include:

- reporting quality (how well or completely a study is reported);
- how credible the findings are based on the design and conduct of the study;
- and how well the study addresses the topic under review (Rooney et al., 2014).

Study quality is first considered on an individual study basis, and the quality is judged. For example, one may have stronger confidence in a well conducted case control study than a poorly conducted cohort study. Credibility of the scientific findings, often called risk of bias, is evaluated using pre-determined criteria for specific domains related to study design and conduct (See Table 2).

OPP initially developed a guidance on using the open scientific literature considerations called the "Guidance for Considering and Using Open Literature Toxicity Studies to Support Human Health Risk Assessment" (USEPA, 2012) and generally continues to follow this guidance. However, with the acceleration of systematic review in risk assessment, some aspects of the literature guidance may need updating in the future.

ED_002061_00044424-00011

Conclusions about the quality of the data are made and can be described in conclusion statements or categories (e.g., acceptable/not acceptable; low, medium, high).

Specific considerations used in evaluating epidemiology studies on pesticide chemicals are provided in Section III.C below. As part of the data review, a concise written review of the study is developed. This written review describes the study design, results, conclusions, and the strengths and weaknesses of the study. The quality of the epidemiologic exposure assessment is an important factor in determining what role epidemiologic data will play in the risk assessment. As such, it is important to fully characterize the assumptions used in the epidemiologic exposure assessment and the degree to which these assumptions affect the interpretation and generalizability of the epidemiologic findings. The evaluation of the epidemiologic exposure assessment may include a consideration of past and present exposure patterns (e.g., exposed populations, pathways, routes, and levels of exposure) and may include significant changes in use patterns (e.g., risk mitigation actions or new use patterns). With regard to evaluating meta-analyses, reporting guidelines for Meta-analysis Of Observational Studies in Epidemiology (MOOSE) have been developed by Stroup et al., (2000) that are useful in evaluating the quality and interpreting meta-analysis.

D. Data Integration: Weight of Evidence (WOE)

OPP's human health characterizations involve the consideration of all available and relevant data, including but not limited to human studies/epidemiology, biomonitoring data, *in vitro* and *in vivo* experimental laboratory toxicological studies, MOA/AOP information, pharmacokinetic studies, and structure-activity relationships (SAR). Once the different types of hazard data are collected and a full evaluation of each relevant study is conducted and documented, the next step is to integrate multiple lines of evidence.

Data integration is based on the principle of reaching a judgment of the totality of the available negative and positive data for relevant hazards. OPP uses a WOE analysis for evaluating epidemiology and human incident data, such conclusions are made on the preponderance of the information rather than relying on any one study. OPP uses the modified Bradford Hill criteria like those in the MOA/human relevance framework as a tool for organizing and integrating information from different sources (Hill, 1965; U.S. EPA, 1999, 2005; Sonich-Mullin et al., 2001; Meek et al., 2003; Seed et al., 2005; OECD AOP Wiki Users Handbook[8]). It is important to note that the Hill Criteria are not intended as a check box approach but instead are points to consider when evaluating the totality of evidence. In addition, the availability of a fully elucidated MOA/AOP is a not requirement for using epidemiology studies in human health risk assessment. However, even in the absence of a fully developed MOA/AOP, collection and evaluation of mechanistic data may provide support for biological plausibility and help explain differences in tissue sensitivity, species, gender, life-stage, or other factor. The MOA/human relevance framework is a flexible tool which provides a foundation for organizing information without rigidity. It is this

[8] https://aopwiki.org/wiki/index.php/Main_Page#OECD_User_Handbook

flexibility that makes it a useful tool for a variety of purposes such as evaluating causality, integrating information across multiple lines of scientific evidence, and identifying data gaps and areas of future research. In this analysis, epidemiologic findings and human incident data can be evaluated in the context of other human information and experimental studies to evaluate biological plausibility, to identify areas of uncertainty and areas of further research. To describe how Bradford Hill aspects are considered in the WOE evaluations, OPP has used some definitions of terms as outlined in EPA's Preamble to the Integrated Science Assessments (ISAs) which serve as a scientific foundation for the review of EPA's National Ambient Air Quality Standards (NAAQS). (USEPA, 2015).

- **Key events.** In cases where the MOA/AOP are established for a particular health outcome, a clear description of each of the key events (i.e., measurable parameters) that underlie the MOA/AOP are given. Data to inform the key events may come from a combination of *in vitro* or *in vivo* data sources (human or animal). These key events can be a combination of PK and PD events. However, it noteworthy that the availability of a fully elucidated MOA/AOP is a not requirement for using epidemiology studies in human health risk assessment.

- **Biological Gradient/Exposure-Response/Dose-Response Concordance & Relationships**. The Preamble to the ISAs notes that "In the context of epidemiology, a well-characterized exposure-response relationship (e.g., increasing effects associated with greater exposure) strongly suggests cause and effect, especially when such relationships are also observed for duration of exposure (e.g., increasing effects observed following longer exposure times) (USEPA, 2015)." When the MOA/AOP is known, dose-response relationships are identified for each key event. Dose-response relationships are compared among key events. In some cases, the earlier key events may be more sensitive than later key events. In other cases, key events may share similar dose-response curves.

- **Temporal association.** Evidence of a temporal sequence between the introduction of an agent and appearance of the effect constitutes another argument in favor of causality (USEPA, 2015). The Preamble to the ISAs notes that "Strong evidence for causality can be provided through 'natural experiments' when a change in exposure is found to result in a change in occurrence or frequency of health."

This analysis considers key events which occur rapidly (e.g., metabolism to an active metabolite which could occur within minutes of exposure) and those which occur after longer durations (e.g., development of a tumor) to ensure coherence of the effects. Specific to considering epidemiology data, the temporal relationship between the exposure and health outcome may be considered.

- **Strength, consistency, and specificity**.

Consistency: An inference of causality is strengthened when a pattern of elevated risks is observed across several independent studies. The reproducibility of findings constitutes one of the strongest arguments for causality. Statistical significance is not the sole criterion by which the presence or absence of an effect is determined. If there are discordant results among investigations, possible reasons such as differences in exposure, confounding factors, and the power of the study are considered (USEPA, 2015).

Consistency of findings across studies is informed by the repeated observation of effects or associations across multiple independent studies. Further support is provided by reproducibility of findings in different populations under different circumstances. However, discordant results among independent investigations may be explained by differences in study methods, random errors, exposure, confounding factors, or study power, and thus may not be used to rule out a causal connection (USEPA, 2015).

Strength of the observed association: The finding of large, precise risks increases confidence that the association is not likely due to chance, bias, or other factors. However, it is noted that a small magnitude in an effect estimate may or may not represent a substantial effect in a population (USEPA, 2015).

Specificity of the observed association: Evidence linking a specific outcome to an exposure can provide a strong argument for causation. However, it must be recognized that rarely, if ever, do environmental exposures invariably predict the occurrence of an outcome, and that a given outcome may have multiple causes (USEPA, 2015).

- **Biological plausibility and coherence**.

Coherence: An inference of causality from one line of evidence (e.g., epidemiologiccontrolled human exposure, animal, or ecological studies) may be strengthened by other lines of evidence that support a cause-and-effect interpretation of the association. There may be coherence in demonstrating effects from evidence across various fields and/or across multiple study designs or related health endpoints within one scientific line of evidence (USEPA, 2015).

When animal and human data show a similar toxic profile, both quantitatively and qualitatively, there is high confidence in the human health risk assessment. Whereas in other cases, animal and human data may show a qualitatively similar toxic profile but quantitative differences are observed. For example, a particular chemical exhibits the same MOA/AOP in animals and humans but there may be species differences in dose-response characteristics. These dose-response differences could be due to tissue dosimetry (i.e., PK) or from different response characteristics (i.e., PD). In contrast, animal and human data can, in some instances, show qualitatively dissimilar outcomes. This situation highlights the need to fully and objectively evaluate all available information in a

ED_002061_00044424-00014

transparent and comprehensive manner to consider factors such as species, gender, and life-stage differences and potential susceptibilities along with study design considers and exposure potential.

Biological plausibility: An inference of causality is strengthened by results from experimental studies or other sources demonstrating biologically plausible mechanisms. A proposed mechanism, which is based on experimental evidence and which links exposure to an agent to a given effect, is an important source of support for causality (USEPA, 2015).

Similarly, information on MOA/AOP for a chemical, as one of many structural analogs, can inform decisions regarding likely causality. Structure activity relationships and information on the agent's structural analogs can provide insight into whether an association is causal (USEPA, 2015).

EPA's Cancer Guidelines (2005) 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]."

However, The Cancer Guidelines further state that "*lack of mechanistic data, however, is not a reason to reject causality* [p. 41]." As such, lack of established MOA/AOP is not necessary knowledge when using epidemiology data and epidemiology associations may still be valid even in the absence of an established MOA/AOP and may also provide insight into potential MOA/AOP.

- **Uncertainties.** Uncertainties are discussed in the WOE transparently and objectively.

E. Overall conclusions, recommendations for risk assessment, statement of areas of confidence and uncertainty

It is important to document a summary of the evidence, the procedures or methods used to weigh the evidence, the basis for the WOE conclusion or recommendation, any uncertainties and areas for further research. Recommendations are made on the role of the epidemiologic or human incident data in the risk assessment. Generally, OPP does not use human incident information for quantitative risk assessment but instead to inform risk assessment/risk management activities such as indicating a potential need for a new risk assessment or new risk management measures, evaluating the success of risk mitigation actions after they are implemented, and targeting possible enforcement activities. In

contrast to more limited role of human incident data, epidemiology studies have the potential to help inform multiple components of the risk assessment in a variety of ways. High quality studies with robust exposure assessment may be used to estimate a risk metric quantitatively. Alternatively, outcomes reported in epidemiologic studies may be compared qualitatively with those seen in *in vitro* and animal studies to evaluate the human relevance of animal findings (Hertz-Picciotto, 1995) and may be useful in assessing the biological plausibility of epidemiologic outcomes. In the final portion of the proposed WOE analysis, the overall conclusions along with statement of areas of confidence and uncertainty. This section also identifies areas of additional research. This section recommends the source of data for regulatory values and the appropriate approach for extrapolating between species (if necessary) and among humans.

IV. REVIEWING EPIDEMIOLOGY STUDIES FOR USE IN PESTICIDE RISK ASSESSMENT

A. Introduction

Epidemiology is a science that seeks to identify and evaluate relationships between exposure to chemical, physical or biological agents, and the health status of populations (Boyes et al., 2007). It has been defined as the "study of how disease is distributed in populations and the factors that influence or determine this distribution" (Gordis, 2009). More broadly, it is considered as "the study of the occurrence and distribution of health-related events, states, and processes in specified populations, including the study of the determinants influencing such processes and the application of this knowledge to control of relevant health problems" (Porta, 2014). The objective of much epidemiologic research is to obtain a valid and precise estimate of the effect of a potential cause on the occurrence of disease. A key objective of epidemiology, like other sciences, is determining cause and effect or - said differently - of identifying the etiology of a disease or health outcome and the risk factors with which it might be associated. Calderon (2000) described four major uses of such studies: 1) describe the health status of a population and discover important time trends in disease and exposure frequency; 2) explain the occurrence of diseases by identifying factors that are associated with specific diseases or trends; 3) predict the number of disease occurrences and the distribution of health states in specific populations; and 4) improving the health status of the population by identifying factors that affect environmental or human health. In the case of pesticides, epidemiology focuses on the relation between exposure and adverse health effects in the general population and in specific sub-populations, such as occupationally exposed workers or applicators.

Epidemiology studies have the potential to help inform multiple components of the risk assessment in a variety of ways. High quality studies with robust exposure assessment may be used to quantitatively estimate risk or an appropriate risk surrogate such as an odds ratio or risk ratio. However, many epidemiology studies that deal with pesticides and pesticide exposure suffer some limitations in size, scope, exposure assessment, or data analysis which prevent or otherwise impede their full use in quantitative risk assessment

ED_002061_00044424-00016

(Ntzani et al., 2013). Pesticide use in the US has changed significantly over the last few decades. As the use changes, so does the exposure to workers. Changes in pesticide use have occurred due to risk mitigation actions by EPA, resistance management activities, introduction of new chemistries, and increased use of genetically modified crops. These significant changes in exposure have to be taken into account when interpreting epidemiology studies and, ultimately, the decision to use such studies in quantitative risk assessment. Even so, epidemiology studies may be used to compare with evidence from experimental animal studies to characterize assumptions used in deriving such values. In other cases, outcomes reported in epidemiologic studies may be compared qualitatively with those seen in *in vitro* and laboratory animal studies to evaluate biological plausibility or human relevance of animal findings (Hertz-Picciotto, 1995). Human information like that found in epidemiology studies are expected to potentially play a significant role in the new vision of toxicity testing recommended by the NRC (2007). Specifically, epidemiology studies can provide insight on health outcomes that may arise from real-world chemical exposures in humans and thus can contribute to problem formulation and hazard/risk characterization. Human information may guide additional studies (e.g., dose and endpoint selection for use in *in vitro* and targeted *in vivo* experimental studies); and identify novel health effects or host susceptibilities which can be investigated with future research.

When laboratory data from animal studies provide the primary source of information for hazard characterization, one potential source of uncertainty is the relevance of animal models to humans. In the absence of data to support the contrary, animal findings are assumed to be relevant to humans. Furthermore, EPA assumes that humans are more sensitive than laboratory animals in the absence of data to support the contrary. In actuality, humans may be more or less sensitive to pesticides than other animal species. Epidemiology and human incident data can provide scientific information and support to inform uncertainties associated with species extrapolation. With respect to population variability, epidemiology studies better characterize potential variability than do animal studies. Specifically, epidemiologic data include the genetic diversity, and variability inherent in human populations and thus can better account for and represent actual population response to environmental chemicals than laboratory animals (Calderon, 2000).

With respect to dose-response characterization, animal toxicology studies have the benefit that studies can be designed to cover a broad range of exposure levels. However, animal toxicology studies generally use exposures which are much larger (sometimes orders of magnitude) than those that occur in the environment. These high exposure levels in animal studies dictate the need for extrapolation from high to low doses. This extrapolation introduces added uncertainty into the risk assessment. Epidemiology studies and human incident data involve actual real-world exposures and thus high dose extrapolation may in many cases not be needed. Epidemiology studies conducted over a range of exposures (from low to high) are most useful.

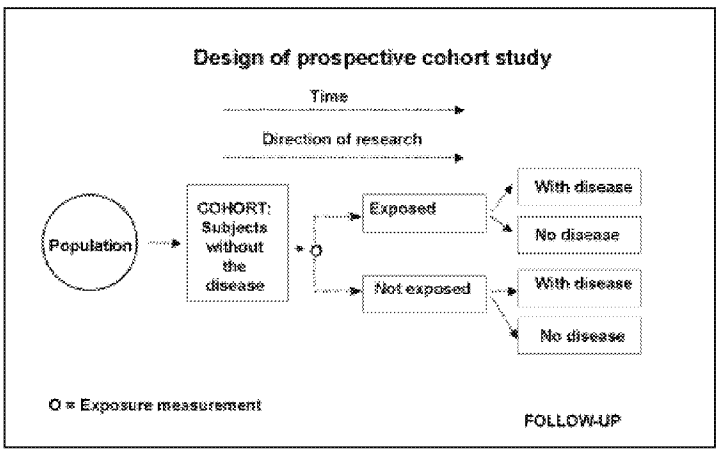
Animal studies do not replicate the length, magnitude, duration, routes of exposure and variability in exposure experienced by humans (Calderon, 2000). Human exposure often occurs through multimedia exposure pathways, including food, water, air, and indoor and outdoor environments. In contrast, controlled laboratory studies typically use a single

route of exposure. In addition, humans may experience exposure to multiple chemicals and/or non-chemical stressors simultaneously, whereas most animal studies involve a single chemical stressor. On one hand, this multi-chemical exposure in epidemiology studies can provide a challenge when attempting to attribute epidemiologic outcomes to a single pesticide chemical. On the other hand, epidemiologic research considers real-world exposures and may help, when considered along with experimental approaches, address questions associated with multiple chemical exposures which can be difficult to evaluate in an experimental setting.

B. Types of Epidemiology Studies

The major types of observational epidemiologic studies are described briefly below with consideration of their strengths and weaknesses (Lilienfeld and Lilienfeld, 1979; Mausner and Kramer, 1985; Kelsey et al., 1996; Rothman and Greenland, 2012; Paddle and Harrington, 2000; USEPA, 2005; Purdue Pesticide Programs, PPP-43).

Cohort studies begin with a group of people that share common characteristics—the cohort—and evaluate their health over an extended follow-up time period during which the occurrence of disease is recorded (see figure box from van den Brandt et al. (2002)). The common characteristic is often the presence vs. absence of "risk factors" (such as exposures)[9]. In such studies, differences in disease occurrence between the "exposed" and "non-exposed" individuals are identified and studied over time to determine differences in the rate of disease[10]. This difference in the rate of disease occurrence is then investigated to determine if the rate of disease differs between the exposed and non-exposed groups. Cohort studies have the ability to simultaneously evaluate multiple disease outcomes under study (which is not true for case-control studies, which are generally limited to evaluating only a single (pre-specified) disease outcome, discussed below). Cohort studies can also be performed either prospectively, like the Agricultural Health Study (AHS, http://aghealth.nci.nih.gov/), or retrospectively from historical records. A prospective cohort design focuses on a group of people from a current point in time through a future point in time. A retrospective cohort design focuses on a group exposed at some point in the past, and compares disease rates after exposure occurred (generally through existing



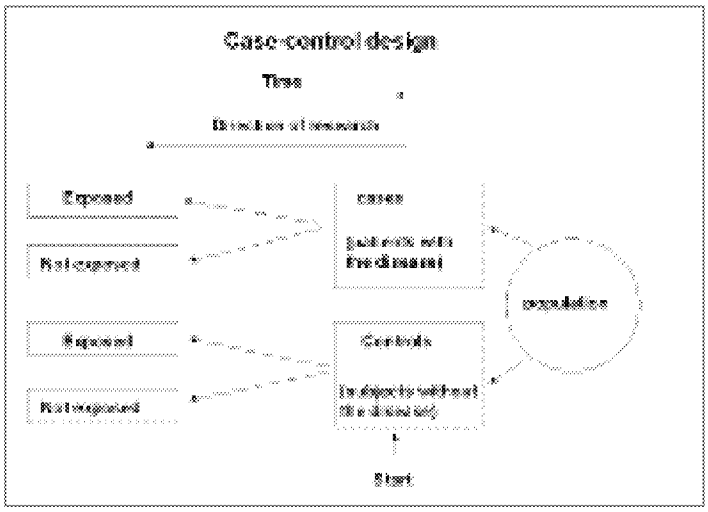
[9] While exposure is often dichotomized on an exposed vs. non-exposed basis in cohort studies, exposure can also be measured on a quantitative scale (e.g., by a continuous measure or by quantiles)

[10] Cohort studies commonly study differences in rates of disease, but these can also include other focal outcomes of interest such as birth weight, mental abilities, blood pressure, etc.

ED_002061_00044424-00018

available exposure databases (or records) available on a person-by-person (individual) basis). Prospective cohort studies can be relatively lengthy and expensive to conduct, particularly for rare diseases, and require a large number of subjects to be under study. Importantly, significant resources and professional staff are required for a long period of time to collect high quality data.

Case-control studies are studies in which groups of individuals with (cases) and generally without (controls) a given disease are identified and compared with respect to (generally past[11]) exposure to determine whether those with the disease of interest are more likely or no more likely to have been exposed to the agent(s) or factor(s) of interest. That is, the analysis of case-control studies contrasts the frequency of exposure of the agent or factor in the cases with those in the controls to determine if these differ and, thus, whether there is a differential association. In case-control studies, determination of the disease status (i.e., cases with the disease; controls without) generally precedes determination of the exposure status (see figure box from van den Brandt et al. (2002)) Because disease has already occurred at the time of selection into the case-control study, this study design is particularly useful in studying uncommon diseases or diseases with long latency and can be utilized to evaluate the relation between many different exposures and a specific (pre-specified) disease outcome of interest . And because case-control studies begin with individuals who have the disease, the studies can involve fewer subjects than cohort studies and can be completed in a comparatively shorter time frame. Challenges in case-control investigations include the selection of an appropriate control group and the assessment of exposures which may have occurred long before the disease was diagnosed (Rothman, 2012; Wacholder et al. 1992a; Wacholder et al. 1992b; Wacholder et al. 1992c; Shultz and Grimes, 2002; Grimes and Schultz, 2005). Case-control studies can be particularly susceptible to "recall bias" in which diseased individuals may remember exposures or events differently (generally better) than those who serve as the controls and are healthy.



Nested case-control studies are an example of a hybrid design and contain the elements of a cohort and a case-control study. These designs can be useful when the analytical costs for determining pesticide exposure are too high for the entire cohort to be studies. For example, a cases that that have developed the disease or health outcome in an

[11] It is possible for case-control studies to be done prospectively in which the cases have not yet developed the disease until after the study begins under which circumstance the cases are enrolled in the study over time.

ED_002061_00044424-00019

ongoing cohort study can be matched with appropriate controls from the study that have not yet developed the disease or outcome of interest at the time of the analysis. One recognized advantage of the nested case-control study (as opposed to a more standard case-control study) is that the issues of selection bias and recall bias are minimized.

Cross-sectional studies focus on the prevalence of disease (e.g., birth defects, small-for-gestational age or SGA), symptoms, biological/physical and physiologic response measurements (e.g., pulmonary function tests, blood pressure, chest X-ray, clinical examinations, liver and kidney biomarkers). A key feature of such studies is that they are observational studies which focuses on the *prevalence* as a frequency measure, with the presence or absence of disease determined at the time of sampling or over a sampling period. Prevalence is the proportion of individuals in a population that has the disease and can either be determined as a "point prevalence" or as a "period prevalence".[12] A prevalence is a proportion not a rate and thus the cross sectional studies do not involve a follow up period. Typically, the exposure status (e.g., exposed or unexposed), disease status/outcome, and demographic characteristics are determined at a point in (or over) time. The major comparison in this study design is a comparison of the prevalence of the outcome in the exposed population vs. the prevalence of that outcome in the non-exposed population, with the risk measure being the prevalence risk ratio or odds ratio. Cross-sectional studies are generally used to identify patterns or trends in disease occurrence over time or in different geographical locations, and can be conducted quickly and relatively inexpensively. However, they measure the prevalence of a disease outcome which is affected by both incidence – the rate of occurrence of new cases – and duration of the disease, and it can be difficult in any analysis to sufficiently separate these factors. Thus, they involve "survivor populations" and do not measure, evaluate, or consider those that have left the population of interest because they became ill. Another important limitation of cross-sectional studies is they do not allow one to determine whether exposure precedes the disease. As such, cross-sectional studies are unable to establish temporal relationships between disease and exposure and typically require additional studies to confirm a hypothesized causal association suggested by a cross-sectional study.

Ecologic studies examine exposure and disease patterns using information reflecting group or population-level data. In an ecologic study, the unit of analysis is a group and not an individual[13]. Here, groups of subjects are sampled, with the exposure, disease, and potential confounding factors measured at this group (or cluster) level. Groups are generally defined on a geographic, administrative, or organizations unit basis (e.g., districts, towns, counties, schools, workplaces, etc.) with all exposure, disease, or confounder measurements made or summarized at the group level rather than at the level of the individual. An ecological (group-based) study contrasts with an individual-level study in that in the former there is no information on whether the cases are the actual individuals

[12] The former involve measurements at a particular place and/or a particular time while the latter involves determinations of the proportion of cases over a given time period.
[13] Some studies can be "partially ecologic" in design in which either the exposure or the disease outcome is measured on a group level but the other variable is measures at an individual level with the researcher making inferences to the individual level.

ED_002061_00044424-00020

with the exposure whereas in the latter exposure information is tied to the individual. As an example, a study of disease rates by contaminant levels in water can be ecologic with respect to evaluation of the exposure, but the health outcome or disease status may have determined on an individual basis. In these instances, the term "semi-ecological" can sometimes be used when exposure is determined at the group level but outcome is determined at the level of the individual.

Using this design, it is not possible to know whether all members of the exposed group are individually exposed (or the individual exposure levels) nor is it possible to infer individual-level effects from the group level effects that result. If the intent of the study is to direct inferences to the *group* (rather than the individual), then this is not a concern and these studies can be appropriate, particularly if measurements are constrained or difficult to perform at the individual level and exposures within the group are generally homogenous. If the intent of the study is instead to direct inferences to the individual, then this study design suffers from what is termed the ecological fallacy: the assumption that an observed relationship in an aggregated or grouped data set will reflect what would have been observed had the sampling occurred at the individual level. In addition to this ecological fallacy issue, an additional bias arises a result of the inability to appropriately control for confounding variables at the level of the individual as opposed to the group when information on confounding factors is only available at the group level.

In most cases, ecologic studies are considered as hypothesis-generating studies and best used for suggesting research hypotheses for future studies and may contribute to problem formulation. Nevertheless, it is important to assess ecological studies on the basis of the quality of their design, and useful information can be gleaned from an ecologic study if it is well-designed (FIFRA SAP, 2010). Ecologic studies alone generally do not have the ability to establish a causal association. When taken with other these studies can be useful under certain circumstances and should be noted in the hazard characterization. In particular, stable populations, clear exposure contrasts, and large differences in risk can be important factors that might increase the utility of these studies.

C. Evaluating epidemiology studies for use in pesticide risk assessment

OPP searches the peer reviewed literature for observational epidemiology studies of potential adverse acute and chronic health effects linked to chemical use. Details regarding literature search protocols and strategies are provided elsewhere. Epidemiologic research utilizing cohort, case-control, or cross-sectional study designs may provide information to OPP to strengthen OPP's understanding of the potential hazards, exposure-response characterization, exposure scenarios. or assessment methods, and – ultimately -- risk characterization (van den Brandt, 2002). In addition, compelling case reports or case series analysis may illumine a health effect or mechanism of action previously unidentified.

Generally speaking, the quality of epidemiologic research, sufficiency of documentation of the study (study design and results), and relevance to risk assessment is considered when evaluating epidemiology studies from the open literature for use in OPP's

risk assessments. It is important that these criteria are endpoint-specific as various methodological details become more or less important given the endpoint of concern. For example, it is important to understand relevant factors that influence outcome ascertainment (*e.g.*, is there a test or a biomarker available to indicate presence of an effect, or are symptoms gradual and non-specific initially leading to physician diagnosis upon advanced disease state). In addition, for environmental and occupational epidemiology studies, the quality of the exposure assessment is vitally important. Prior consideration must be given to aspects of exposure and confounder measurement to the question under consideration.

When considering individual study quality, various aspects of the design, conduct, analysis and interpretation of the epidemiology studies are important. These include:

1. Clear articulation of the hypothesis, even if the study is hypothesis-generating in nature;
2. Adequate assessment of exposure for the relevant critical windows of the health effects, the range of exposure of interest for the risk assessment target population, and the availability of a dose/exposure-response trend from the study, among other qualities of exposure assessment,
3. Reasonably valid and reliable outcome ascertainment (the correct identification of those with and without the health effect in the study population),
4. Appropriate inclusion and exclusion criteria that result in a sample population representative of the target population, and absent systematic bias,
5. Adequate measurement and analysis of potentially confounding variables, including measurement or discussion of the role of multiple pesticide exposure, or mixtures exposure in the risk estimates observed,
6. Overall characterization of potential systematic biases in the study including errors in the selection of participation and in the collection of information; this can include performing sensitivity analysis to determine the potential influence of systematic error on the risk estimates presented (*e.g.,* Greenland's formula)
7. Evaluation of the statistical power of the study to observe health effects with appropriate discussion and/or presentation of power estimates,
8. Use of appropriate statistical modeling techniques, given the study design and the nature of the outcomes under study

Other Federal and non-Federal entities have offered such guides (*e.g.*, OHAT, Navigation Guide, National Toxicology Program [NTP] Report on Carcinogens [ROC[14]], IRIS, Cochrane ACROBAT-Non-Randomized Studies of Interventions) (Sterne et al., 2015 as well as the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) statement for observational epidemiological studies (see www.strobe-statement.org and Vandenbroucke et al., 2007; Von Elm, 2014) As OPP gains experience with integrating epidemiology studies into human health risk assessment, relevant adjustments to its evaluation approach will be made.

[14] http://ntp.niehs.nih.gov/pubhealth/roc/index.html

ED_002061_00044424-00022

Independent study evaluation is performed and documented prior to the development of evidence- tables of detailed summary tables which are informative to hazard identification and exposure response assessment. Table 2 provides a structure to the major considerations evaluated and the associated weight (low, medium, high) for each consideration. Table 2 provides a generic set of considerations and should not be considered a checklist. The specific scientific considerations appropriate for particular science analysis are adjusted on a case by case basis.

The culmination of the study evaluation process would be to provide professional/expert opinion as to the nature of the potential bias that may result from systematic errors in each specific study identified through study specific evaluations, and an assessment of overall confidence in the epidemiological database. In this way, data integration (animal, human, mechanistic, other) would be informed by level of confidence in the human epidemiological studies that inform human health effects of environmental and occupational exposures.

ED_002061_00044424-00023

Table 2. Study Quality Considerations [a] (Adapted from Munoz-Quezada et al., 2013; LaKind et al., 2014)

Parameter	High	Moderate	Low
Exposure assessment	Accurate and precise quantitative relationship with external exposure, internal dose, or target dose, possibly associated with an MOA/AOP. If questionnaire utilized, questionnaire and/or interview answered by subjects for chemical-specific exposure	Evidence exists for a relationship between biomarker in a specified matrix and external exposure, internal dose, or target dose. Questionnaire and/or interview for chemical-specific exposure answered by subjects or proxy individuals	Poor surrogate Low-quality questionnaire and/or interview; information collected for groups of chemicals rather than chemical-specific; no chemical-specific exposure information collected; ever/never use of pesticides in general evaluated
Outcome Assessment	Standardized tool, validated in study population; medical record review/diagnosis confirmation by trained staff; appropriate consideration of prevalence/incidence of cases	Standardized tool, not validated in population, or screening tool; or, medical record review, methods unstated	Selected sections of test, or maternal report, other; or, maternal/paternal self-report; unclear/no consideration for whether prevalent or incident cases are appropriate
Confounder control	Good control for important confounders relevant to scientific question, and standard confounders	Moderately good control confounders, standard variables, not all variables relevant for scientific question	Multi-variable analysis not performed no adjustments; no stratification, restriction, or matching
Statistical Analysis	Appropriate to study question and design, supported by adequate sample size, maximizing use of data, reported well (not selective)	Acceptable methods, questionable study power (especially sub-analyses), analytic choices that lose information, not reported clearly	Minimal attention to statistical analyses, comparisons not performed or described clearly
Risk of (other) bias (selection, differential misclassification, effect size magnification, other)	Major sources of other potential biases not likely present, present but analyzed, unlikely to influence magnitude and direction of the risk estimate	Other sources of bias present, acknowledged but not addressed in study, may influence magnitude but not direction of estimate	Major study biases present, unacknowledged or unaddressed in study, cannot exclude other explanations for study finding

[a] Overall study quality ranking based on comprehensive assessment across the parameters.

ED_002061_00044424-00024

1. *Exposure Assessment*

Exposure assessment can be defined as the "process of estimating or measuring the magnitude, frequency and duration of exposure to an agent, along with the number and characteristics of the population exposed. Ideally, it describes the sources, pathways, routes, and the uncertainties in the assessment. (Zartarian et al., 2005)." In environmental epidemiology, exposure assessment poses a unique challenge, particularly for toxicants that are found in low concentrations in environmental media (NRC, 1991; NRC, 1997). Given the complexity of exposure pathways, researchers have developed a number of different approaches to assess exposure, which vary in accuracy, precision, and resource requirements (Niewenhuijsen, 2003). Some of these approaches are not specific to epidemiologic research but may be used to inform exposure assessment in a variety of scientific analyses. These approaches include indirect methods, based on historical records, questionnaires, and environmental monitoring, and direct methods, based on personal monitoring and biomonitoring. A brief description of each method and its strengths and limitations is summarized below.

Table 3. Summary of indirect and direct exposure assessment methods.

Approach	Method/Tools	Example	Exposure Estimation
Indirect	Historical Records	Estimating proximity to agricultural crops using address information	Dichotomous or ordinal exposure
	Questionnaires	Determine potential for exposure based on pesticide-use responses	Dichotomous or ordinal exposure
	Environmental Monitoring	Measuring pesticide levels in community water drinking system	Dichotomous or ordinal exposure, although exposure can be estimated using modeling
Direct	Personal Monitoring	Measuring pesticide inhalation and dermal contact	Quantified exposure
	Biomonitoring	Measuring pesticide levels in blood and urine	Quantified internal dose

Historical records and questionnaires are used to characterize key characteristics which may be associated with chemical exposure. When used in epidemiologic studies, historical records and questionnaires are not typically used to predict quantitative levels of exposure. Rather, historical record information or questionnaire responses are used to assign categorical levels of exposure. Examples of historical record information that can be used to assign exposure levels includes address in proximity to an agricultural crop and employment history information on job title and history. Similarly, questionnaires can be used to determine if individuals recall using pesticides or identify individuals that perform specific job functions that increase their potential for exposure. While historical records and questionnaires can be cost-effective sources of data on potential exposure, they do have limitations. Data collected from historical records and questionnaires is only a surrogate of exposure. As a result, these

ED_002061_00044424-00025

data sources may be an oversimplification of exposure and not accurately rank individual's exposure potential.

Environmental monitoring is used to characterize the levels of contaminants in environmental media, including air, water, soil, food, and home and work environments. Many state and Federal programs collect environmental monitoring data that may be useful in epidemiologic studies. Environmental monitoring is particularly useful for exposure that can be defined by geographic boundaries, such as air pollution and drinking water. As such, many epidemiologic studies have utilized ambient air monitoring data and community drinking water system data to characterize exposure to air pollution and drinking water contamination, respectively. While environmental monitoring data is useful for estimating exposures defined by geographic boundaries, it can be less reliable for the purposes of assigning individual-levels exposures, particularly when individuals live, work, and spend time in many different locations.

Personal monitoring is used to characterize exposure at the point of contact of a body boundary. Examples of personal monitoring include the use of dosimeters to assess dermal contact with pesticides, personal air sampling devices to assess inhalation exposure, and collection of duplicate diet samples to determine pesticide levels in food. The advantage of personal monitoring is that it is likely to provide more accurate estimates of individual-level exposure than indirect methods. Personal monitoring also makes it possible to quantify exposure levels that can be useful for prioritizing the relevance of different routes of exposure. Additionally, personal monitoring can also be used to assess longitudinal exposure when repeated measurements are taken over time. While personal monitoring offers many advantages over indirect approaches, it also tends to be labor and resource intensive (Niewenhuijsen, 2003). As a result, it is not typically feasible to conduct large-scale epidemiologic studies that assess exposure using personal monitoring. Furthermore, personal monitoring is highly dependent on the measurement techniques and analytic tools used to obtain samples and it is less likely that information that characterizes exposures during the relevant time period (usually in the past) will be available. In addition, it is unlikely that the full range of exposures over the time period of interest will be captured, and sampling may not be over a sufficient time period to capture peaks and fluctuations As such, it is extremely important to consider the scientific rigor and reliability of personal monitoring methodologies that are used in epidemiologic studies, and such monitoring may need to be supplemented by other monitoring (e.g., environmental, biological, and/or interview/questionnaire data).

Biomonitoring is used to characterize exposure by measuring a chemical, its metabolite(s), or reactive product(s) in biological samples, such as blood, urine, saliva, milk, adipose, and other body tissues (Needham et al., 2007). Zartarian et al. (2005) state that "a biomarker/biological marker has been defined as an "indicator of changes or events in biological systems. Biological markers of exposure refer to cellular, biochemical, analytical, or molecular measures that are obtained from biological media such as tissues, cells, or fluids and are indicative of exposure to an agent". Thus, biomarkers can be used to assess exposure or as indicators of health effects (LaKind et al., 2014). Table 4 provides scientific considerations for evaluating the quality and relevance of biomonitoring data

ED_002061_00044424-00026

collected from epidemiology studies. Assessing exposure using biomonitoring has expanded rapidly as analytical tools have become more cost-effective and more biomarkers are identified. Compared with self-reported questionnaire or interview data, biomonitoring may reduce exposure misclassification and enhance the precision of the risk estimates. Similarly, biomonitoring integrates exposures from different routes and can be used to determine the amount of exposure that is absorbed into the body (Checkoway et al., 2004). Furthermore, knowledge as to the role of the biomarker in the natural history of disease is known in certain instances, such that biomarkers may help resolve temporality of exposure issues.

While biomonitoring has many advantages over others exposure assessment methods, it also has its own limitations. In many studies, biological sample are only taken from a single point in time and may not reflect accurately reflect longitudinal patterns, particularly if exposures are highly variable. Furthermore, evaluation of biomarkers also requires an understanding of degradation and metabolism of chemicals in both the environment and human body. As such, biomarkers of exposure may differ between individuals for reasons other than exposure level. Differences in metabolism, co-morbidities such as kidney disease in relation to urinary measurements, uncertainty as to whether the biomarker measures exposure to the active ingredient or the environmental degradates may all account for apparent differences in biomarkers of exposure among individuals, and possibly between comparison groups.

ED_002061_00044424-00027

Table 4. Considerations of biomonitoring data from environmental epidemiology research (Adapted from LaKind et al. (2014).

Biomarker Consideration	Tier 1	Tier 2	Tier 3
Exposure biomarker	Biomarker has accurate and precise quantitative relationship with external exposure, internal dose, or target dose.	Biomarker has an unknown quantitative relationship with external exposure, internal dose, or target dose or is poor surrogate (low accuracy and precision) for exposure/dose.	NA
Effect biomarker	Bioindicator of a key event in a MOA/AOP.	Biomarkers of effect for which the relationship to health outcome is understood	Biomarker has undetermined consequences (e.g., biomarker is not specific to a health outcome).
Specificity	Biomarker is derived from exposure to one parent chemical.	Biomarker is derived from multiple parent chemicals with similar toxicities.	Biomarker is derived from multiple parent chemicals with varying types of adverse endpoints.
Method sensitivity	Limits of detection are low enough to detect chemicals in a sufficient percentage of the samples to address the research question.	Frequency of detection too low to address the research hypothesis.	NA
Biomarker stability	Samples with a known history and documented stability data.	Samples have known losses during storage but the difference between low and high exposures can be qualitatively assessed.	Samples with either unknown history and/or no stability data for analytes of interest.
Sample contamination	Samples are contamination-free from time of collection to time of measurement (e.g., by use of	Study not using/documenting these procedures.	There are known contamination issues and no documentation that the issues were addressed

ED_002061_00044424-00028

Biomarker Consideration	Tier 1	Tier 2	Tier 3
	certified analyte-free collection supplies and reference materials, and appropriate use of blanks both in the field and lab). Research includes documentation of the steps taken to provide the necessary assurance that the study data are reliable.		
Method requirements	Instrumentation that provides unambiguous identification and quantitation of the biomarker at the required sensitivity (e.g., GC-HRMS, GC-MS/MS, LC-MS/MS)	Instrumentation that allows for identification of the biomarker with a high degree of confidence and the required sensitivity (e.g., GC-MS, GC-ECD).	Instrumentation that only allows for possible quantification of the biomarker but the method has known interferants (e.g., GC-FID, spectroscopy)
Matrix adjustment	Study includes results for adjusted and non-adjusted concentrations	Study only provides results using one method (matrix-adjusted or not).	NA

FP = false positive; FN = false negative; GC-HRMS = gas chromatography/high-resolution mass spectrometry; GC-MS = gas chromatography/mass spectrometry; GC-ECD = gas chromatography-electron capture detector; GC-FID = gas chromatography-flame ionization detector], ICC = intra-class correlation coefficient ; NA = not applicable; PFP = probability of false positive

ED_002061_00044424-00029

Indirect exposure assessment methods are common in retrospective studies and based on factors that are surrogates of chemical exposure. As described above, indirect exposure data cannot generally be used to estimate quantitative exposure levels without additional modeling. For example, a questionnaire can be used to determine if an individual has ever used a pesticide, but can less reliably collect data on all the environmental and behavioral factors that are needed to calculate that individual's exposure. As such, indirect exposure data are often used to classify exposure using a dichotomous exposure variable (i.e. exposed/unexposed) or ordinal exposure scale. In contrast, direct exposure assessment methods are based on data on actual individual-level exposure through personal monitoring and biomonitoring. Thus, direct methods can be used to estimate individual exposure or internal dose levels. Direct methods are more common in prospective studies, but are also used in retrospective studies when existing biological samples are available from well-defined population groups.

Quantified personal measurements, such as personal monitoring and biomonitoring, are generally considered the best source of data for estimating actual exposure levels (NRC, 1991; NRC, 1997). While this is the case, accurate qualitative measures of exposure (e.g. dichotomous and ordinal exposure metrics) from indirect methods can be just as accurate for the purpose of epidemiology. Moreover, indirect methods are often easier to interpret and may require less additional research and development to demonstrate their utility in exposure assessment.

Regardless of the approach, exposure assessment methods should be able to provide exposure estimates that are reliable and valid. In the context of epidemiology, *reliability* general refers to the ability to reproduce results and *validity* generally refers to the extent that exposure estimates reflect true exposure levels (Checkoway et al., 2004). When evaluating a particular exposure assessment's reliability and validity, it is important to consider the exposure assessment's strengths and weaknesses in the context of the study's research objectives. Less refined exposure assessment may be suitable for exploratory studies. This is because exploratory studies help raise awareness about potential hazards that can encourage investment in more focused research. Conversely, studies with more focused hypotheses can be greatly strengthened through the use of more refined exposure assessment methods. Therefore, indirect and direct exposure assessment methods represent a spectrum of tools that are complimentary and can be used at different stages of research when exploring exposure-disease relationships.

2. Confounding Factors

Confounding occurs when the relationship between the exposure and disease is to some extent attributable to the effect of a second (confounding) risk factor. This can happen when this second (i.e., confounding) risk factor is an independent, causally-associated risk factor for the disease but is also associated -- causally or non-causally -- with the exposure under analysis and does not also serve as an intermediate variable in the causal pathway between the exposure and the outcome of interest. If not properly measured and accounted

ED_002061_00044424-00030

for, confounders have the ability to change the magnitude (and potentially the direction) of the estimated association between an exposure and health outcome. This can result in an over- or under-estimation of the relationship between exposure and disease because the effects of the two risk factors have not been appropriately separated, or "disentangled". As an example: a given pesticide may be associated with lung cancer in a given study, but this may be due to a confounding effect of farm tractor diesel fumes: here, this second factor – farm tractor diesel fumes – would be a confounder if it was causally associated with the disease outcome (here, lung cancer) but also associated with pesticide exposure. Confounding factors may include less intuitive lifestyle exposures such as cigarette smoking, dietary factors (e.g., high energy/calorie laden diet), and physical activity (e.g., lack of physical activity) genetics, comorbidity, medication use, alcohol consumption, etc., all of which may adversely affect health and may be statistically associated with pesticide use. In epidemiological analyses, confounding factors are measured in the study sample and typically "adjusted for" in the final risk estimate in either the design phase of the study or the analysis phase. With respect to the former, the epidemiological researcher can "restrict" the study population to individuals that share a characteristic which the researcher wishes to control; this has the result of removing the potential effect of confounding caused by that (now controlled) characteristic. A second available method – also applicable to the design phase of the study -- is for the researcher to control confounding by "matching" individuals based on the confounding variable. This ensures that the confounding variable is evenly distributed between the two comparison groups and effectively controls for this. It is important to note that the relationship between the confounder and the exposure or outcome does not need to be found to be statistically significant in order for it to have an impact on the risk estimate for the main effect[15].

At the analysis stage, one method by which confounding can be controlled is by stratification. Under this means of control, the association is measured separately under each of the (potentially) confounding variables; the separate estimates are "brought together" statistically -- if determined to be appropriate -- to produce a common odds ratio or other effect size measure by using Mantel-Haenszel approaches which weight the estimates measured in each stratum. Stratification can be difficult if there are multiple potential confounders that need to be controlled simultaneously. In such cases, confounding is typically dealt with by means of statistical modelling. (e.g., logistic regression).

It is important that careful consideration be given to confounders prior to any epidemiological studies being initiated in the field and it is important that any study adequately describe how this was done: epidemiological studies are frequently critiqued for ignoring or paying insufficient attention to potential confounders. For this reason, a sensitivity analysis can be helpful to demonstrate the potential effects that a missing or unaccounted for confounder may have on the observed effect sizes (see Gustafson and

[15] This is why it is generally considered inappropriate to "statistically test" for a confounder to determine whether the confounder needs to be adjusted for. Instead, some consider a change in the effect size of 10% or more after adjustment for (inclusion of) a potential confounder to be sufficient evidence for the confounder to be incorporated into the analysis.

ED_002061_00044424-00031

McCandless, 2010). If unmeasured confounders are thought to affect the results, researchers should conduct sensitivity analyses to estimate the range of impacts and the resulting range of adjusted effect measures. Such sensitivity analyses -- generally not uniformly conducted in most published epidemiological studies – can be used when available to estimate the impact of biases and potential confounding by known but unmeasured risk factors.

Depending upon the specific exposure-disease association under study, a factor may or may not be a confounding factor that is necessary to control: in order for a substantial distortion in the effect size estimate to occur due to confounding, the confounder must be not only a relatively strong risk factor for the disease of interest[16], but also be strongly associated with the exposure of interest. Assessment of potential confounding is made on a study specific basis and – if unmeasured confounders are thought to affect the results -- researchers should conduct a sensitivity analysis to estimate the range of impacts and resulting range of adjusted effect measures. When evaluating the quality of observational epidemiology studies, OPP will consider whether relevant confounding factors are properly identified, described, measured and analyzed such that an unbiased estimate of the specific association under study can be made, and, when possible, may consider sensitivity analysis as a potential tool to assist in determining the degree to which such confounding might potentially affect the estimate of the effect size. It should be emphasized that a confounder must be a relatively strong risk factor for the disease to be strongly associated with the exposure of interest to create a substantial distortion in the risk estimate. In such cases, it is not sufficient to simply raise the possibility of confounding; one should make a persuasive argument explaining why a risk factor is likely to be a confounder, what its impact might be, and how important that impact might be to the interpretation of findings. (p. 23-25, FIFRA SAP Report, 22 April 2010)

Finally, it is important to distinguish between confounding, effect modification, synergy, and other mediating effects of covariates. Confounding is a bias that results from not controlling for a variable that is associated causally with the disease and associated – causally or non-causally -- with the exposure of interest. Epidemiologic researchers seek to minimize this bias. Effect modifiers -- on the other hand -- are variables that differentially affect the magnitude of the effect size, by strata (e.g., age, race/ethnicity, SES status, genetic polymorphisms). Effect modifiers may or may not also be confounders. Typically, they are modelled by either introducing interaction terms in multivariable models or by evaluating effect sizes by strata after stratifying the data by levels of the effect modifier. A study frequently needs to be specifically designed to evaluate effect modifiers in order to have a sufficient sample size in each population strata of interest. Epidemiologic researchers seek to understand effect modifiers (not minimize them, as they do with confounders) because they can be important in evaluating risk differences across population strata, in evaluating the association between exposure and the effect of interest, and in identifying susceptible

[16] Consideration needs to be given not only to ensuring that the confounding factor is indeed a risk factor on its own but also to ensuring not only related to the exposure of interest. Adjusting for a factor that has an association with the disease of interest wholly or partly because of its association with the exposure of interest will lead to attenuation of the exposure-disease relationship if it truly exists.

subpopulations. Effect modifiers may or may not also be confounders. For example, smoking may be a confounder in a study associating lung cancer with a pesticide often used on tobacco, but it may also be an effect modifier if the risk of exposure to this pesticide is higher among smokers than non-smokers. Synergy is often introduced as a biological or pharmacological/toxicological concept rather than an epidemiological one and relates to the ability of two chemicals, together and acting jointly, to magnify or exaggerate the effect beyond that which would be seen considering the (mathematical) sum of each chemical's effects alone. In epidemiological and statistical terms, this is often expressed as effect modification or interaction.

3. Statistical Analysis

Epidemiologic studies are designed to measure an association between a specific exposure and a disease. When evaluating the quality of pesticide epidemiology studies, OPP will also consider the statistical methods used. Specifically, OPP will consider the extent to which the analytic methods described in the study are appropriate to the research question; the completeness of the description of the statistical methods utilized; the appropriateness of the methods for identification, assessment and adjustment of potentially confounding variables in the exposure-disease relation; and, the description, extent of, and presentation of any sub-group analyses which may have been performed (including whether statistical corrections for multiple comparisons have been made).

Epidemiologic investigations typically utilize statistical modeling to estimate risk (e.g. generalized linear models such as logistic (for odds ratios) or Poisson (for count data) regression. To do so, researchers must consider not only the relevant main exposure and outcome variables, but also consider relevant confounding factors, and whether the association under investigation may differ by level of these factors, i.e., effect modification or interaction (Szklo et al., 2004). Upon identification of a potentially confounding variable -- one that substantively changes the magnitude and/or direction of the association under study -- adjustment through regression modeling can help to isolate the risk estimate of interest, i.e., the association under study. In addition, OPP will evaluate the stratification of the association by the level of the potential effect modifier under study or evaluation of statistical interaction. If the magnitude and direction of the association of interest differs greatly by level of a third variable, then the stratified results should be considered primary.

When performing statistical modeling when the outcome is rare or the sample size is relatively small, it is important to be cautious about including too many covariates in the model. Any resulting effect size estimate may be too high or too low and is unlikely to reflect the true estimate of effect. Such issues due to rare events or low sample sizes are also possible when conditional methods are used (e.g., conditional logistic regression when the design includes matching of the comparison group under study): if too few discordant pairs (or discordant sets) are observed, the estimated effect size may also be unreliable. Thus: while controlling for confounders and other covariates is important, the assessor must take care not to over-control or end up with too few degrees of freedom to produce a

reliable test. In these cases, it may be more important to seek parsimonious models that adjust for only a smaller number of the most influential confounders and other covariates so that the effective sample size remains adequate.

Finally, it is important in any statistical modeling exercise to consider statistical significance in the context of clinical/biological/scientific significance of the result. It may be that some results are statistically significant but unimportant in a clinical/biological/ scientific context. The reverse can be true: it may be that results are not statistically significant but may be important in a clinical/biological/scientific context. The former may suggest a sample size that is larger than necessary while the latter may suggest one than is smaller than needed. The latter case may be important from a public health perspective and warrant further exploration, especially when the association is strong (despite it being imprecise)

4. Potential Bias in Observational Research

Bias is a systematic error in the design or conduct of a study that gives rise to study results that are systematically different from the (unobserved) true situation. This contrasts with random errors which relate to sampling variability and precision (or, equivalently, confidence bounds) around the effect size measure, but which do not "drive" or "push" the result in one particular direction (e.g., either toward or away from the null).

Bias is a reflection of methodological imperfections in the design or conduct of the study and should be addressed or discussed by researchers as part of their analysis. There are a number of ways that bias can be introduced into a study: studies may be biased in the way in which participants are selected into the study (selection bias), or the way in which information about exposure and disease status is collected (information bias, including recall bias discussed earlier for case-control studies). One example of a common occupational selection bias is the "healthy worker effect" which can create an important bias in occupational epidemiology studies, leading to bias toward the null, and even below (creating the interpretation that the exposure is "protective") No study is totally devoid of bias and one should consider the extent to which authors of published studies described potential bias in the study, and how (if at all) they attempted to address it and characterize it in the study. Bias can result from differential or non-differential misclassification (Greenland, 1998). Differential misclassification (bias) means that misclassification has occurred in a way that depends on the values of other variables, while non-differential misclassification (bias) refers to misclassifications that do not depend on the value of other variables. Misclassification biases – either differential or non-differential – depend on the sensitivity and specificity of the study's methods used to categorize such exposures and can have a predictable effect on the direction of bias under certain (limited) conditions: this ability to characterize the direction of the bias based on knowledge of the study methods and analyses can be useful to the regulatory decision-maker since it may allow the decision maker to determine the extent to which, if any, the epidemiological effect sizes being considered (e.g., OR, RR) are likely underestimates or overestimates of the true effect

ED_002061_00044424-00034

size[17]. It is not atypical to find degrees of misclassification in the range of 10 to 20 percent and it can be helpful in reviewing epidemiological studies to consider a form of sensitivity (or "what if") analysis which evaluates such a degree of misclassification -- and whether it is differential or non-differential – and the degree to which such misclassification might impact the odds ratio or relative risk with respect to both magnitude and direction[18]. (p.25, FIFRA EPA SAP report, 22 April, 2010). As mentioned earlier with respect to confounding, such quantitative sensitivity analysis is only rarely performed or practiced in published epidemiology studies, with bias instead more typically evaluated in a narrative manner without any quantitative assessment of its potential magnitude and the effect it may have on the epidemiological effect size estimates (Jurek at al., 2006). This may be due – in part -- to a general lack of availability of computational tools for such analysis by epidemiologists or their unfamiliarity with them. Such tools are becoming increasingly available and may be valuable in developing more rigorous quantitative methods for evaluation of potential biases.

5. *Interpretation of Null studies*

"Null" studies -- or well-conducted studies which report no association between exposure to the pesticide and an adverse health outcome -- will be evaluated carefully for their potential usefulness in human health risk assessment. The study may report a null result either because the investigated association indeed does not in reality exist, or because the study was conducted failed to detect an association at a given predetermined level of significance. This latter result –the failure to detect an association -- should not necessarily be interpreted to mean that no association exists, but rather as simply one was not found in the particular study[19,20]. To evaluate which of these two conditions may be correct when reviewing "null" studies, one should consider other research reported concerning the same or similar research question, the manner in which exposure and outcome were assessed, the extent to which exposure misclassification may have biased the study to the null, the statistical methods used including the identification and analysis of confounding variables in the association, the extent to which the exposure is below a threshold at which an effect would occur or be detected, as well as the power of the study and its ability to detect an effect size of substantive interest. Statistical power refers to the probability that researchers may correctly identify that there is a difference between the two comparison groups, i.e., there is an association between exposure and disease, when in

---

[17] The direction of bias that results from the degree of non-differential misclassification will also depend on the categorization of exposure (either dichotomous or polytomous).

[18] Such sensitivity analyses might be especially recommended for exposure misclassification biases which in many cases are expected to result in more substantive effects on the effect size estimate than those from confounding.

[19] The old adage that "the absence of evidence does should not be interpreted as the evidence of absence" is true here.

[20] See also the American Statistical Association's Statement on Statistical Significance and P-values at https://www.amstat.org/asa/files/pdfs/P-ValueStatement.pdf

ED_002061_00044424-00035

fact there is in fact a true difference (or association). Studies that are "low powered" may falsely conclude there is no association, when an association actually exists[21].

Finally, it is important to consider the effects of publication bias in any systematic review of the literature with respect to interpretation of null studies. The term publication bias refers to the tendency for the available published literature to disproportionately exclude such null studies. Studies that demonstrate such a "null" association between a disease or health outcome can be as equally informative as those that do provided that the study in question meets the quality criteria established as part of the epidemiological review process. These may include such factors as study design; the existence of an *a priori* hypothesis vs. an exploratory analysis; sample size and statistical power to detect an effect size of interest; proper ascertainment of outcome *vis-à-vis* sensitivity and specificity; the quality of the exposure assessment and the potential for differential and non-differential misclassification; adequacy of the measurement of key potential confounders and other forms of bias (information, selection, etc.); and evaluation of effect modifiers; appropriate statistical analyses, including consideration of and possible correction for multiple comparisons that a unsupported by a priori hypotheses, biological plausibility, or other supporting information.

6. External Validity (Generalizability)

As noted above, *validity* generally refers to the extent that exposure estimates reflect true exposure levels (Checkoway et al., 2004). *External validity*, or *generalizability*, refers to the ability to extend the epidemiologic study results derived from a sample of the population (e.g., pesticide applicators) to other populations (e.g., all agricultural workers). To assess external validity, comparison of characteristics in the sample to the larger population (if known) can be made. Such evaluation should include not only demographic factors, but also whether exposures (e.g., dose, timing, duration) are similar and whether important effect modifiers (e.g., sensitivity of vulnerable populations) were considered. Generalizability is of particular importance because it is important to understand whether and how individual study results may be applied to the larger group or targeted sub-groups in regulatory risk assessment. For example, the AHS has reported statistical associations between some cancer and non-cancer health outcomes for some pesticide chemicals. OPP has an interest in evaluating the extent to which the reported findings may apply to pesticide applicators in states other than North Carolina and Iowa or to farm workers who primarily do post-application activities.

[21] Studies that are low-powered but find statistically significant effects may also be subject to the phenomenon of effect size magnification and this can be important to investigate as well. (Ioannidis, 2008).

ED_002061_00044424-00036

V. HUMAN INCIDENT SURVEILLANCE DATA

Generally speaking, epidemiology studies on pesticides such as those described above focus on lower exposures (over a longer time period) that are less likely to result in acute clinical symptoms. OPP is also interested in exposures that are higher and occur over shorter-intervals (often on an acute "one-time" basis). This "human incident," or poisoning data can be useful for evaluating short term, high exposure scenarios that can be readily attributed to the pesticide in question.

OPP uses such "human incident information" for several purposes. Most broadly, the program uses incident data to inform risk assessment/risk management activities; this forms an integral part of our registration review activities under our Pesticide Registration Improvement Act (PRIA) responsibilities. To this end, OPP evaluates human incident data for trends over time and examines patterns in the severity and frequency of different pesticide exposures. In some cases, incident information can indicate need for additional information or additional risk management measures. Incident information can also help assess the success of risk mitigation actions after they are implemented, and incident information is an important part of OPP's performance accountability system to ensure the effectiveness of risk management actions that OPP has taken to protect human health and the environment. Lastly, incident information can be useful in providing real world use information with respect to usage practices and also in potentially targeting enforcement or educational activities, where appropriate.

OPP obtains this information from a variety of sources. Sources of human incident data include both (human) **medical case reports** appearing in the medical and toxicological literature as well as information from a variety of national **toxico-surveillance activities** for acute pesticide poisonings which are considered jointly to aid acute and chronic hazard identification and as an integral part of the risk assessment process.[22]

Medical case reports (first-hand accounts written by physicians) or medical case series (a compendium of medical case reports across individuals that share common source or symptomology) are valuable tools for analyzing all available evidence of health effects, and to complement the findings of animal studies and epidemiological studies. In addition, they can identify unusual or novel occurrences of an adverse health effects plausibly associated with use of a specific pesticide providing "advance notice" to the agency for toxico-vigilance purposes. Published case reports for pesticides typically describe the effects from an atypical (high exposure/dose, illegal, off-label) acute or short-term exposure. The reports are often anecdotal and can be highly selective in nature. They can, however, can be particularly valuable in identifying previously unidentified toxic effects in humans and in learning about the effects, health outcomes, and medical sequelae following high exposures. They frequently have more detailed medical information (including sequelae), detailed follow-up, and generally higher quality and/or quantitative

[22] OPP is aware of efforts by IPSC to consider human incident data in risk assessment. http://www.who.int/ipcs/publications/methods/human_data/en/index.html

information about dose. If similarities are seen across multiple medical case studies or patterns emerge – in symptoms, exposure scenarios or usage practices -- these can provide valuable information for the risk assessment process and strengthen any findings. Medical case studies and series that include quantitative exposure information can be compared to exposure estimates in the risk assessment (which are based on labeled application rates and surrogate exposure information) to characterize margins of exposure expected from typical use, when appropriate.

The following considerations are evaluated in assessing medical case reports and medical case series:

- A detailed history of exposure (when, how, how much); time of onset of adverse effects; and signs and symptoms of the patient, are reported.
- Information on the product/chemical/pesticide, such as name, pesticide label, registration number, etc.
- Patient information (e.g. age, race, sex); underlying health conditions and use of any medications that can produce similar signs and symptoms; relevant medical history; and the presence of any risk factors.
- Description of events and how the diagnosis was made.
- Management and treatment of the patient, and laboratory data (before, during and after the therapy), including blood levels of pesticides and chemicals.
- Whether the medical report is reliable, reasonable and whether it is consistent with current knowledge, including other research, reviews and guidelines.
- Clinical course of the event and patient outcome (e.g. patient recovered and discharged from hospital; condition of patient after the discharge, any chronic health effects or premature death related to the pesticide or chemical exposure).

In addition to using medical case reports/series as a source of real-world exposure and toxicological information, OPP also engages in toxico-surveillance activities using a variety of pesticide poisoning incident databases are also available. Specifically, OPP has access to the following five human incident data sources: the *OPP Incident Data System* (IDS); the American Association of Poison Control Centers (PCC) summary reports from their *National Poison Data System* (NPDS); data from the EPA-funded *National Pesticide Information Center* (NPIC), currently at Oregon State University; the Centers for Disease Control and Prevention/National Institute for Occupational Safety and Health *Sentinel Event Notification System for Occupational Risk-Pesticides* (NIOSH SENSOR-Pesticides) and the *California Pesticide Illness Surveillance Program* (PISP). Each of these are described, in turn below:

- **OPP Incident Data System (IDS)** is maintained by OPP and incorporates data submitted by registrants under FIFRA section 6(a)(2)[23], as well as other incidents reported directly to EPA. OPP has compiled the pesticide related

[23] Under FIFRA 6(a)(2), pesticide registrants are required to notify EPA if and when they become aware of "factual information regarding unreasonable adverse effects on the environment of the pesticide."

incident reports in the IDS since 1992. The IDS includes reports of alleged human health incidents from various sources, including mandatory FIFRA Section 6 (a) (2) reports from registrants, other federal and state health and environmental agencies and individual consumers. IDS include information on incidents involving humans, plants, wild and domestic animals where there is a claim of an adverse effect. The vast majority of IDS reports are received by the agency in paper format. IDS entries act as a "pointers" to copies of original reports retained on microfilm and scanned images in OPP's Information Service Center.

While IDS includes both occupational and non-occupational incidents, the majority of incidents reported relate to non-occupational/residential scenarios The reports are obtained from across the U.S. and most incidents have all relevant product information (such as the EPA Registration Number) recorded. As IDS is populated mostly by information provided by pesticide registrants under their FIFRA 6(a)(2) reporting requirements, the agency has relatively high confidence in the identification of the specific product which is involved. Severity rankings are included for each incident (as specified by CFR §159.184). Symptom information is sometimes included in the narrative portion of the incident, but this information is usually not validated/confirmed by a healthcare professional. IDS also includes narrative information on exposure scenario and hazard information. Many companies use standardized, industry-developed Voluntary Incident Reporting Forms.

OPP collects and evaluates the data from the IDS and identifies potential patterns with respect to the extent and severity of the health effects due to pesticides exposure. While IDS reports are broad in scope and can in some cases contain detailed information, the system does not necessarily consistently capture detailed information about incident events, such as occupational exposure circumstances or medical outcome.

In addition, most cases data going into IDS is not validated or verified, though some reports are collected from calls to contract poison control centers. Nevertheless, incident information can provide an important post-marketing feedback loop to the agency following initial registration of the product: IDS incidents of a severe nature, or a suggested pattern or trend among less severe incidents can signal the agency to further investigate a particular chemical or product. Because IDS has such extensive coverage, it can assist in providing temporal trend information and determining whether risk mitigation has helped reduce potential pesticide exposure and decreased the number of potential incidents reported to IDS. Overall, IDS provides good information about national trends and frequency of incidents for pesticides and can provide valuable insights into the hazard and/or exposure potential of a pesticide.

- **The National Poison Data System (NPDS)** -- formerly called the Toxic Effects Surveillance System (TESS) -- is maintained by the American Association of Poison Control Centers (AAPCC) and is supported with funding from several federal agencies. NPDS is a computerized information system with geographically specific and near real-time reporting. Although the main mission of Poison Control Centers is in helping callers respond to emergencies, NPDS data can help identify emerging problems in chemical product safety. Hotlines at 61 PCC's nationwide are open 24/7, 365 days a year and are staffed by specially trained nurses, pharmacists, and other clinical health care specialists to provide poisoning information. Using computer assisted data entry, standardized protocols, and strict data entry criteria, local callers report incidents. These reported incidents are retained locally and are updated in summary form to the national database maintained by AAPCC. Information calls are tallied separately and not counted as incidents. The PCC system covers nearly all the US and its territories and has undergone major computer enhancements since 2001.

  NPDS includes mainly non-occupational incidents. NPDS does not include narrative information and the product information may not be complete. NPDS provides severity rankings and symptom information that are designated/recorded by trained specialists, and the agency has relatively high confidence in this information. NPDS also provides some information on the likelihood of the adverse effect being a result of the reported exposure. Overall, NPDS provides good information about national trends, frequency of incidents for pesticides, as well as the hazard potential for particular pesticides. However, resource limitations permit the agency to only access AAPCC summary reports published each year (e.g., see http://www.aapcc.org/annual-reports/) and these serve as a supplement to other data sources for which the agency has more complete access.

- **The National Pesticide Information Center (NPIC)** (http://npic.orst.edu/index.html) is funded by EPA to serve as a source of objective, science-based pesticide information in response to inquiries and to respond to incidents. NPIC functions nationally during weekday business hours and is a cooperative effort between Oregon State University (currently) and EPA; it is intended to serve as a source of objective, science-based pesticide information and to respond to inquiries from the public and to incidents. Similar to Poison Control Centers, NPIC's primary purpose is not to collect incident data (about 10% of NPIC's annual calls are considered "incident" related), but rather to provide information to inquirers on a wide range of pesticide topics, and direct them to other sources for pesticide incident investigation and emergency treatment. Nevertheless, NPIC does collect information about incidents (approximately 4000 incidents per year) from inquirers and records that information in a database. NPIC is a source of national incident information, but generally receives fewer reports than IDS. Regardless, if a high frequency is observed in IDS for a given pesticide or

product, NPIC provides a source of information that can prove valuable in determining consistency across national data sets.

As with IDS and PCC, the incidents in NPIC are mainly non-occupational. NPIC incidents include narratives and product information when the caller provides the information. Although the scope is national, there are significantly fewer incidents reported to NPIC than to NPDS or IDS but considerably more information is provided and the agency can request custom reports on an as-needed basis. Hazard information includes severity rankings, route of exposure and symptoms – which are recorded by trained personnel. NPIC also provides information on how likely the link between exposure and adverse effect is (which they call a certainty index). NPIC also publishes annual reports and analyses in the open literature which are valuable resources.

- The Center for Disease Control and Prevention National Institute for Occupational Health (CDC/NIOSH) manages a pesticide surveillance program and database entitled the **Sentinel Event Notification System for Occupational Risk (SENSOR)-Pesticides.**[24] This database includes pesticide illness case reports in 12 states from 1998-2013. Participating states are: California, Florida, Iowa, Louisiana, Michigan, Nebraska, New Mexico, New York, North Carolina, Oregon, Texas and Washington. The participating states for a given year vary depending on state and federal funding for pesticide surveillance.

  Cases of pesticide-related illnesses in the SENSOR-Pesticides database are ascertained from a variety of sources, including: reports from local Poison Control Centers, state Department of Labor workers' compensation claims when reported by physicians, reports from state Departments of Agriculture, and physician reports to state Departments of Health. Although both occupational and non-occupational incidents are included in the database, the SENSOR coordinators primarily focus their follow-up case investigation efforts on the occupational pesticide incidents. The SENSOR coordinator at the state Department of Health will follow-up with cases and work to obtain medical records in order to verify exposure scenario, symptoms, severity, and health outcome. Using standardized protocol and case definitions, SENSOR coordinators at state Departments of Health enter the incident interview description provided by the case, medical report, physician and patient into the SENSOR data system.

  All SENSOR-Pesticides cases must report a minimum of two health effects in order to be included in the aggregate database that EPA uses for incident

[24] SENSOR-Pesticides webpage: http://www.cdc.gov/niosh/topics/pesticides/overview.html

ED_002061_00044424-00041

analyses. Evidence for each case is evaluated, based on the NIOSH case classification matrix, for its causal relationship between exposure and illness. 98% of SENSOR-Pesticides cases are classified as definite, probable, or possible, and 2% of the cases are classified as suspicious. Unlikely, asymptomatic, and unrelated cases, as well as those with insufficient information, are not included in the SENSOR-Pesticides database.

Overall, SENSOR-Pesticides provides very useful information on both occupational and non-occupational incidents, and sometimes valuable insights into the hazard and/or exposure potential of a pesticide. SENSOR-Pesticides also conducts analyses of its own data and publishes these in the Morbidity and Mortality Weekly. Unlike the aforementioned databases and although it contains both non-occupational/residential and occupational incidents, SENSOR's has traditionally focused on occupational pesticide incidents, and is of particular value in providing that information. SENSOR-Pesticides data from 1998-2011 is available online at: http://wwwn.cdc.gov/Niosh-whc/Home/Pesticides.

- **The California Pesticide Illness Surveillance Program (PISP)** is maintained by the State of California. This database documents pesticide-related illnesses and injuries. Case reports are received from physicians and via workers' compensation records. The local County Agricultural Commissioner investigates the circumstances of the exposure. Medical records and investigative findings are then evaluated by California's Department of Pesticide Regulation (DPR) technical experts and entered into an illness registry. All reported pesticide illnesses in the California PISP program are investigated by the county agricultural commissioners, and the DPR evaluates the reports and compiles them into a database, which is used to improve the state's program to protect workers and others from the adverse effects of pesticide exposure (http://apps.cdpr.ca.gov/calpiq/).

Currently, OPP evaluates human incident data on a chemical-specific basis. Incidents from each database are analyzed for hazard potential (deaths, frequency of more severe incidents, and patterns/trends of reported symptoms) and exposure potential (frequency of incidents/ trends over time, patterns/trends of exposure scenarios, of factors affecting exposure or of products). When evaluating human incident data from the above databases, OPP considers several general criteria. OPP considers the relative severity and frequency of symptoms. Additionally, OPP generally has greater confidence in reports in which temporal association can be verified or are at least plausible. Lastly, other factors that are used to evaluate human incident data include evidence of an exposure response association, consistency in reported health effects, biological plausibility of reported health effects, elimination of alternative causes of health effect such as pharmaceutical use, and the specificity of the observed symptoms and health effects. Additionally, narratives of more severe incidents are often evaluated for any temporal association between time-of-exposure and effects reported to determine whether an association is supported by the circumstances. For example, a heart attack in an elderly individual that occurs three

months following an indoor pesticide application may be determined not to be a likely causal association. On the other hand, a severe incident occurring at or shortly after the time of exposure with symptoms consistent with known symptomology for the pesticide class and that occurs without prior medical history may suggest that causal inference is more justified.

In sum, then, incident data -- consisting of both medical case reports/case series appearing the medical and human toxicological literature and toxico-surveillance data derived from the databases that EPA either maintains, funds, or accesses -- can provide useful, complementary information that assists OPP in evaluating the real-world risks of pesticides.

VI. SUMMARY & CONCLUSIONS

This framework describes important factors in reviewing epidemiology and human incident data and describes a proposed WOE analysis for incorporating such data in pesticide human health risk assessment. OPP uses the best available data across multiple lines of evidence and from *in vitro*, *in vivo*, and *in silico* data sources. OPP uses a WOE approach when integrating data from multiple sources to take into account for quality, consistency, relevancy, coherence and biological plausibility using modified Bradford Hill criteria as an organizational tool. Application of WOE analysis is an integrative and interpretive process routinely used by EPA according to in scientific analysis outlined in its risk assessment guidelines. The WOE analysis also evaluates the quality of the combined data set and is consistent with the level of effort and complexity that is appropriate for a particular scientific assessment (U.S. EPA, 2002). OPP acknowledges that toxicology and risk assessment are currently undergoing transformational changes towards implementing the new vision of 21st century toxicity testing. As these transformation changes occur, OPP will update this approach as appropriate.

ED_002061_00044424-00043

VII. REFERENCES

American Statistical Association. 2016 "AMERICAN STATISTICAL ASSOCIATION RELEASES STATEMENT ON STATISTICAL SIGNIFICANCE AND P-VALUES" March 7. Available at: https://www.amstat.org/asa/files/pdfs/P-ValueStatement.pdf

Ankley, GT, Bennett RS, Erickson RJ et al. 2010. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. Environ Toxicol Chem 29(3):730–741.

Balshem H, Helfand M, Schünemann HJ, Oxman AD, Kunz R, Brozek J, Vist GE, Falck-Ytter Y, Meerpohl J, Norris S, and Guyatt GH. 2011. GRADE guidelines: 3. Rating the quality of evidence. J Clin Epidemiol. 2011 Apr;64(4):401-6. doi: 10.1016/j.jclinepi.2010.07.015. Epub 2011 Jan 5.

Blair A, Tarone R, Sandler D, Lynch C, Rowland A, Wintersteen W, Steen W, Dosemeci M, and Alavanja M. 2002. Reliability of reporting on lifestyle and agricultural factors by a sample of participants in the agricultural health study from Iowa. Ann Epidemiol. Oct 1;10(7):478.

Borenstein M, Hedges LV, Higgins JPT, and Rothstein HR. 2009. Introduction to Meta-analysis. John Wiley and Sons, Chichester, UK.

Boyes WK, Moser VC, Geller AM, Benignus VA, Bushnell PJ, and Kamel F. 2007. Integrating epidemiology and toxicology in neurotoxicity risk assessment. Hum. Exp. Toxicol. 26(4):283-93.

Calderon RL 2000. Measuring risks in humans: the promise and practice of epidemiology. Food and Chemical Toxicology. 38:S59-S63.

Carlile DJ, Zomorodi, K , and Houston, JB. 1997. Scaling factors to relate drug metabolic clearance in hepatic microsomes, isolated hepatocytes, and the intact liver: studies with induced livers involving diazepam. Drug Metab. Dispos. 25(8):903-911.

Checkoway H, Pearce, N, and Kriebel D. 2004. Research Methods in Occupational Epidemiology, 2nd Edition. Oxford University Press, New York.

Clark LH, Setzer RW, and Barton, HA. 2004. Framework for evaluation of physiologically-based pharmacokinetic models for use in safety or risk assessment. Risk Anal. 24(6):1697-1717.

FIFRA Scientific Advisory Panel. (2010). February 2 - 4, 2010: Incorporation of Epidemiology and Human Incident Data into Human Risk Assessment.

Glymor, MM and Greenland, S. 2012. "Causal Diagrams" in Rothman, KJ, Greenland, S, Poole, C, Lash, TL. Modern epidemiology. 3rd ed. Lippincott Williams & Wilkins, Philadelphia. pp. 183-212.

Gordis, L. 2009. Epidemiology. 4th Edition. Saunders-Elsevier, New York.

Greenland, S. 1998. "Basic Methods for Sensitivity Analysis and External Adjustment" in Rothman, KJ and Greenland, S. Modern Epidemiology. 2nd ed. Lippencott-Raven Publishers, Philadelphia. pp.343-357.

Greenland, S. and Lash T. 2012. "Bias Analysis" in Rothman KJ, Greenland S, Poole C, Lash TL. Modern epidemiology. 3rd ed. Lippincott Williams & Wilkins, Philadelphia. pp. 345-380.

Greenland, S and O'Rourke, K. 2012. "Meta-analysis" in Rothman, KJ, Greenland S, Poole C, and Lash, TL. Modern epidemiology. 3rd ed. Lippincott Williams & Wilkins, Philadelphia. pp. 652-682.

Grimes, DA and Schultz, KF. 2005. Compared to What? Finding controls for case-control studies. Lancet 365: 1429-1433.

Gustafson P[1], and McCandless LC. 2010. Probabilistic approaches to better quantifying the results of epidemiologic studies. Int J Environ Res Public Health. 2010 Apr;7(4):1520-39. doi: 10.3390/ijerph7041520..

Hartung T. 2010. Evidence-based toxicology - the toolbox of validation for the 21st century? ALTEX. 2010;27(4):253-63.

Hertz-Picciotto I. 1995. Epidemiology and quantitative risk assessment: a bridge from science to policy. American Journal of Public Health. 85(4): 484-491.

Hill AB. 1965. The Environment and Disease: Association or Causation? President's Address. Proceedings of the Royal Society of Medicine 58:293-300

Hoppin JA, Yucel F, Dosemeci M, and Sandler DP. 2002. Accuracy of self-reported pesticide use duration information for licensed pesticide applicators in the Agricultural Health Study. Journal of Exposure Analysis and Environmental Epidemiology, 12: 313-318.

IPCS (2005). Chemical-specific adjustment factors for interspecies differences and human variability: guidance document for use of data in dose/concentration response assessment. Harmonization Project Document 2. World Health Organisation, International Programme on Chemical Safety, Geneva, Switzerland.

Ioannidis JP. 2008. Why Most Discovered True Associations Are Inflated. Epidemiology. 19(5): 640-8.

ED_002061_00044424-00045

Jurek AM, Maldonado G, Greenland G, and Church TR. 2006. Exposure-measurement Error is Frequently Ignored When Interpreting Epidemiological Study Results. Europ. J. Epid. 21: 871-876.

Kelsey JL, Whittemore AS, Evans AS, and Thompson WD. 1996. Methods in Observational Epidemiology. 2nd ed. Oxford University Press, New York.

LaKind JS, Sobus JR, Goodman M, Barr DB, Fürst P, Albertini RJ, Arbuckle TE, Schoeters G, Tan YM, Teeguarden J, Tornero-Velez R, and Weisel CP. 2014. A proposal for assessing study quality: Biomonitoring, Environmental Epidemiology, and Short-lived Chemicals (BEES-C) instrument. Environ Int. Dec;73:195-207. doi: 10.1016/j.envint.2014.07.011.

Lash, TL, Fox, MP, and Fink, AK. 2009. Applying Quantitative Bias Analysis to Epidemiologic Data. Springer, New York.

Lash TL, Fox MP, MacLehose RF, Maldonado G, McCandless LC, and Greenland S. 2014. Good practices for quantitative bias analysis. International Journal of Epidemiology p. 1-17.

Lilienfeld AM and Lilienfeld D. 1979. Foundations of epidemiology, 2nd ed. Oxford University Press, New York.

Mausner JS and Kramer S. 1985. Epidemiology, 2nd ed. W.B. Saunders, Philadelphia.

Meek, ME, Bucher, JR, Cohen, SM et al. 2003. A framework for human relevance analysis of information on carcinogenic modes of action. Crit. Rev. Toxicol. 33:591-653.

Meek ME, Boobis A, Cote I, Dellarco V, Fotakis G, Munn S, Seed J, and Vickers, C. 2014. New developments in the evolution and application of the WHO/IPCS framework on mode of action/species concordance analysis. J Appl Toxicol. Jan;34(1):1-18.

Muñoz-Quezada MT[1], Lucero BA, Barr DB, Steenland K, Levy K, Ryan PB, Iglesias V, Alvarado S, Concha C, Rojas E, and Vega C. 2013. Neurodevelopmental effects in children associated with exposure to organophosphate pesticides: a systematic review. Neurotoxicology. Dec;39:158-68. doi: 10.1016/j.neuro.2013.09.003.

Needham LL, Calafat AM, and Barr DB. 2007. Uses and issues of biomonitoring. Int. J. Hyg. Environ. Health. 210: 229-238.

Nieuwenhuijsen MJ. 2003. Exposure Assessment in Occupational and Environmental Epidemiology. Oxford University Press, New York.

NRC (National Research Council). 1983. Risk Assessment in the Federal Government: Managing the Process. National Academy Press, Washington, DC.

ED_002061_00044424-00046

NRC (National Research Council). 1991. Environmental Epidemiology, Volume 1: Public Health and Hazardous Wastes. National Academy Press, Washington, DC.

NRC (National Research Council). 1994. Science and Judgment in Risk Assessment. National Academy Press, Washington, DC.

NRC (National Research Council). 1997. Environmental Epidemiology, Volume 2: Use of the Gray Literature and Other Data in Environmental Epidemiology. National Academy Press, Washington, DC.

NRC (National Research Council). 2007. Toxicity Testing in the 21st Century: A Vision and a Strategy. National Academy Press, Washington, DC.

NRC (National Research Council). 2009: Science and Decisions: Advancing Risk Assessment. National Academy Press, Washington, DC.

NRC (National Research Council). 2011. Review of the Environmental Protection Agency's draft IRIS assessment of formaldehyde. National Academies Press, Washington, DC. http://www.nap.edu/catalog/13142.html

NRC (National Research Council). 2014. Review of EPA's Integrated Risk Information System (IRIS) process. The National Academies Press, Washington, DC. http://www.nap.edu/catalog.php?record_id=18764

Ntzani EE, Chondrogiori MNG, Evangelou E and Tzoulaki I. 2013. Literature review of epidemiological studies linking exposure to pesticides and health effects. External Scientific Report. EFSA supporting publication 2013-EN-497. 159 pp. Available online at www.efsa.europa.eu/publications.

Organisation for Economic Co-operation and Development. 2013. GUIDANCE DOCUMENT ON DEVELOPING AND ASSESSING ADVERSE OUTCOME PATHWAYS, Series on Testing and Assessment, No. 184, ENV/JM/MONO(2013)6, April 17, 2013. http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2013)6&doclanguage=en

Paddle GM, and Harrington JM. 2000. Environmental epidemiology--strengths and weaknesses. Int Arch Occup Environ Health. 73:7-14.

Porta MJM. 2014. A Dictionary of Epidemiology. 6th ed. Oxford University Press, New York.

Purdue Pesticides Programs. 2003. Pesticides and Epidemiology: Unraveling Disease Patterns. Purdue University Cooperative Extension Service. http://www.btny.purdue.edu/Pubs/PPP/PPP-43.pdf.

Rothman KJ and Greenland S. 2012. Modern epidemiology. 3rd ed. Lippincott Williams & Wilkins, Philadelphia.

ED_002061_00044424-00047

Rothman, KJ, Greenland S, Poole C, and Lash TL. 2012a. "Causation and Causal Inference" in Rothman, KJ, Greenland S, Poole C, and Lash TL. Modern epidemiology. 3rd ed. Lippincott Williams & Wilkins, Philadelphia. pp. 5-31.

Rooney AA, Boyles AL, Wolfe MS, Bucher JR, and Thayer KA. 2014. Systematic review and evidence integration for literature-based environmental health science assessments. Environ Health Perspect. Jul;122(7):711-8. doi: 10.1289/ehp.1307972.Seed, J., E.W.

Carney, RA, Corley, et al. 2005. Overview: Using mode of action and lifestage information to evaluate the human relevance of animal toxicity data. Crit. Rev. Toxicol. 35(8-9):664-672.

Schultz, KF and Grimes DA. 2002. Case-control studies: research in reverse. Lancet: 359:431-434.

Sonich-Mullin C, Fielder R, Wiltse J, et al. 2001. IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. Regul Toxicol Pharmacol. 34:146-152.

Sterne JAC, Higgins JPT, and Reeves, BC. on behalf of the development group for ACROBAT-NRSI. A Cochrane Risk Of Bias Assessment Tool: for non-randomized studies of interventions (ACROBAT-NRSI), Version 1.0. 0, 24 September 2014." *www. riskofbias. info* (2015)

Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Bennie D, Moher D, Becker BJ, Sipe TA, and Thacker SB for the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) Group. 2000. J. American Medical Association 283(15): 2008-2012.

Szklo M and Nieto FJ. 2004. Epidemiology: Beyond the Basics. Jones and Bartlett Publishers, Boston, MA.

U.S. Environmental Protection Agency. (1999). Guidelines for carcinogen risk assessment. Risk Assessment Forum. SAB review draft. Washington, DC: U.S. Environmental Protection Agency. www.epa.gov/ncea/raf/crasab.htm.

U.S. EPA (U.S. Environmental Protection Agency). 2000. Science Policy Council Handbook: Risk Characterization. U.S. Environmental Protection Agency, Office of Research and Development, Office of Health and Environmental Assessment, Washington, DC. EPA/100/B-00/002. Available at http://www.epa.gov/iris/backgr-d.htm.

U.S. EPA (U.S. Environmental Protection Agency). 2001. General Principles For Performing Aggregate Exposure And Risk Assessments Washington, DC. Available at https://www.epa.gov/sites/production/files/2015-07/documents/aggregate.pdf

U.S. Environmental Protection Agency. 2001a. "Guidance on Cumulative Risk Assessment of Pesticide Chemicals that Have a Common Mechanism of Toxicity." Office of Pesticide Programs, Office of Prevention, Pesticides, and Toxic Substances, U.S. Environmental Protection Agency. Washington, DC.

ED_002061_00044424-00048

U.S. EPA (U.S. Environmental Protection Agency). 2002a. Guidelines for Ensuring and Maximizing the Quality, Objectivity, Utility and Integrity for Information Disseminated by the Environmental Protection Agency. Office of Environmental Information, Washington, DC. EPA/260/R-02/008. Available at http://www.epa.gov/quality/informationguidelines/documents/EPA_InfoQualityGuidelines.pdf.

U.S. EPA (U.S. Environmental Protection Agency). 2002b. A Review of the Reference Dose and Reference Concentration Processes. December. Risk Assessment Forum. Washington, DC. EPA/630/P-02/002F.

U.S. Environmental Protection Agency. 2002c. "Guidance on Cumulative Risk Assessment of Pesticide Chemicals That Have a Common Mechanism of Toxicity"; January 14, 2002.
U.S. EPA (U.S. Environmental Protection Agency). 2004. An Examination of EPA Risk Assessment Principles & Practices. Staff Paper Prepared for the U.S. Environmental Protection Agency by members of the Risk Assessment Task Force. Office of the Science Advisor. U.S. Environmental Protection Agency, Washington, DC. EPA/100/B-04/001.

U.S. EPA (U.S. Environmental Protection Agency). 2005. Guidelines for Carcinogen Risk Assessment. U.S. Environmental Protection Agency, Risk Assessment Forum, Washington, DC. EPA/630/P-03/001F. Federal Register 70(66):17765-17817. Available at http://www.epa.gov/raf.

U.S. EPA. (U.S. Environmental Protection Agency). 2006a. Harmonization in Interspecies Extrapolation: Use of $BW^{3/4}$ as Default Method in Derivation of the Oral RfD (External Review Draft). U.S. Environmental Protection Agency, Washington, DC. EPA/630/R-06/001. Available at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=148525.

U.S. EPA (U.S. Environmental Protection Agency). 2006b. Approaches for the Application of Physiologically Based Pharmacokinetic (PBPK) Models and Supporting Data in Risk Assessment (Final Report). U.S. Environmental Protection Agency, Washington, DC. EPA/600/R-05/043F.

U.S. EPA (U.S. Environmental Protection Agency). 2009. Scientific Issues Associated with Field Volatilization of Conventional Pesticides. U.S. Environmental Protection Agency, Washington, DC. OPP Regulatory Public Docket EPA-HQ-OPP-2009-0687.

U.S. EPA (U.S. Environmental Protection Agency). 2010. Draft Framework for Incorporating Human Epidemiologic and Incident Data in Health Risk Assessment, January 7, 2010.

U.S. EPA (U.S. Environmental Protection Agency). 2012. Guidance for Considering and Using Open Literature Toxicity Studies to Support Human Health Risk Assessment U.S. Environmental Protection Agency, Washington, DC. Office of Pesticide Programs. Available at: https://www.epa.gov/sites/production/files/2015-07/documents/lit-studies.pdf

ED_002061_00044424-00049

U.S. EPA (U.S. Environmental Protection Agency). 2014a. Framework for Human Health Risk Assessment to Inform Decision Making. https://www.epa.gov/sites/production/files/2014-12/documents/hhra-framework-final-2014.pdf

U.S. EPA (U.S. Environmental Protection Agency). 2014b. Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation. http://www2.epa.gov/osa/guidance-applying-quantitative-data-develop-data-derived-extrapolation-factors-interspecies-and

U.S. EPA (U.S. Environmental Protection Agency). 2015. Preamble to the Integrated Science Assessments. National Center for Environmental Assessment, RTP Division, Office of Research and Development, USEPA. https://yosemite.epa.gov/sab/sabproduct.nsf/0/33E1AD305287588F85257D20006BE8CC/$File/ISA_PREAMBLE_FINAL2015.PDF

van den Brandt P, Voorrips L, Hertz-Picciotto I, Shuker D, Boeing H, Speijers G, Guittard C, Kleiner J, Knowles M, Wolk A, and Goldbohm A. 2002. The contribution of epidemiology. Food Chem Toxicol. Feb-Mar;40(2-3): 387-424.

Vandenbroucke JP, Van Elm E, Altman DG, Gotzsche PC, Mulroew CD, Pockock SJ, Pool C, Schlesseman JJ, and Egger, M. 2007. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): Explanation and Elaboration. Ann. Int. Med. Vol 147(8): W163-193

Von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, and Vandenbroucke JP. 2014. The Strengthening of the Reporting of Observational Studies in Epidemiology (STROBE) Statement: Guidelines for Reporting Observational Studies. Int. J. Surgery 12: 1495-99.

Wacholder S, McLaughlin JK, Silverman DT, and Mandel JS. 1992a. Selection of Controls in Case-Control Studies I. Principles. American J. Epid. 135(9): 1019-1028.

Wacholder S, McLaughlin JK, Silverman DT, and Mandel JS. 1992b. Selection of Controls in Case-Control Studies II. Types of Controls. American J. Epid. 135(9): 1029-1041.

Wacholder, S, McLaughlin, JK, Silverman, DT, and Mandel, JS. 1992c. Selection of Controls in Case-Control Studies III. Design Options. American J. Epid. 135(9): 1042-1050.

Woodruff TJ[1] and Sutton P. 2014. The Navigation Guide systematic review methodology: a rigorous and transparent method for translating environmental health science into better health outcomes. Environ Health Perspect. Oct;122(10):1007-14. doi: 10.1289/ehp.1307175.

Zartarian V., Bahadori T, and McKone T. 2005. Adoption of an official ISEA glossary. Journal of Exposure Analysis and Environmental Epidemiology 15:1-5

ED_002061_00044424-00050

ED_002061_00044424-00051

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

OFFICE OF
CHEMICAL SAFETY AND
POLLUTION PREVENTION

MEMORANDUM

Date: December 29, 2016

SUBJECT: Updated Literature Review on Neurodevelopment Effects & FQPA Safety Factor Determination for the Organophosphate Pesticides

PC Code: See Below
Decision No.: 524105
Petition No.: None
Risk Assessment Type: None
TXR No.: 0057561
MRID No.: None

DP Barcode: 437043
Registration No.: None
Regulatory Action: None
Case No.: None
CAS No.: See Below
40 CFR: None

FROM: Ashlee Aldridge, MPH, Epidemiologist
Health Effects Division (7509P)
Office of Pesticide Programs

Anna Lowit, Ph.D., Senior Science Advisor
Immediate Office
Office of Pesticide Programs (7501P)

Virginia C Moser, PhD, DABT, Fellow ATS, Toxicologist
Toxicity Assessment Division
Office of Research and Development

THROUGH: Dana Vogel, Director
Health Effects Division (7509P)

TO: Yu-Ting Guilaran, Director
Pesticide Re-Evaluation Division (7508P)

This paper supports the use of the 10X FQPA Safety Factor in the individual organophosphate human health risk assessments. This paper updates the September, 2015 paper based on comments from the public and addition of new epidemiology papers from the open literature. No additional studies have been added to the laboratory animal toxicology sections of the literature review.

Chemical	PC Code	CAS No.
Dicrotophos	035201	141-66-2
Fosthiazate	129022	98886-44-3
Coumaphos	036501	56-72-4
Terbufos	105001	13071-79-9
Profenofos	111401	41198-08-7
Bensulide	009801	741-58-2
Diazinon	057801	333-41-5
Ethoprop	041101	13194-48-4
Dimethoate	035001	60-51-5
Malathion	057701	121-75-5
Phosmet	059201	732-11-6
Chlorethoxyfos	129006	54593-83-8
Acephate/ Methamidiphos	103301/ 101201	30560-19-1/ 10265-92-6
Pirimiphos-methyl	108102	29232-93-7
TCVP	083701	961-11-5
Tribufos	074801	78-48-8
Phorate	057201	298-02-2
Phostebupirim	129086	96182-53-5
DDVP	084001	62-73-7
Naled	034401	300-76-5
Trichlorfon	057901	52-68-6
Fenamiphos	100601	22224-92-6
AZM	058001	86-50-0
Methidathion	100301	950-37-8
Propetamphos	113601	31218-83-4
ODM	058702	301-12-2
Disulfoton	032501	298-04-4
Methyl parathion	053501	298-00-0
Temephos	059001	3383-96-8
Chlorpyrifos-methyl	059102	5598-13-0

Updated Literature Review on Neurodevelopment Effects & FQPA Safety Factor Determination for the Organophosphate Pesticides

Office of Pesticide Programs
US Environmental Protection Agency

December 29, 2016

ED_002061_00044425-00003

Table of Contents

1.0 Introduction and Background 6
2.0 Literature Review 8
2.1 Developmental Neurotoxicity (DNT) Research on OPs other than Chlorpyrifos: Laboratory Animal Studies 8
2.1.1 Literature Search Strategy & Results 9
2.1.2 Integration of Literature: Neurobehavioral Domains 11
2.1.3 Integration with AChE Inhibition 22
2.1.4 Summary of Findings from the Developmental Neurotoxicity (DNT) Guideline Studies 23
2.1.5 Conclusions on *In Vivo* Laboratory Animal Studies 24
2.2 Epidemiology Research on OPs other than Chlorpyrifos 25
2.2.1 Overview of Literature Reviews: 2012/2014, 2015, and updated in 2016 25
2.2.2 Literature Search Methodology 26
2.2.3 Breadth and Depth of the 2015/2016 Literature Review 27
Table 2.2-aStudy Designs, Exposure Measurement Methods, and Outcome Measurement Methods Used across the 2015/2016 31
Table 2.2-b Detailed Neurological Outcome Measurement Methods Used across the 2015/2016 Review Studies 33
2.2.4 Study Designs & Considerations for Study Quality Evaluation 35
2.2.4.1 Study Designs 35
2.2.4.2 Considerations for Study Quality 37
Table 2.2.4-1 Study Quality Considerations 38
2.2.4.2.1 Exposure Measures 39
Table 2.2.4-2 CDC Table of Organophosphate Pesticides and Their Dialkyl Phosphate Metabolites (2008) 41
2.2.4.2.2 Neurological and Other Outcome Measures 42
2.2.4.2.3. Statistical Analysis 42
2.2.4.2.4 Confounding 43
2.2.4.2.5 Risk of Bias 43
2.2.5 Review of Quality Results 44
2.2.5.1 "High" Quality Group 44
Table 2.2.5.1-1. High Quality Studies from 2015 and 2016 reviews: Summary of Study Design Elements Impacting Study Quality Assignment 45
Table 2.2.5.1-2 High Quality Studies from 2012/2014 literature search of Children's Environmental Health Epidemiology Studies 49
2.2.5.2 "Moderate" Quality Group 64
Table 2.2.5.2-1. Moderate Quality Studies: Summary of Study Design Elements Impacting Study Quality Assignment 65
2.2.5.3 "Low" Quality Group 68
2.2.6 Assessment of Epidemiological Studies for Relevance to Analysis 68
2.2.7 Birth Outcome Epidemiologic Studies 70
2.2.8 Neurodevelopment Outcome Epidemiologic Studies 71
2.2.8.1 CCCEH, CHAMACOS, & Mt. Sinai Cohorts 72

2.2.8.2 HOME Cohort 77
2.2.8.3 Meta-analysis (Engel et al, 2015) 78
2.2.8.4 Other Neurodevelopmental Studies 79
3.0 Weight of Evidence Analysis: Integration Across Multiple Lines of Evidence 80
3.1 Dose-Response Relationships & Temporal Concordance 82
3.2 Strength, Consistency & Specificity 83
3.2.1 Strength 83
3.2.2 Consistency 84
3.2.3 Specificity 89
3.3 Biological Plausability & Coherence 91
4.0 10X FQPA Safety Factor for Infants and Children 92
5.0 References 94
6.0 Appendices 106
Appendix 1. Table of *In Vivo* Developmental Neurotoxicity Studies of OPs. 107
Appendix 2. Summary of Guideline DNT Studies Submitted to the Agency for OPs other than Chlorpyrifos. 112
Appendix 3. Low Quality Studies: Summary of Study Design Elements Impacting Study Quality Assignment 114
Appendix 4. Table of Systematic Review Analysis: Initial Search Research & Initial Exclusion Criteria. 118
Appendix 5. Table of Systematic Review Analysis: Second Tier Exclusion Criteria. . 157
Appendix 6. Plausible hypotheses on MOA/AOP for neurodevelopmental outcomes (Extracted from Section 4.4.3. RHHRA for chlorpyrifos) 171

Literature Review on Neurodevelopment Effects & FQPA Safety Factor Determination for the Organophosphate Pesticides

1.0 Introduction and Background

Organophosphate pesticides (OPs), widely used in agricultural and household pesticidal applications, act by inhibiting acetylcholinesterase (AChE) in nerve cells. OPs share the ability to inhibit AChE via phosphorylation of the active site of the enzyme leading to accumulation of acetylcholine and ultimately neurotoxicity, this class of pesticides is subject to assessment of cumulative risk (USEPA, 1999; 2006). Historically the agency has used inhibition of AChE as the point of departure for OP human health risk assessments (HHRAs).

Newer lines of research on OPs in the areas of potential modes of action/adverse outcome pathways (MOAs/AOPs), [1] *in vivo* animal studies, and notably epidemiological studies in mothers and children, have raised uncertainty about the agency's risk assessment approach with regard to the potential for neurodevelopmental effects in fetuses and children. Many of these studies focus on chlorpyrifos and have been the subject of review by the agency since 2008 (See Appendix 6).

The agency has taken a stepwise, objective and transparent approach in evaluating, interpreting, and characterizing the strengths and uncertainties associated with all of the available lines of scientific information related to the potential for adverse neurodevelopmental effects in infants and children. The stepwise evaluation began with the September 2008 FIFRA Scientific Advisory Panel (SAP) meeting involving a preliminary review of the epidemiology studies on three children's cohorts, with a particular focus on women and children and exposure to chlorpyrifos (USEPA, 2008), followed by the draft "Framework for Incorporating Human Epidemiologic & Incident Data in Health Risk Assessment" for integration of epidemiology with other types of experimental data (USEPA, 2010; FIFRA SAP 2010a,b). In December, 2016, OPP's Framework for Incorporating Human Epidemiologic & Incident Data in Risk Assessments for Pesticides (USEPA, 2016c) was finalized based on input from the 2010 FIFRA SAP, public comment and experience gained over the last few years.

In 2012, the agency convened another meeting of the FIFRA SAP focused on chlorpyrifos which incorporated the newest experimental data related to AChE inhibition and both cholinergic and non-cholinergic adverse outcomes, including neurodevelopmental studies on behavior and cognition effects (FIFRA SAP 2012). Similarly, the agency also performed a more in-depth analysis of the biomonitoring data and of epidemiological studies from three major children's health epidemiology cohort studies in the U.S., as well as plausible hypotheses on MOAs/AOPs

[1] Mode of action (MOA) and adverse outcome pathways (AOPs) describe a set of measureable key events that make up the biological processes leading to an adverse outcome and the causal linkages between such events.

ED_002061_00044425-00006

leading to neurodevelopmental outcomes (USEPA 2012; Appendix 6). Following the 2012 SAP meeting, the agency solicited additional input from federal experts in the areas of Magnetic Resonance Imaging (MRI) and neurobehavioral testing in children to further clarify results obtained by examination of the epidemiological studies.[2] In December, 2014, the agency released "Chlorpyrifos: Revised Human Health Risk Assessment for Registration Review" which included the use of a physiologically-based pharmacokinetic/pharmacodynamic (PBPK-PD) model to derive human PODs, which obviated the need for the animal to human extrapolation factor, and refined intra-species factors for some lifestages (USEPA 2014). The chlorpyrifos 2014 revised HHRA also included retention of the 10X FQPA Safety Factor due to uncertainty regarding the degree of protection the endpoint of AChE inhibition provides for potential neurodevelopmental effects (USEPA, 2014).

A review of the scientific literature on potential MOA/AOP leading to effects on the developing brain was conducted for the 2012 FIFRA SAP meeting (USEPA, 2012) and updated for the 2014 chlorpyrifos revised HHRA (USEPA 2014; Summarized in Appendix 6). In short, multiple biologically plausible hypotheses and pathways are being pursued by researchers including: AChE as a morphogen; cholinergic system; endocannabinoid system; reactive oxygen species; serotonergic system; tubulin, microtubule associated proteins and axonal transport. However, no one pathway has sufficient data to be considered more plausible than the others. Among the available studies, there are effects which are either as sensitive as or more sensitive than AChE inhibition. The fact that there are, however, sparse data to support the *in vitro* to *in vivo* extrapolation, or the extrapolation from biological perturbation to adverse consequence, significantly limits their quantitative use in risk assessment. The SAP concurred with the agency in 2008 and 2012 about the lack of definable key events in a MOA/AOP leading to developmental neurobehavioral effects. Since the 2014 literature review, there have been no substantive changes in the ability to define and quantify steps in an MOA/AOP leading from exposure to effects on the developing brain. The lack of an established MOA/AOP makes quantitative use of the epidemiology study in risk assessment challenging, particularly with respect to dose-response, critical duration of exposure, and window(s) of susceptibility. The agency will continue to monitor the scientific literature for studies on the AOP for neurodevelopmental effects but this document does not include an updated literature review on this line of evidence.

This document (Section 2.0) provides the literature review of *in vivo* laboratory animal studies and epidemiology studies for OPs other than chlorpyrifos to support the single chemical HHRAs. It also provides an integrated weight of evidence (WOE) analysis for all the OPs to support retention of the 10X FQPA Safety Factor (Section 3.0). Section 4.0 states that the 10X FQPA Safety Factor is being retained for all the OPs listed in the table on page 2 above.

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

2.0 Literature Review

In recent years, the National Academy of Sciences has encouraged the agency to move towards systematic review processes to enhance the transparency of scientific literature reviews that support chemical-specific HHRAs to inform regulatory decision making[3]. The NRC defines systematic review as "a scientific investigation that focuses on a specific question and uses explicit, pre-specified scientific methods to identify, select, assess, and summarize the findings of similar but separate studies".[4] Consistent with NRC's recommendations, EPA's Office of Chemical Safety and Pollution Prevention (OCSPP) is currently developing systematic review policies and procedures. In short, OCSPP employs fit-for-purpose systematic reviews that rely on standard methods for collecting, evaluating and integrating the scientific data supporting the agency's decisions. The literature review described here uses concepts consistent with systematic review such as detailed tracking of search terms and which literature have been included or excluded.

2.1 Developmental Neurotoxicity (DNT) Research on OPs other than Chlorpyrifos: Laboratory Animal Studies

The literature on neurobehavioral effects of developmental exposure to chlorpyrifos was summarized and discussed at the 2012 FIFRA SAP. More recent studies were added to this summary for the 2014 chlorpyrifos revised HHRA (USEPA, 2014). At that time, the conclusions were that the animal studies clearly showed neurobehavioral outcomes following developmental exposure to chlorpyrifos, but there were inconsistencies in the types of effects reported (neurological domain altered, direction of change, gender specificity). Furthermore, the studies were conducted with doses that most likely produced at least some amount of AChE inhibition at some time during the exposure based on results of guideline studies submitted for registration. The impact of these observations has lead the agency to evaluate whether or not these conclusions extend to other OP pesticides. In this review, the studies of a number of OP pesticides are summarized.

The search aimed to focus on rodent studies involving prenatal/perinatal exposure to OPs in which the offspring were evaluated with *in vivo* neurobehavioral tests. The search methods and analytical scope for this analysis are consistent with the chlorpyrifos analysis from the 2012 FIFRA SAP and 2014 HHRA. Preweaning measurements of behavioral development were noted but not compiled, since these could reflect effects of current exposure to the pesticide rather than long-term neuronal changes. Information on AChE inhibition in either fetuses/pups or dams during this exposure period was evaluated where available. Sections 2.1.1-2.1.3 describe the studies from the open scientific literature. Section 2.1.4 summarizes relevant results from the DNT guidelines studies submitted for pesticide registration (US EPA guideline 870.6300 and/or OECD guideline 426).

---

[3] NRC 2011. "Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde" ; NRC 2014. "Review of EPA's Integrated Risk Information System (IRIS) Process"

[4] http://dels.nas.edu/Report/Review-Integrated-Risk/18764

2.1.1 Literature Search Strategy & Results

To review and evaluate the developmentally neurotoxic effects of other OPs, a search of the open literature was undertaken. Due to the limited number of studies available, the agency did not limit the search to currently registered OPs. The agency is aware that some OPs listed below are no longer registered for use in the US. In addition, the data evaluation records (DERs) for existing guideline DNT studies were collected from OPP files and summarized. The literature search strategy was developed and conducted by a US EPA reference librarian. Databases searched were PubMed, Web of Science (WoS) and ScienceDirect using key words described below. Duplicates were eliminated after the total database was generated.

1. PubMed (751 results)
(((((organophos* OR Cholinesterase Inhibitors OR acetylcholinesterase inhibitor))) AND ((prenatal OR neonatal OR perinatal OR in utero OR fetal OR foetale OR newborn OR infant* OR postnatal OR gestational OR pregnancy[MeSH Terms]))))) AND ((neurodevelop* OR attention OR birth outcome* OR health outcome* OR cognitive OR cognition OR developmental disability* OR fetal growth OR fetal growth OR foetal development OR fetal development OR sex OR social OR intelligence OR memory OR neurological functioning OR psychomotor OR neurotoxicity OR neurobehavior OR behavior OR learning OR Nervous System[MeSH Terms]) OR Neurotoxicity Syndromes[MeSH Terms]) AND ((((((guinea pigs[MeSH Terms]) OR rabbits[MeSH Terms]) OR mice[MeSH Terms]) OR rats[MeSH Terms]) NOT fishes[MeSH Terms])

2. Web of Science (427 results)
#5 #4 AND #3 AND #2 AND #1
DocType=All document types; Language=All languages;
#4 TS=(guinea pig* OR rabbit* OR mice OR mouse OR rat* OR rodent*) NOT TS=(fish*)
DocType=All document types; Language=All languages;
#3 TS=(neurodevelop* OR attention OR birth outcome* OR health outcome* OR cognitive OR cognition OR developmental disabilit* OR fetal growth OR fetal growth OR foetal development OR fetal development OR sex OR social OR intelligence OR memory OR neurological functioning OR psychomotor OR neurotoxicity OR neurobehavior OR behavior OR learning OR Nervous System OR Neurotoxicity Syndromes)
DocType=All document types; Language=All languages;
#2 TS=(prenatal OR neonatal OR perinatal OR in utero OR fetal OR foetale OR newborn OR infant* OR postnatal OR gestational OR pregnan*)
DocType=All document types; Language=All languages;
#1 TS=(organophos* OR Cholinesterase Inhibitors OR acetylcholinesterase inhibitor OR chlorpyrifos)
DocType=All document types; Language=All languages;

3. Science Direct (19 results)
(ALL(organophos* OR Cholinesterase Inhibitors OR acetylcholinesterase inhibitor OR chlorpyrifos) and ALL(prenatal OR neonatal OR perinatal OR in utero OR fetal OR foetale OR

newborn OR infant* OR postnatal OR gestational OR pregnan*)) AND (neurodevelop* OR attention OR birth outcome* OR health outcome* OR cognitive OR cognition OR developmental disabilit* OR fetal growth OR fetal growth OR foetal development OR fetal development OR sex OR social OR intelligence OR memory OR neurological functioning OR psychomotor OR neurotoxicity OR neurobehavior OR behavior OR learning OR Nervous System OR Neurotoxicity Syndromes) AND (ALL(guinea pig* OR rabbit* OR mice OR mouse OR rat* OR rodent) and not ALL(fish*)).

This broad literature search identified 1012 potential papers, which were reviewed individually. Specific criteria were applied to select suitable studies, as was previously done with chlorpyrifos. Since the literature on chlorpyrifos has been previously reviewed, those papers were excluded here. This resulted in 19 relevant papers with the following specifications:

- Exposure occurred during gestation and/or the postnatal time frame, ending no later than weaning.
- Dosing included maternal and/or pup administration.
- Dosing was via oral or subcutaneous injection. One paper with intracisternal injection was excluded.
- Behavioral testing of the offspring occurred after weaning and/or into adulthood.
- Studies involved only single-chemical exposure, and where two or more chemicals were administered together, only the single-chemical data were included in the summaries.
- Test subjects were rats or mice. Several papers in pigs and rabbits were excluded due to the lack of comparative database for those species.
- The test measures of interest were neurobehavioral endpoints. At least two studies involved only electrophysiological measures, and those were excluded. No neurochemical, genomic, or other molecular endpoints were included.

The OPs examined, and the number of papers for each, are listed below. Of particular interest are studies from one laboratory (Duke University) that included parathion and diazinon, and can be directly compared to studies with chlorpyrifos using similar experimental designs. The majority of studies used rats (13), and exposures periods varied about evenly between gestational and postnatal stages.

- Parathion (5)
- Diazinon (5)
- Methyl parathion (3)
- Methamidophos (2)
- Chlormephos (1)
- Dichlorvos (1)
- Fenitrothion (sumithion) (1)
- Oxydemeton-methyl (demeton-S-methyl, metasystox-R) (1)

These papers dated back to 1968, and there was a wide range in study quality. Shortcomings were noted in almost all papers, including cursory methodological information and presentation of results, inappropriate statistical analyses, contradictory statements, and problematic interpretation of the data. Regardless, the literature is summarized below in terms of the functional domains organized by each neurobehavioral evaluation.

ED_002061_00044425-00010

Below are study descriptions and summaries organized by neurological domain. Appendix 1 is an overall summary for each chemical, presenting each endpoint and outcome.

2.1.2 Integration of Literature: Neurobehavioral Domains

Cognition

Fifteen studies measured some aspect of cognition: tests included mazes (radial arm maze, Lashley maze, T-maze spontaneous alternation, M-maze), conditioned response (passive avoidance, conditioned avoidance, operant responding, T-maze), and recognition (novel object). Most of these showed adverse effects of OP exposure, although not always in a consistent or dose-responsive manner.

Rats treated with diazinon (0.5, 2 mg/kg/d, postnatal day (PND) 1-4) showed no differences alternating in a T-maze (Timofeeva *et al.*, 2008a). The same rats were tested several months later in a radial arm maze, and showed increased working memory errors (both males and females), but only at the low dose (0.5 mg/kg/d) with no effect on reference memory performance. Diazinon (1 mg/kg/d) was given to rats on gestational day (GD) 15-18 or PND1-4, and there was no change in the trials to criterion in a passive avoidance test; however, there was clearly decreased step-down latency when tested 24 hr later (Vatanparast *et al.*, 2013). This finding suggests a change in memory but not learning. After *in utero* exposure this effect was only seen in females. In contrast, both genders (greater effect in males) were affected following postnatal exposure. Using a novel object test, male mice (females not tested) exposed postnatally (PND8-11) to diazinon (0.5, 5 mg/kg/d) showed less exploration and discrimination of the new object (Win-Shwe *et al.*, 2013). This was significant at both doses (but no dose-response) when tested at PND49, and only the high dose group showed effects at PND84. Mice exposed to diazinon (0.18, 9 mg/kg/d) throughout gestation were tested in a Lashley III maze, with no changes in the number of errors, suggesting no effect on learning (Spyker and Avery, 1977). Thus, these data on diazinon suggest an effect on learning and/or memory (radial arm maze, passive avoidance, novel object recognition) but no changes in learning a maze task. There was a lack of dose-response across studies.

Parathion exposure in rats (0.1, 0.2 mg/kg/d, PND1-4) produced no changes in T-maze spontaneous alternation (Timofeeva *et al.*, 2008b). These rats showed decreased working memory errors, indicative of improvement, at the low dose only (both males and females), and no changes in reference memory errors when tested at about 3 months of age. However, Levin *et al.* (2008) tested littermates from the Timofeeva study beginning at 14 months, and reported increased working memory errors in male rats treated with the low dose only. Interestingly, there is a difference in direction of change and gender specificity compared to the Timofeeva data. Reference memory errors were also increased at both doses (but no dose-response) in male rats only. When the rats were tested again at 17 months, working memory errors were increased at both doses (but no dose-response), again only in males. There was no effect on

reference errors at 17 months, and no change in either parameter when the rats were again tested at 19 months. In a study by Stamper *et al.* (1988), rats (only males were tested) exposed postnatally to parathion (1.3, 1.9 mg/kg/d, PND5-20) showed decreased spontaneous alternations in a T-maze at both doses (dose-response evident), and increased working memory errors in a radial arm maze at both doses (but no dose-response). Reference memory errors were not altered. Al-Hachim and Fink (1968) administered parathion (3 mg/kg/d) during either the first, second or third week of gestation in mice, and reported no effect on conditioned avoidance (sex not mentioned) following any exposure period. Overall, the radial arm maze showed effects of parathion in several studies, but the direction of change, specificity of errors, and gender selectivity differed. The results with the T-maze were contradictory, with one study out of two reporting effects.

Radial arm maze performance was affected by methyl parathion given directly to pups PND1-21 using an incrementally increasing dose schedule (Johnson *et al.*, 2009). The middle (0.2 to 0.6 mg/kg/d) and high (0.3-0.9 mg/kg/d) dose groups increased both working and reference memory errors. The lowest dose group (0.2 mg/kg/d throughout dosing) also increased reference memory errors; however, on this measure all dose groups had similar averages. Only males were affected on all measures. Rats dosed with methyl parathion (1 mg/kg/d, GD7-15) were trained to go to a specific side in T-maze, and the correct side reversed five times (Crowder *et al.*, 1980). Treated rats had more trials to criterion only on second and fifth switch (note, the text claimed there was an effect on the 4th switch, but the figure does not show it as significant). Effects on only certain reversals is difficult to interpret, and may be a reflection of the multiple t-tests used to analyze the data. Males and females were tested but data were not provided for each sex separately. There were no effects on passive or active avoidance in rats (no mention of gender) exposed to methyl parathion (1, 1.5 mg/kg/d, GD6-20) (Gupta *et al.*, 1985). The low-dose group only showed slower latency to bar press during operant shaping, and more days to asymptote; however, details of operant training and schedule were not provided, the sample size was extremely small (n=4/group), and high variability was mentioned. Thus, the most consistent effect was seen in the radial arm maze (even though there was no dose-response), and other tests were either negative or the data were inconclusive.

Several other OPs were tested in different cognitive tasks, but there is no more than one report for any specific pesticide. After single-trial passive avoidance training, male rats (females not tested) treated with dichlorvos (8 mg/kg/d, GD6-15) showed faster latency to cross when tested 7 days later, suggesting delayed retention (Lazarini *et al.*, 2004). With fenitrothion, male rats (females not tested) exposed gestationally (5, 10, 15 mg/kg/d, GD7-15) were conditioned to climb a pole to escape shock (Lehotzky *et al.*, 1989). The mid and high dose groups (dose-response evident) showed more escapes, were faster, and reached criterion faster than controls. This same pattern seen with reacquisition after a period of extinction, during which time there were no group differences. There was no effect of oxydemeton methyl (0.5, 1.5, 4.5 mg/kg/d, GD6-15) on M-maze learning or memory in rats (Clemens *et al.*, 1990). There was also no effect on retention of single trial passive avoidance in mice exposed to methamidophos (1 mg/kg/d, PND3-9); however, the retention trial occurred at 3 hr instead of the more standard 24 hr or greater (Lima *et al.*, 2013).

Table 2.1.1 Summary of Cognitive Outcomes

	Early gestation ~GD1-7, ~GD8-14, GD6-15 GD7-15	Late gestation ~GD15-21, GD6-20, GD15-18	Gestation GD1-birth	Early postnatal PND1-4, PND3-9	Late postnatal PND8-11, PND5-20	Postnatal PND1-21
Radial arm maze				Diazinon: cognitive deficit – rat, low dose only, M & F[1] Parathion: improved cognition – rat, low dose only, M & F[2] Parathion: cognitive deficit – rat, M not F[3]	Parathion: cognitive deficit – rat, no dose-response, only M tested[4]	Methyl parathion: cognitive deficit – rat, dose-response, M only[5]
T-maze spontaneous alternation				Diazinon: no effect – rat, M & F[1] Parathion: no effect – rat, M & F[2]	Parathion: cognitive deficit – rat, dose-response, only M tested[4]	
T-maze learning	Methyl parathion: cognitive deficit – rat, sex not specified[6]					
Lashley III maze			Diazinon: no effect – mouse, sex not specified[7]			
M-maze	Oxydemeton methyl: no effect – rat, M & F[8]					
Active avoidance	Fenitrothion: improved	Methyl parathion: no				

ED_002061_00044425-00013

	cognition – rat, dose-response, only M tested[9] Parathion: no effect – mouse, sex not specified[11]	effect – rat, sex not specified[10] Parathion: no effect – mouse, sex not specified[11]				
Passive avoidance	Dichlorvos: cognitive deficit – rat, only M tested[12]	Diazinon: cognitive deficit – rat, F not M[13] Methyl parathion: no effect – rat, sex not specified[10]		Diazinon: cognitive deficit – rat, M & F[13] Methamidophos: no effect – mouse, sex not specified[14]		
Novel object recognition					Diazinon: cognitive deficit – mouse, no dose-response, only M tested[15]	
Operant responding		Methyl parathion: cognitive deficit – rat, sex not specified[10]				

[1] Timofeeva *et al.*, 2008a
[2] Timofeeva *et al.*, 2008b
[3] Levin *et al.*, 2008
[4] Stamper *et al.*, 1988
[5] Johnson *et al.*, 2009
[6] Crowder *et al.*, 1980
[7] Spyker and Avery, 1977
[8] Clemens *et al.*, 1990
[9] Lehotzky *et al.*, 1989
[10] Gupta *et al.*, 1985
[11] al-Hachim and Fink, 1968
[12] Lazarini *et al.*, 2004
[13] Vatanparast *et al.*, 2013
[14] Lima *et al.*, 2013
[15] Win-Shwe *et al.*, 2013

ED_002061_00044425-00014

Motor Activity

Despite being a commonly tested measure in these studies, only a few of the tested OPs produced changes in motor activity. Rats exposed to dichlorvos (8 mg/kg/d, GD6-15) showed decreased open field locomotion at weaning (males not females), and decreased locomotion and increased immobility as adults (age not specified, only males tested) while rearing was not affected at either age (Lazarini *et al.*, 2004). Male rats exposed to fenitrothion (5, 10, 15 mg/kg/d, GD7-15) showed decreased horizontal activity in the high-dose group only on PND104, with an apparent but not significant effect at PND26, but no effect at PND36 (Lehotsky *et al.*, 1989). Open field testing of rats exposed to methyl parathion (1 mg/kg/d, GD7-15) showed what appeared to be increases only on PND23 and 54, with no differences on PND30, 44, 65 (also not PND18); however, the data are not compelling since statistics are not provided, and the text refers to the data as "a possible change" (Crowder *et al.*, 1980). Methyl parathion-treated rats (1 mg/kg/d, GD6-20) showed a decrease in locomotor activity "accommodation" (apparently the period that is 15-30 min into the activity session) in the low dose group only (Gupta *et al.*, 1985).

Several OPs consistently produced no changes on motor activity, regardless of exposure or test species. No effects were seen with diazinon in rats exposed gestationally (1 mg/kg/d, GD15-18, Vatanparast *et al.*, 2013) or postnatally (0.5, 2 mg/kg/d, PND1-4, Timofeeva *et al.*, 2008a, or 1 mg/kg/d, PND1-4, Vatanparast *et al.*, 2013), or in mice exposed gestationally (0.18, 9 mg/kg/d, GD1-birth, Spyker and Avery, 1977). As with diazinon, there were no motor activity changes following parathion exposure postnatally in rats (0.1, 0.2 mg/kg/d, PND1-4, Timofeeva *et al.*, 2008b, or 1.3, 1.9 mg/kg/d, PND5-20, Stamper *et al.*, 1988) or in mice treated during either the first, second, or third week of gestation (3 mg/kg/d, Al-Hachim and Fink, 1968). There was no effect on measures of activity reported in rats treated with methamidophos (1 mg/kg/d, GD6-15), although high variability of the measures was discussed (deCastro *et al.*, 2000). Methamidophos (1 mg/kg/d, PND3-9) also produced no effect in mice (Lima *et al.*, 2013). Finally, there was no effect of oxydemeton methyl exposure (0.5, 1.5, 4.5 mg/kg/d, GD6-15) on open field activity (Clemens *et al.*, 1990).

In this review, effects on activity are only considered in tests designed specifically for that purpose. During the course of other behavioral tests, e.g., radial arm or T-maze, speed or latency is often measured. These ancillary activity measures were sometimes altered by treatment, but are not included in this domain, since they are not designed to specifically target motor activity.

ED_002061_00044425-00015

Table 2.1.2. Summary of Motor Activity Outcomes

Task/Test Apparatus	Early gestation ~GD1-7, ~GD8-14, GD6-15 GD7-15	Late gestation ~GD15-21, GD6-20, GD15-18	Gestation GD1-birth	Early postnatal PND1-4, PND3-9	Late postnatal PND8-11, PND5-20
Open field	Dichlorvos: decreased activity – rat, only M tested[1] Fenitrothion: decreased activity – rat, dose-response, only M tested[2] Methamidophos: no effect – rat, sex not specified[3] Methyl parathion: increased activity – rat, sex not specified[4] Oxydemeton methyl: no effect – rat, M & F[5] Parathion: no effect – mouse, sex not specified[6]	Diazinon: no effect – rat, M & F[7] Parathion: no effect – mouse, sex not specified[6]	Diazinon: no effect – mouse, sex not specified[8]	Diazinon: no effect – rat, M & F[7] Methamidophos: no effect – mouse, sex not specified[9]	Parathion: no effect – rat, only M tested[10]
Figure-Eight				Diazinon: no effect – rat, M & F[11] Parathion: no effect – rat, M & F[12]	
Donut		Methyl parathion: decreased activity – rat, no dose-response, sex not specified[13]			

ED_002061_00044425-00016

[1] Lazarini *et al.*, 2004
[2] Lehotzky *et al.*, 1989
[3] deCastro *et al.*, 2000
[4] Crowder *et al.*, 1980
[5] Clemens *et al.*, 1990
[6] al-Hachim and Fink, 1968
[7] Vatanparast *et al.*, 2013
[8] Spyker and Avery, 1977
[9] Lima *et al.*, 2013
[10] Stamper *et al.*, 1988
[11] Timofeeva *et al.*, 2008a
[12] Timofeeva *et al.*, 2008b
[13] Gupta *et al.*, 1985

ED_002061_00044425-00017

Anxiety/Depression

In a series of tests in rats, Roegge *et al.* (2008) showed that early postnatal exposure to diazinon (0.5, 2 mg/kg/d, PND1-4) produced behaviors suggesting higher anxiety at the high dose (decreased open arm time in an elevated plus maze), lower fearfulness (decreased time to start eating in novel environment) in both dose groups, and anhedonia (decreased chocolate milk preference) at the low dose only, but not depression (forced swim test). These effects occurred only in males and did not show a clear dose-response for the novelty eating and chocolate milk preference tests. Using the same tests (same laboratory; Timofeeva *et al.*, 2008b), parathion exposure (0.1, 0.2 mg/kg/d, PND1-4) in rats (high dose, both sexes) increased time in the open arm in an elevated plus maze (suggesting decreased anxiety); however, there was also an increase in center crossings, indicating hyperactivity, that may confound overall interpretation. These same rats showed no changes in novelty-suppressed feeding or in chocolate milk preference. Thus, in these two studies the pesticides appear to have different effects on this functional domain.

There are only a few reports of these behaviors with other pesticides. Rats treated with methyl parathion (1, 1.5 mg/kg/d, GD6-20) showed faster emergence from a cage, interpreted by the authors as lowered anxiety, in the low dose group only (Gupta *et al.*, 1985). With chlormephos exposure (~0.06, 0.6 mg/kg/d in drinking water, one week premating to weaning), adult mice (both males and females) in the high-dose group showed decreased time spent in the open arms and increased time in the closed arms (no change in latency) of an elevated plus maze, suggesting increased anxiety (Ceh *et al.*, 2012). Mice exposed to methamidophos (1 mg/kg/d, PND3-9) showed no differences in time spent in either arm of an elevated plus maze, but spent less time in center (Lima *et al.*, 2013). This was interpreted by the authors as effect on choosing arms, which they say is a cognitive effect; however, this measure is often interpreted to reflect only activity levels. The same mice showed increased immobility in forced swim test, suggesting depressive-like behaviors. Overall, these results are varied and did not consistently show a dose-response.

Table 2.1.3. Summary of Anxiety/Depression Outcomes

	Late gestation ~GD15-21, GD6-20, GD15-18	Perinatal premating-weaning	Early postnatal PND1-4, PND3-9
Elevated plus maze		Chlormephos: increased anxiety – mouse, M & F[1]	Diazinon: increased anxiety – rat, dose-response, M not F[2] Methamidophos: no effect –mouse, sex not specified[3] Parathion: decreased anxiety – rat, dose-response, M & F[4]
Chocolate milk preference			Diazinon: increased anhedonia – rat, no dose-response, M not F[2] Parathion: no effect – rat, M & F[4]
Novelty suppressed feeding			Diazinon: decreased fearfulness – rat, dose-response, M not F[2] Parathion: no effect – rat, M & F[4]
Forced swim			Diazinon: no effect – rat, M & F[2] Methamidophos: increased despair – mouse, sex not specified[3]
Open field behaviors	Methyl parathion: decreased anxiety – rat, no dose-response, sex not specified[5]		

[1] Ceh *et al.*, 2012
[2] Roegge *et al.*, 2008
[3] Lima *et al.*, 2013
[4] Timofeeva *et al.*, 2008b
[5] Gupta *et al.*, 1985

ED_002061_00044425-00019

Social Behavior

Only one study has used tests of social behavior using these OPs. With exposure to fenitrothion (5, 10, 15 mg/kg/d, GD7-15), rats in mid and high dose (dose-response) spent more time actively interacting in conspecific pairs (Lehotsky *et al.*, 1989). The scarcity of data on this measure prevents any conclusions across OPs.

Sensory Function

In a study measuring response to a tactile stimulus, with and without an acoustic prepulse, male (but not female) rats treated with diazinon (0.5, 2 mg/kg/d, PND1-4) showed less prepulse inhibition at both doses; however, no dose-response was evident (Timofeeva *et al.*, 2008a). Using the same paradigm, rats treated with parathion (0.1, 0.2 mg/kg/d, PND1-4) showed a different pattern: lower response to the stimulus alone (high dose, both sexes), but no change in the inhibition produced by the prepulse (Timofeeva *et al.*, 2008b). Mice treated with diazinon through gestation showed no change in response to noise (auditory startle) or smell (olfactory orientation), but did show change in visual cliff behavior (more steps off a "cliff") which occurred only in females in the low dose group (Spyker and Avery, 1977). Additional studies are needed for general conclusions regarding the effects of these pesticides on sensory function.

Table 2.1.4. Summary of Sensory Outcomes

	Gestation GD1-birth	Early postnatal PND1-4, PND3-9
Auditory	Diazinon: no effect – mouse, sex not specified[1]	
Tactile with or without prepulse		Diazinon: decreased sensory gating – rat, no dose-response, M not F[2] Parathion: decreased startle response – rat, dose-response, M & F[3]
Visual	Diazinon: decreased function – mouse, no dose-response, F not M[1]	
Olfactory	Diazinon: no effect – mouse, sex not specified[1]	

[1] Spyker and Avery, 1977
[2] Timofeeva *et al.*, 2008a
[3] Timofeeva *et al.*, 2008b

ED_002061_00044425-00020

Neuromotor Function

One study assessed neuromotor function in mice exposed to diazinon (0.18, 9 mg/kg/d, GD1-birth), and reported some changes in motor abilities measured at about 2 months of age; however, these were not consistent in dose or direction of change (Spyker and Avery, 1977). Increased ability was suggested by a longer time to cling to a rod (both doses, no dose-response). In contrast, there was less ability to stay on an increasingly inclined plane (both doses, no dose-response) or perhaps on a rotarod (group means not significant due to large variability, suggestive of effect at both doses).

Rats in the high-dose group exposed to fenitrothion (5, 10, 15 mg/kg/d, GD7-15) fell off a rotarod faster on PND26 and PND104, but not PND36. Only males were tested (Lehotsky *et al.*, 1989).

There were no neuromotor changes in terms of rotarod performance in rats following exposure to parathion (1.3, 1.9 mg/kg/d, PND5-20) (Stamper *et al.*, 1988) or methyl parathion (1, 1.5 mg/kg/d, GD6-20) (Gupta *et al.*, 1985). Overall, there is little support for conclusions of neuromotor outcomes following these pesticides, but more studies are needed.

Table 2.1.5. Summary of Neuromotor Outcomes

	Early gestation ~GD1-7, ~GD8-14, GD6-15 GD7-15	Late gestation ~GD15-21, GD6-20, GD15-18	Gestation GD1-birth	Late postnatal PND8-11, PND5-20
Rotarod	Fenitrothion: decreased performance – rat, dose-response, only M tested[1]	Methyl parathion: no effect – rat, sex not specified[2]	Diazinon: no effect – mouse, sex not specified[3]	Parathion: no effect – rat, only M tested[4]
Inclined plane			Diazinon: decreased performance – mouse, dose-response, sex not specified[3] (Spyker)	
Rod cling			Diazinon: increased performance – mouse, no dose-response, sex not specified[3]	

[1] Lehotzky *et al.*, 1989
[2] Gupta *et al.*, 1985
[3] Spyker and Avery, 1977
[4] Stamper *et al.*, 1988

2.1.3 Integration with AChE Inhibition

There are some data with which to compare effective doses in these DNT studies with doses producing AChE inhibition. A number of studies in this literature review included AChE inhibition (brain and/or blood) in their measurements. Information on diazinon and parathion can be taken from a separate (non-behavioral) study (Slotkin *et al.*, 2006) conducted in the same laboratory with the same dosing paradigm used in several studies (Levin *et al.*, 2008; Roegge *et al.*, 2008, Timofeeva *et al.*, 2008a, 2008b).

Neurobehavioral effects of diazinon were reported in rats at doses of 0.5-2 mg/kg/d (Roegge *et al.*, 2008; Timofeeva *et al.*, 2008a). Slotkin *et al.* (2006) reported that a dose of 0.5 mg/kg/d produced some (<10%, statistically significant) brain AChE inhibition when measured the day after the last dose. At 2 hr after a higher dose (2 mg/kg/d; lower doses were not tested at 2 hr), there was greater brain inhibition (25-30%) compared to 24 hr (10-20%). Thus it is probable that for diazinon, inhibition during and shortly after the dosing period (i.e., within hours) was greater at lower doses. While there is no direct AChE data following diazinon exposure at 1 mg/kg/d (Vataparast *et al.*, 2013), it can be assumed from the Slotkin data that this dose also inhibited brain AChE at some time during/after dosing. No AChE activity was measured in mice by Spyker and Avery (1977), but the high dose of 9 mg/kg/d resulted in depressed weight gain, a sign of maternal toxicity. There is no mention of toxicity at the lower doses (0.5, 5 mg/kg/d) used in mice by Win-Shwe *et al.* (2013).

The parathion studies showing effects at 0.1 and 0.2 mg/kg/d (Timofeeva *et al.*, 2008b; Levin *et al.*, 2008) are also informed by the AChE inhibition presented in Slotkin *et al.* (2006) in which a dose of 0.1 mg/kg/d inhibited brain AChE 5-15% on the day after the last dose. There are no AChE data available for a higher dose, 0.2 mg/kg/d, but Timofeeva noted 5% mortality in that dose group. Much higher doses (1.3, 1.9 mg/kg/d in rats) were reported to produced 35, 68% brain inhibition on the day after the last dose (PND5-20), with 26, 36% inhibition persisting a week later (Stamper *et al.*, 1988).

The methyl parathion postnatal incrementing dose paradigm in rats used by Johnson *et al.* (2009) produced brain AChE inhibition in all dose groups at the end of dosing, persisting for 10 to 20 days later; recovery was evident 30-40 days later. The low dose (0.2 mg/kg/d throughout) produced 13-15% inhibition. During gestational exposure to higher doses of methyl parathion at 1, 1.5 mg/kg/d, the dams had 20, 60% brain inhibition at the end of dosing (GD6-20) (Gupta *et al.*, 1985). At the high dose, cholinergic signs and increased resorptions were noted. Furthermore, the offspring (fostered to control dams) showed brain inhibition as high as 50% in both dose groups when measured at birth and also PND7, 14, 21, and 28. The low dose showed recovery at PND28, but not the high dose.

In the study of rats treated with fenitrothion, there was postnatal mortality of 16-17.5% at all doses (5, 10, 15 mg/kg/d, GD7-15), compared to 5% in controls (Lehotsky *et al.*, 1989). In separate study of rats, 5, 25 mg/kg on GD19 produced 40, 80% brain AChE inhibition in dams, and fetal brains showed about 90% inhibition (no dose response) (Sochaski *et al.*, 2007).

Assuming similar responses, the postnatal mortality observed in the Lehotsky study could be at least partly due to this high degree of AChE inhibition in both dams and fetuses.

In rats, nonpregnant females dosed methamidophos 1 mg/kg/d for 10 days showed 16% plasma AChE inhibition, but brain AChE was apparently not measured. This suggests that dams treated at the same dose in a study by deCastro *et al.* (2000) most likely experienced some plasma inhibition. Mouse pups dosed with methamidophos 1 mg/kg/d PND3-9 showed ~36, 46% brain inhibition 1, 4 hr after first dose, 53, 61% inhibition at 1, 4 hr after last dose, and ~19% brain inhibition the day after last dose (Lima *et al.*, 2013). The mice therefore experienced considerable brain AChE inhibition throughout dosing.

In the oxydemeton methyl study, dams showed 22-68% brain AChE inhibition on the day after the last dose (0.5-4.5 mg/kg/d, dose response), and 5 days later (GD20) there was 20-54% brain AChE inhibition. Fetal brains taken the day after dosing showed no inhibition (Clemens *et al.*, 1990); however, there were no fetal AChE tissues collected during or shortly after the dosing period when AChE inhibition would be greatest.

Overall, in the studies for which there are direct or comparable data, it is clear that the dosing paradigms produced AChE inhibition and in some cases maternal toxicity. Indeed, there are no studies reporting or even suggesting a lack of AChE inhibition in the dam and/or fetus/pup at any time during dosing. Thus, it is not known whether exposure paradigms that do not inhibit AChE would produce any neurobehavioral effects.

2.1.4 Summary of Findings from the Developmental Neurotoxicity (DNT) Guideline Studies

DNT studies have been submitted for 20 OPs, summarized in Appendix 2. These studies follow the US EPA guideline 870.6300 and/or OECD guideline 426 which require testing of motor activity, acoustic startle response, learning and memory, and brain morphometrics in the offspring around weaning and also in adulthood. In general, these studies provide exposure during development either via diet or oral gavage dosing, including direct dosing of the pup preweaning. As with the literature studies, these submitted studies have shortcomings such as inappropriate statistical analyses, limited methodological information and presentation of results. Many measures tend to show high variability, which reduces their interpretability and utility.

In order to compare the submitted guideline and published studies under the scope identified under Section 2.1 and to be consistent with the chlorpyrifos 2012/2014 review, only changes that occurred after dosing had ended (i.e., shortly after weaning or as adults) were considered here. Across the seven submitted studies that reported effects, there are mostly changes in acoustic startle reactivity, cognitive function, and to a lesser extent, motor activity. Some OPs altered multiple domains, others only one. There are both submitted guideline studies and literature studies for only four OPs: diazinon, methyl parathion, methamidophos, and

dichlorvos. Diazinon produced cognitive changes but no effects on motor activity or acoustic startle in the DNT: these results are generally in agreement with published studies. Male offspring in the high dose group (~33 mg/kg/d in the dam diet) showed increased errors and longer latency in Biel maze performance at both PND24 and PND62, and similar effects were seen in females but only in the middle dose group (~3.4 mg/kg/d via diet) at PND24. Following methamidophos exposure, female rats in the middle and high dose groups (~1.7, 5.2 mg/kg/d in the dam diet) showed decreased peak amplitudes of the startle response, which was statistically significant at PND38 and apparent but not significant at PND60. In contrast to the literature reports of cognitive and motor effects of methyl parathion, there were no reported changes in the submitted guideline study. The submitted study of dichlorvos was uninterpretable due to high pup mortality in all groups, including control.

In these studies, AChE activity was assessed as part of the DNT itself, or by means of a separate study comparing the response in pups and adults (comparative cholinesterase, or CCA, studies). Thus, in almost all guideline DNT studies there are adequate data describing AChE inhibition in the pups at some time during development. It is clear from these DNT studies that the doses used did produce AChE inhibition in the offspring, sometimes at all doses tested. It was noted that in these studies, most of the reported effects occurred before weaning, which is the period during which there was likely to be ongoing AChE inhibition.

Thus, as with the literature studies, there are scant data that could inform potential neurodevelopmental changes occurring at doses lower than those needed to inhibit AChE. Furthermore, there is little consistency in patterns of effects across studies or chemicals. Thus, there is uncertainty as to whether lower, non-inhibiting exposures are developmentally neurotoxic; this uncertainty was described in the chlorpyrifos reviews and remains applicable for the available data for other OPs as well.

2.1.5 Conclusions on *In Vivo* Laboratory Animal Studies

For chlorpyrifos, there are >30 papers on developmental neurotoxicity; for the remaining OPs, the literature is sparse with very few studies for each OP (including DNT guideline studies). The studies span over decades, and many of the lower quality studies were the earlier ones; however, some very recent papers also have significant deficits. Methodological detail is lacking, inappropriate statistical analyses are applied, results are cursorily described and/or inaccurately presented, and interpretation of some behavioral changes is faulty. Overall, most studies have significant shortcomings and/or are of low quality.

The most commonly tested behaviors considered aspects of cognition. In the majority of studies, some sort of cognitive deficit was detected, especially with working memory performance (radial arm maze) and conditioned response retention (passive avoidance). However, in many cases there was no dose-response, there was some gender specificity which did not replicate in multiple studies, and cognitive improvement instead of deficit was noted in a few papers. Changes in motor activity in offspring were generally not reported, and the direction of change differed in the papers reporting such effects. There is generally not enough

information to make definitive statements about OP effects on other types of neurological disorders.

Few published papers included AChE measurements of the dams and/or offspring, but where measured, all doses used inhibited AChE to some degree. Some papers even reported overt maternal and fetal toxicity. This was also the case in the guideline studies, most of which included concurrent or supplemental data on AChE inhibition. Since there are no studies with low doses that definitively do not inhibit AChE, there is no information in the animal literature that shows whether or not there would be developmentally neurotoxic outcomes at those lower exposures.

2.2 Epidemiology Research on OPs other than Chlorpyrifos

2.2.1 Overview of Literature Reviews: 2012/2014, 2015, and updated in 2016

In April 2012, EPA presented to the FIFRA Scientific Advisory Panel (SAP) its review and assessment of several epidemiological investigations of the potential adverse neurodevelopmental outcomes of *in utero* and early life exposure to chlorpyrifos. In this effort, EPA limited its review to studies conducted within three major US based prospective birth cohort studies: 1) Mother's and Newborn Study of North Manhattan and South Bronx conducted by Columbia University, referred to in this document as CCCEH 2) Mount Sinai Inner-City Toxicants, Child Growth and Development Study, or the "Mount Sinai Study/Cohort;" and 3) Center for Health Assessment of Mothers and Children of Salinas Valley (CHAMACOS) conducted by the University of California Berkeley, or "CHAMACOS Study/ Cohort." The conclusion of EPA's evaluation, supported by the FIFRA SAP (2008, 2012), was that "chlorpyrifos likely played a role in the neurodevelopmental outcomes observed in these studies."

In 2015, the agency expanded its consideration of the epidemiological data to include studies of any OP pesticide; several different types of development and neurological, neurodevelopmental, and neurobehavioral health outcomes; studies performed in non-U.S. countries as well as US based studies; and non-cohort studies. This 2016 updated literature review includes edits made in response to public comments on the 2015 literature review and addition of new epidemiology papers: Yolton et al. (2013), Cartier et al. (2015), Rauh et al (2015), Ranaan et al, (2015), Engel et al. (2015), Fiedler et al. (2015), Harley et al (2016), Stein et al (2016), Rauh et al (2015), Ranaan et al, 2015; Engel et al. (2015), and Donauer et al (2016).The agency has developed individual study reviews for each study in the 2015 and 2016 updated literature review; these study reviews contain details about study design, results, strengths, uncertainties, and interpretation. The study reviews can be found in USEPA, 2015b, USEPA, 2016, and USEPA,2016b. This literature review document summarizes key information, strengths and uncertainties and provides a concise, coherent weight of evidence analysis.

The 2012/2014 literature review was limited to studies from CCCEH, Mt. Sinai, and CHAMACOS; all studies from these cohorts are considered high quality. In the CCCEH, Mt. Sinai, and

CHAMACOS studies, mother-infant pairs were recruited for the purpose of studying the potential health effects of environmental exposures during pregnancy on subsequent child development. Importantly, each of these cohorts evaluated the association between prenatal chlorpyrifos or OP exposure with adverse neurodevelopmental outcomes in children through age 7 years. For those studies in 2012/2014 literature review detailed summaries and evaluation, associated strengths and limitations, and accompanying detailed evidence table for the CCCEH, CHAMACOS, and Mt. Sinai cohorts can be found in the white paper for the 2012 SAP review, and the 2014 chlorpyrifos revised HHRA. Limited summary information is provided here for consideration with the studies identified in the 2015 and updated 2016 literature reviews.

Publication bias is a type of bias associated with the kinds of academic research likely to be published in the open literature; generally, this bias tends towards lack of publishing of null findings. However, in the case of the neurodevelopmental epidemiology studies on OPs, as noted by commenter, there are already significant amount of null findings reported in this body of evidence. Moreover, these studies are derived from existing cohorts of mothers and children which are well-known to the science community; these kinds of cohorts are expensive and resource intensive to develop and maintain making the number of studies from other groups unlikely. Given the amount of null findings already reported in the literature, the agency believes that publication bias with regard to lack of publishing of null findings does not impact the interpretation of the existing studies on neurodevelopmental outcomes associated with OPs.

The findings from the birth outcome studies are summarized in 2.2.7. The major findings of CCCEH, Mt. Sinai, and CHAMACOS on neurodevelopmental outcomes are briefly summarized in Section 2.2.8.1. Results from other studies on neurodevelopmental outcomes are summarized in 2.2.8.2.

2.2.2 Literature Search Methodology

To identify the epidemiological investigations of the association between OP exposure and adverse neurological, neurodevelopmental or neurobehavioral effects, EPA scientists queried PubMed/Medline and Web of Science directly on January 21 and January 23, 2015. In this literature search, emphasis was placed upon identification of all possible epidemiological studies available, and the ability to use the identical search string in both PubMed/Medline and Web of Science The following search string was utilized:

> ((Chlorpyrifos OR Organophosphates) AND (prenatal OR neonatal OR perinatal OR in utero OR fetal OR foetal OR newborn OR infant* OR infancy OR preschool OR child* OR maternal OR mother* OR pregnan*) AND (neurodevelop* OR attention OR birth outcome* OR health outcome* OR birth height OR birth weight OR birth length OR cephalometry OR head circumference OR child development OR cognitive OR cognition OR developmental disability* OR fetal growth OR foetal growth OR foetal development

OR fetal development OR intelligence OR memory OR neurological functioning OR psychomotor) AND (human)) (Filter: English language only)

With the aid of an EPA reference librarian, EPA also searched the following databases: PsycInfo, Agricola, Biosis, Embase, Enviroline, Gale Health and Wellness, Global Health, Pascal and Pollution Abstracts. EPA used similar, but modified search terms as listed above. Upon identification of the final set of relevant articles (n=38), limited hand-searching of the reference lists and citation mapping *(Science citation index)* of articles deemed to be most relevant to the review question was performed.

In 2015, EPA identified 299 articles across these several biomedical search engines. The determination of relevance to the study question was made by two EPA epidemiologists who agreed by consensus as to article disposition in the 2015 literature search. Removing duplicates (56), there were 243 articles, and 79 were determined to be epidemiological investigations of potential relevance. The 164 studies excluded from the analysis comprised 57 exposure only studies; 51 review articles; 33 reports of acute OP intoxication; 20 studies in non-human systems; and 3 were otherwise not relevant (See Appendix 4). Among the 79 potentially relevant epidemiologic studies, 41 were excluded; 17 articles were previously reviewed in 2012; 16 were epidemiological methods papers including exposure validation studies without an original epidemiological risk estimate; and 8 were otherwise not relevant for various reasons. Among the 40 remaining studies, 2 were additionally excluded (one was a duplicate study published a second time; the other did not make a measure of an OP pesticide. Therefore, 38 articles are included in the 2015 narrative literature review (referred to herein as "2015 Literature Review/Studies"). (See Appendix 5).

No additional formal literature search was conducted prior to the development of this 2016 update. However, the agency maintains active inquiry of the open scientific literature on issues related to OPs and the agency believes the added studies represent the available, relevant information. Of the 9 new epidemiology studies, two are meta-analyses.

The following sections provide the results of this literature review. Section 2.2.3 describes the breadth and depth of the 2015/2016 literature review, with Section 2.2.4 summarizing the approach for assigning a quality ranking and Section 2.2.5 providing the results of this quality ranking. In Section 2.2.6, these studies were further analyzed with focus on identification of the most appropriate exposure assessment and relevant outcomes for this assessment. Studies focusing solely on birth outcomes are discussed in Section 2.2.7. However, the emphasis in this assessment is on those studies focusing on neurodevelopmental outcomes, which are discussed in detail in Section 2.2.8 and summarized in Section 2.2.9.

2.2.3 Breadth and Depth of the 2015/2016 Literature Review

Key features of each of the 47 articles in the 2015/2016 literature review are summarized in Table 2.2.5.1-1, 2.2.5.1-2, and 2.2.5.2-1 as well as Appendix 3. These articles cover wide range

of study designs, study locations and time periods, and exposure and outcome measurement approaches and are listed in Table 2.2-a. The majority of the studies utilized a cross-sectional or a prospective birth cohort study design.

- Mothers and Newborn Study of North Manhattan and South Bronx conducted by Columbia University, New York, NY (See tables 2.2.5.1-1 & 2.2.5.1-2)
- Mount Sinai Inner-City Toxicants, Child Growth and Development Study, New York, NY (See tables 2.2.5.1-1 & 2.2.5.1-2)
- Center for Health Assessment of Mothers and Children of Salinas Valley (CHAMACOS) conducted by the University of California Berkeley (See tables 2.2.5.1-1 & 2.2.5.1-2)
- Denmark, Birth Cohort (Andersen *et al.* 2015)
- Saint Peter's University Hospital, New Brunswick, New Jersey, Birth Cohort (Barr *et al.* 2010)
- Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT), Mexico City, Mexico, Birth Cohort (Fortenberry *et al.* 2014, 2014a)
- EcoSalud Project, Cayambe-Tabacundo region, Ecuador, Infant and Young Child Cohort (Handal *et al.* 2007, 2007b, 2008)
- University Hospital of Heraklion, Crete, Greece, Birth Cohort (Koutroulakis *et al.* 2014)
- Children's Pesticide Survey (CPS), Yuma County, Arizona (Lizardi *et al.* 2008)
- Infancio y Medio Ambiente (INMA) (Environment and Childhood), Spain, Birth Cohort (Llop *et al.* 2013)
- Perturbateurs endocriniens: Étude Longitudinale sur les Anomalies de la Grossesse, l'Infertilité et l'Enfance (PELAGIE), Brittany, France, Birth Cohort (Petit *et al.* 2010; Cartier et al, 2015)
- Health Outcomes and Measure of the Environment (HOME), Cincinnati, OH, Birth Cohort (Rauch *et al.* 2012; Yolton et al, 2013; Donauer et al, 2016; Harley et al, 2016; Engel et al, 2015)
- Embilipitiya Base Hospital, Southern Sri Lanka, Birth Cohort (Samarawickrema *et al.* 2008)
- Ontario Farm Family Health Study, Ontario, Canada, Birth Cohort (Savitz *et al.* 1997)

ED_002061_00044425-00028

- Childhood Autism Risks from Genetics and the Environment (CHARGE), California, Child Cohort (Shelton *et al.* 2014)

- Secondary Exposure to Pesticides Among Infants, Children and Adolescents (ESPINA), Pedro Moncayo County, Pichincha, Ecuador, Child Cohort (Suarez-Lopez *et al.* 2012, 2013, 2013a)

- Shenyang, China, Birth Cohort (Zhang *et al.* 2014)

- Rice and aquaculture farming regions outside of Bangkok, Thailand (Fiedler et al, 2015)

Exposures were assessed via environmental samples, biomarkers, and/or proxy methods.

- Only one study used environmental samples as an exposure measure - methyl parathion in household wipe samples (Ruckart *et al.*, 2004).

- Among the studies, 11 biomarkers were used to assess exposure. One study utilized a direct measure of OP pesticide – chlorpyrifos (CPF) in maternal and cord serum (Barr *et al.*, 2010). Several studies looked at specific OP parent metabolites (IMPY, 3,5,6-trichloro-2-pyridinol [TCPy], PNP, MDA). The majority of studies used non-specific OP biomarkers: dialkyl phosphates (DAPs), diethyl phosphates (DEPs), and dimethyl phosphates (DMPs). Two effect biomarkers, AChE and butyl cholinesterase (BuChE), were used as the exposure metric.

- Proxy exposure methods included questionnaire and non-questionnaire approaches. For example, questionnaire-based exposures included maternal and or paternal self-report of living with an exposed worker, occupational exposure/ employment, and home pesticide use and child outdoor play exposure. Non-questionnaire-based exposures included Community/area of residence, distance to treated area/farm, percent of area treated with pesticide, level of urbanization, pounds OP pesticide used/year, and pesticide spray season.

Likewise, there were numerous outcome measures examined across the studies, falling into six broad categories: birth characteristics, autonomic nervous system (ANS) effects, Attention Deficit Hyperactivity Disorder (ADHD)/attention problems, autism, general neurodevelopment (cognitive, behavioral, IQ), and physiological effects. The most common outcome measures were birth characteristics (with birth weight, birth length, head circumference and gestational age being the most frequent) and neurodevelopment tests and test batteries. Table 2.2-b lists the many specific neurodevelopment tests employed in the studies. Most studies utilized more than one test, and few tests were utilized in more than one study.

Other features that varied widely among the studies include:

- Study periods ranged from 1991 to 2012 (reports were published from 1997 to 2016).
- Study sizes varied from 25 to 3,159 participants.
- Children's ages ranged from newborns to age 15 years.

Pre-natal exposures were assessed in 23 reports, post-natal exposures in 14 reports, and both pre- and post-natal exposures in 7 reports.

Table 2.2-a Study Designs, Exposure Measurement Methods, and Outcome Measurement Methods Used across the 2015/2016

Study design (# studies used)	Exposure measurement (# studies used*)	Outcome measurement (# studies used*)
Prospective Cohort (16) Retrospective Cohort (3) Case-control (2) Cross-sectional (16) Ecological (1)	**Biomarkers** **AChE** - acetyl cholinesterase (maternal 1, child 2) **BuChE** - butyl cholinesterase (child 1) **CPF** -chlorpyrifos parent (maternal 1, cord serum 1) **DAP** – dialkyl phosphate (maternal 9, child 10, amniotic fluid 1) **DCCA** - dimethylvinylcyclopropane carboxylic acid (child 1) **DEP** – diethyl phosphate (maternal 3, child 8, amniotic fluid 1) **DETP** – diethylthiophosphate (maternal 2) **DEDTP** – diethyldithiophosphate (maternal 2) **DMP** – dimethyl phosphate (maternal 8, child 8, amniotic fluid 1) **DMTP** – dimethylthiophosphate (maternal 1) **DMDTP** – dimethyldithiophosphate (maternal 2) **DZN** – diazinon parent (1) **IMPY** – diazinon metabolite (child 1) **MAL** - malathion parent (0) **MDA** - malathion metabolite (maternal 1) **OP** - organophosphate (cord blood 1) **PNP** - para-nitrophenol (child 2) **TCPy** - chlorpyrifos metabolite (maternal 1, child 2) **Environmental**	**Birth characteristics** (10) • Birth Weight/LBW/FGR (records 7, NP 1, report 2) • Birth Length (records 3) • Head circumference (records 3, NP 1) • Abdominal circumference (records 1) • Gestational age (GA)/preterm (report 2, records 3) • Ponderal index (records 1) • Placental maturity index (1) • Spontaneous abortion/miscarriage (report 1) • Altered sex ratio (report 1) **Autonomic Nervous System** (4) **ADHD/attention problems** (2) • Diagnosis DISC-IV (1) • Use of medication (1) • Screening (CPRS-R, CPT, BASC-PRS) (1) **Autism** (2) • CA Department of Developmental Services (CDDS) reports • US Individuals with Disabilities Education Act (IDEA) reports • Autism spectrum disorders (ASD) • Autism Diagnostic Observation Schedule (ADOS) combined with ADI-R. **Neurodevelopmental (ND) test/battery** (19) – see Table 2.2-2b for details **IQ** (57) - see Table 2.2-2b for details

ED_002061_00044425-00031

Study design (# studies used)	Exposure measurement (# studies used*)	Outcome measurement (# studies used*)
	Methyl parathion - Household wipe (1) **Proxy (questionnaire)** • Maternal occupational exposure/ employment (6) • Paternal occupational exposure/ employment (2) • Living with exposed worker (1) • Home and outdoor play exposure (1) **Proxy (non-questionnaire)** • Community/area of residence (3) • Distance to treated area/farm (2) • Percent of area treated with pesticide (1) • Level of urbanization (1) • Pounds OP used/year (1) • Pesticide spray season (1)	**Physiological** (3) • AChE activity, child • BuChE activity, maternal • Antioxidant status: superoxide dismutase (SOD) activity • Fetal oxidative stress: malondialdehyde (MDA) concentrations) • Fetal DNA fragmentation: electrophoresis • Respiratory symptoms, child

*A single study may include biomarkers at multiple endpoints and / or multiple outcome measurements; thus the total number of studies used may exceed the total number of studies in this manuscript

ED_002061_00044425-00032

Table 2.2-b Detailed Neurological Outcome Measurement Methods Used across the 2015/2016 Review Studies

Outcome measurement

Neurodevelopmental (ND) test/battery (USA-based studies)
• Pediatric Environmental Neurobehavioral Test Battery (PENTB) : cognitive, motor, sensory, and affect domains
  ○ Developmental Test of Visual-Motor Integration (VMI)
  ○ Kaufman Brief Intelligence test (K-BIT)
  ○ Purdue Pegboard
  ○ Story Memory and Story Memory-Delay from Wide Range Assessment of Memory and Learning
  ○ Trail-Making test, Part A and Part B
  ○ Verbal Cancellation test
• Children's Memory Scale (CMS)
• Bayley Scales of Infant Development, Second Edition (Bayley-II)
• Mental Developmental Index (MDI)
• Psychomotor Developmental Index
• Clinical Evaluation of Language Fundamentals-Preschool, Second Edition
• Archimedes spirals
• NICU Network Neurobehavioral Scale (NNNS)
• Behavioral measures:
  ○ The Child Behavior Checklist/4-18
  ○ The Teacher Report Form
• Developmental delay (DD):
  ○ Mullen Scales of Early Learning (MSEL)
  ○ Vineland Adaptive Behavioral Scale (VABS)
  ○ Reciprocal social interaction: Social Responsiveness Scale

ND test/battery (Non-USA-based studies)
• Bayley Scales of Infant Development - mental and psychomotor development
• Behavioral Assessment and Research System (BARS): Memory and attention, response speed and coordination, visual memory, attention, divided attention, recall and recognition memory, dexterity, hand-eye coordination
• Figure drawing task: child's perception and dexterity
• Long-term memory test
• Ages and Stages Questionnaire (ASQ) – communication, fine motor, gross motor, problem solving, and personal–social skills
• Strengths and Difficulties Questionnaire, parent version (SDQ): behavioral problems
• NEPSY-II test (trained examiners): general assessment battery:
  ○ attention and executive functioning
  ○ language
  ○ memory and learning
  ○ sensorimotor (visuomotor precision),
  ○ visuospatial processing
  ○ Statue and Knock Tap
• Neonatal Behavioral Neurological Assessment (NBNA)
  ○ Behavior
  ○ Passive Tone
  ○ Active Tone
  ○ Primary Reflexes
  ○ General Assessment
• Reach-and-grasp, bi-manual coordination: Prehension abilities
• UC Berkeley Preferential Looking Test Cards: Visual acuity skills
• Visual Motor Integration (VMI), Beery-Buktenica, 4th Ed.
• Finger Tapping test: Manual motor speed
• Catsys equipment: Simple reaction time
• Conners' Continuous Performance Test II (CPT II, v5): Attention
• Woodcock-Johnson III Tests of Cognitive Abilities (WJ-III) Verbal Comprehension test: Long-term memory and language function
• Visuospatial performance and memory functions:
  ○ Raven's Colored Progressive Matrices

ED_002061_00044425-00033

- Stanford–Binet Copying Test, 4th ed
- Physical examination (social response, spontaneous motility, involuntary movements, Romberg's sign, walking straight line, standing on one leg, number hops, biceps and patellar reflexes, finger opposition, diadochokinesis, finger–nose coordination, hearing, vision)
- Gesell Development Schedule (GDS): motor, adaptive, language, and social
- Santa Ana Form Board: Motor coordination
- Child's developmental delay - Parent interview

IQ

- Wechsler Intelligence Scale for Children-Revised (WISC-R) Digit Span Test, Card sorting Test
- Wechsler Intelligence Scale for Children, 4th edition (WISC-IV)
- Wechsler Preschool and Primary Scale of Intelligence, Third Edition
- Stanford-Binet Memory for Sentences and Digit String test
- Recall and recognition test

2.2.4 Study Designs & Considerations for Study Quality Evaluation

2.2.4.1 Study Designs

Besides the two meta-analyses, four basic study designs were used in the literature reviewed for this document: cohort study, case-control study, cross-sectional study, and ecologic study. The first of these two constitute the two basic types of observational (i.e. non-interventional) studies used to evaluate relative incidence of health and disease outcomes by exposure status. The latter two are generally considered descriptive or hypothesis generating study designs, though they too can be used to test hypotheses regarding relative prevalence of health outcomes and, under certain conditions, incidence as well.

Cohort Study

A commonly used design in this literature was the cohort study (See Tables 2.2.5.1-1, 2.2.5.1-2, and Appendix 3 for examples). In a typical cohort study, individuals are classified according to exposure status (i.e., presence, absence, or magnitude of exposure), and then followed over time to quantify and compare the development (i.e., incidence) of the health outcome of interest by exposure group. Conceptually, the non-exposed comparison group in a cohort study provides an estimate of the incidence of the outcome among the exposed, had they, counter-to-fact, not been exposed. Apart from chance variations, a valid cohort study comparing exposed individuals to non-exposed individuals provides an estimate of the relative risk (or rate) of the disease associated with exposure. Ideally, the exposed and non-exposed groups are exchangeable, in the sense that switching the exposed to non-exposed, and non-exposed to exposed would yield the same measure of association (e.g., relative risk). If this were the case then, apart from chance, a cohort study would yield a measure of association equivalent to that produced in a corresponding (intervention) study where exposure status was randomly assigned.

The chief advantage of the cohort study design is that it affords the investigator the opportunity to avoid and/or adjust for potential biases (i.e., selection bias, information bias, and confounding). Cohort studies also allow for discernment of the chronological relationship between exposure and outcome, and can be particularly efficient for studying uncommon exposures. The primary disadvantage of the cohort study design is logistical inefficiency with respect to the necessary time, expense, and other resources needed to conduct them. Cohort studies are particularly inefficient for evaluating associations with rare outcomes and diseases with long induction or latency periods. Though prospective studies are often logistically less efficient relative to other study designs (e.g., the case-control study), these logistical concerns can be minimized in cohort studies of short duration, such as those used to evaluate prenatal OP pesticide exposure effects on birth outcomes or other outcomes of neonatal development assessed shortly after birth.

Two sub-categories of cohort studies – prospective and retrospective - are often applied to distinguish between studies in which the health outcome has occurred (retrospective study), or

ED_002061_00044425-00035

has not occurred (prospective study) at the time the investigators initiate the study. This distinction is important primarily as it pertains to the potential differences in the quality (e.g., completeness, accuracy, and precision) of information that can be ascertained by the investigators, and also as it relates to potential sources of bias. Although not always true, the prospective study design is considered the preferable of the two, as investigators can potentially have more choices in determining how exposure, outcome, and covariate information is collected. In a retrospective study conducted to evaluate the same hypothesis, by contrast, the investigators would have to rely on exposure information such as maternal self-report. Such reporting is subject to (human) errors in recall. Moreover, the outcome status of the child (i.e., whether a child has developmental delays that are known to the mother) may influence the recall of prenatal OP pesticide exposure by the mother.

Case-control Study

In a typical case-control study (see, for example, Dawbrowski *et al.*, 2003 in Appendix 3 and Shelton *et al.* 2014 in Table 2.2.5.2-1), individuals are classified according to their outcome status (i.e., cases who have developed the outcome of interest, and controls who represent the population from which the cases arise). The relative odds of exposure are then compared between cases and controls. The primary advantage of case-control studies is that they are logistically efficient relative to cohort studies. In fact, properly conducted case-control study can be conceptualized as a cohort study with efficient sampling of exposure among the cohort, yet they can often be conducted at a fraction of the cost, in a fraction of the time as a corresponding cohort study. Case-control studies can be used to examine associations between multiple exposures and a given health outcome. They are particularly efficient for evaluating rare outcomes but are inefficient for studying uncommon exposures. The primary weakness of the case-control study is the potential for selection bias, which arises if the exposure distribution among the control subjects is not representative of the exposure distribution among the population that gave rise to the cases. Case-control studies that rely on self-reported exposure measures are also susceptible to information bias.

Cross-sectional study

Cross-sectional studies (see, for example, Suarez-Lopez *et al.* in Table 2.2.5.2-1and Grandjean *et al.* 2006 in Appendix 3) are used to evaluate associations between exposure and outcome prevalence in a population at a single point in (or period of) time. The primary advantage of a cross-sectional study is logistical efficiency; they are relatively quick and inexpensive to conduct, as a long period of follow-up is not required, and exposure and outcome assessments occur simultaneously. Cross sectional studies have three primary *potential* disadvantages: 1) potential difficulty in discerning the temporal relationships (i.e., whether the exposure precedes the outcome); 2) estimating outcome prevalence rather than incidence of the outcome; and 3) the possible overrepresentation of cases of the outcome with long duration relative to the average in the population, and often with a better prognosis.

Ecological study

Ecological studies are used to evaluate associations between exposures and outcomes using population-level rather than individual-level data. For example, Nevison (2014, Appendix 3)

uses annual estimates of pesticides applied to crops and population level autism prevalence to assess the association between OP pesticide exposure and autism. The primary advantages of ecological studies are related to logistical efficiency, as they often rely on pre-existing data sources and require no individual-level exposure, outcome, or covariate assessments. The primary weakness of the ecologic study is the potential for confounding and resultant inappropriate extrapolation of associations observed on the aggregate-level to associations on an individual level. The mistaken belief that associations observed at the population level exist at the individual level is referred to as the ecological fallacy.

In judging an individual study's contribution to the strength of evidence in the epidemiologic literature base, the following hierarchy of observational study designs was considered (from most to least preferred): prospective cohort study, retrospective cohort study, case-control study, cross-sectional study, ecological study. It is important to note, however, that this hierarchy of study designs reflects the *potential* for the collection of high quality information (related to exposure, outcome, confounders, and effect modifiers) and *potential* for efficient and valid estimation of the true association. Thus, in deliberating on quality, care has been taken to consider the circumstances and particulars of each individual study. For example, a well-conducted case-control study of a rare outcome can provide much higher quality evidence vis-à-vis the association of interest than a poorly conducted prospective cohort study of the same relationship. For this report, the placement of the study design in the aforementioned hierarchy of observational study designs was but one facet of the judgment of study design quality. Additional consideration was given to whether the study was *well conducted*, independent of study design type. The particulars of a study's design, specifically the design elements employed to minimize and adjust for biases, were also considered. Finally, the relevance of each study with respect to the association of primary interest in this initiative, namely the relationship between prenatal (and early life) OP pesticide exposures and fetal and child neurodevelopment was considered.

2.2.4.2 Considerations for Study Quality

This section summarizes how specific study characteristics factored in overall quality category. [Note: these study quality considerations are specific to issue of relevance to this document, namely potential for neurodevelopmental effects of OPs. These considerations are considered 'fit for purpose' under this context and could differ in another regulatory or scientific context.]

The literature base evaluated is heterogeneous, as noted in Section 2.2.3. Pesticide exposure assessments variously relied on, for example, exposure biomarkers, maternal self-reports, and other proxy indicators of OP pesticide exposures. Outcome assessments were similarly varied, relying, for example, on biomarkers of biological effects, birth records, maternal self-reports, and clinical instruments designed to evaluate neurocognitive and neurobehavioral development. These design elements have potential impacts on study quality and relevance to this document. Each study was therefore judged to be of high, moderate, or low quality in five

ED_002061_00044425-00037

domains effecting study quality: exposure assessment, outcome assessment, confounder control, statistical analysis, susceptibility to bias (See Table 2.2.4-1 for general considerations under each domain).

Table 2.2.4-1 Study Quality Considerations

Parameter	High	Moderate	Low
Exposure assessment	Exposure assessment includes information on specific OP a.i.'s (e.g., CPF, MAL), or urinary metabolite (TCPy, IMPy,), or high quality questionnaire based chemical specific exposure assessment during relevant exposure window (pre-natal, early life)	Non-specific biomarker of exposure (DAP), or effect (AChE/BuChE), or questionnaire based individual level information on the OP class, or sub-class	Low quality questionnaire based exposure assessment, or ecologic exposure assessment, with or without validation
Outcome Assessment	Standardized tool, validated in study population; or, medical record review with trained staff (birth characteristics)	Standardized tool, not validated in population, or screening tool; or, medical record review, methods unstated (birth characteristics)	Selected sections of test, or maternal report, other; or, maternal/paternal self-report (birth characteristics)
Confounder control	Good control for important confounders relevant to OP-ND question, and standard confounders	Moderately good control confounders, standard variables, not all variables for OP-ND question	Multi-variable analysis not performed, no adjustments
Statistical Analysis	Appropriate to study question and design, supported by adequate sample size, maximizing use of data, reported well (not selective)	Acceptable methods, questionable study power (especially sub-analyses), analytic choices that lose information, not reported clearly	Minimal attention to statistical analyses, comparisons not performed or described clearly
Risk of (other) bias (selection, differential misclassification, effect size magnification, other)	Major sources of other potential biases not likely present, present but analyzed, unlikely to influence magnitude and direction of the risk estimate	Other sources of bias present, acknowledged but not addressed in study, may influence magnitude but not direction of estimate	Major study biases present, unacknowledged or unaddressed in study, cannot exclude other explanations for study finding

ED_002061_00044425-00038

(Adapted from Munoz-Quezada *et al.* 2013)

2.2.4.2.1 Exposure Measures

There were three major categories of exposure assessment employed in this literature: exposure biomarkers, participant-reported proxy exposure via questionnaire, and objectively obtained proxy indicators of exposure. Although one study included environmental wipe sampling results in the exposure assessment, urinary biomarker measures were also included and no differentiation of the two approaches was presented, so this exposure assessment category has not been included. The merits and the disadvantages of the three primary exposure assessment strategies are discussed below. For this evaluation, studies employing exposure assessments that quantified biomarkers of specific OP pesticides (e.g., chlorpyrifos, malathion, diazinon), or urinary metabolites of these pesticides (e.g., TCPy, malathion dicarboxylic acid, 2-isopropyl-4-methyl-6-hydroxypyrimidine), or high-quality chemical-specific exposure quantitation during relevant exposure-time windows (i.e. pre-natal, early life) were given the highest weight. Studies that quantified levels of non-specific biomarker of OP pesticide exposure (e.g., DAPs), or exposure effects (e.g., AChE, BuChE,) were given a moderate weight. Studies relying on high-quality survey-based individual-level information on pesticide exposure were also assigned a moderate weight. Exposure assessments that only crudely or subjectively classified pesticide exposures and ecologic and other proxy measures of exposure were assigned a low weight.

Many studies used questionnaire-based exposure assessments in which study participants (typically mothers) self-reported their exposures, in addition to, or instead of, quantifying OP pesticide biomarkers in samples of biological media. These exposure assessments typically include querying OP pesticide exposure directly, or asked study participants to report on behaviors and conditions associated with pesticide use (e.g., occupation, tasks). Such reporting likely misclassifies actual OP pesticide exposure. If conducted as part of a prospective exposure assessment, these errors are likely to be non-differential with respect to the outcome(s) of interest. In the context of a retrospective assessment in which the mother has knowledge of the outcome status of the child, these errors may be differential or non-differential.

Several studies used proxy measures (including ecological indicators) of pesticide exposure. These included, for example questions about occupational use and exposure to pesticides, distance from residence to fields where pesticides were applied, the proportion of land in a specified area dedicated to agricultural uses, and occurrence of pregnancy during the pesticide application season. Again, substantial non-differential exposure measurement error/misclassification of exposure is likely.

The CHARGE study (Shelton *et al.*, 2015) used a different method for exposure assessment. This study used geospatial analysis to focus on the residential proximity to OP exposure and the association of this exposure with autism spectrum disorders. OP exposure was assessed by Shelton *et al* (2015) using data from the California Department of Pesticide Regulation, with five OPs accounting for a total of 73% of the exposure and each accounting for 10% or more of

the exposure (chlorpyrifos, acephate, diazinon, bensulide, and dimethoate); eight OPs accounting for a total of 25% of the exposure and each accounting for 1% or more of the exposure (malathion, methyl parathion, azinphos-methyl, phosmet, oxydemeton-methyl, ethephon, naled, and methidathion); and eight OPs accounting for a total of 2% of the exposure and each accounting for 0.1% or more of the exposure (methamidophos, phorate, disulfoton, fenamiphos, coumaphos, parathion, ethoprop, and sulfotep).

Most of the studies reviewed herein assessed biomarkers of exposure quantified in samples of biological media (most often urine, but also blood, serum, and breast milk). These biomarkers were of three types: 1) OP pesticide residues, 2) metabolites of specific OP pesticides, and 3) non-specific OP metabolites.

Two studies focusing on neurodevelopmental outcomes measured OP exposure using both DAPs and malathion dicarboxylic acid (a metabolite of malathion) (Eskenazi *et al.*, 2007; Engel *et al.*, 2007), with TCPy exposure also being measured in one of these studies (Eskenazi *et al.*, 2007). Additionally, two studies focusing on birth outcomes measured OP exposure by testing for specific OPs, with Whyatt *et al.* (2004) measuring chlorpyrifos and diazinon; and Eskenazi *et al.* (2004) testing for DAPs and for seven pesticide specific metabolites (MDA - derived from malathion; PNP - derived from methyl parathion, parathion, and other nonpesticide chemicals; TCPy - from chlorpyrifos, chlorpyrifos methyl, and triclopyr; DEAMPY - from pirimiphos methyl; IMPY - from diazinon; CMHC - from coumaphos and coumaphos methyl; CIT - from isazophos and isazophos methyl). Finally, several method validation studies tested for specific OP pesticides, but did not evaluate the association between these exposures and specific adverse health outcomes (Whyatt *et al.*, 2007; Whyatt *et al.*, 2009; Bradman *et al.*, 2003).

The most commonly measured biomarkers were urinary DAPs. These non-specific markers are easily quantified using gas chromatography/mass spectrometry and related methods. Though objective, use of urinary DAPs as biomarkers for OP pesticide exposures has limitations, including substantial temporal variability, often varying substantially over short time scales (i.e., day-to-day). Quantification of DAPs in a single urine sample may not represent an individual's usual exposure to OP pesticides over the time period of interest (e.g., pregnancy) in their utility as biomarkers of OP pesticide exposure. Urinary DAP metabolite levels may also reflect exposure to ambient metabolites in addition to exposure to OP parent compounds. For example, Chen et al (2012) and Zhang et al (2008) have shown that direct exposure to DAPs can occur from consumption of DAPs in food. Lu et al. (2005) observed that OP pesticides in fortified fruit juice samples degraded into DAPs. In this literature, errors in DAP as a biomarker of OP pesticide exposure are likely to be non-differential with respect to outcomes. Epidemiologists often distinguish between two mechanisms or types of misclassification – those that are non-differential (or random) and those that are differential (non-random).

Total DAPs is a non-specific measure of OP exposure and is the sum of six separate molecules - three dimethyl alkylphosphate (DMAP) molecules of DMP, DMTP, DMDTP, and three diethyl alkylphosphate (DEAP) molecules of DEP, DETP, and DEDTP. Each metabolite is a breakdown product from multiple OPs (Table 2.2.4-2). Specifically, DMP, DMTP, and DMDTP are associated

with 18, 13, and 5 OPs, whereas DEP, DETP, and DEDTP are associated with 10, 10, and 4 OPs, respectively. Thus, using DAPs as an exposure measure, it is not possible to separate the exposure and associated effects for single, specific OPs. For studies evaluating TCPy (e.g., Fortenberry *et al.*, 2014; Eskenazi *et al.*, 2007; Whyatt *et al.*, 2009), this molecule is a metabolite of chlorpyrifos, chlorpyrifos-methyl, and the herbicide triclopyr and thus is not entirely specific to chlorpyrifos. TCPy can be found on directly food. Studies focusing solely on chlorpyrifos could assess exposure to only this OP (i.e., measure chlorpyrifos directly) (e.g., Lovasi *et al.*, 2011; Whyatt *et al.*, 2004; Rauh *et al.*, 2011).

Table 2.2.4-2 CDC Table of Organophosphate Pesticides and Their Dialkyl Phosphate Metabolites (2008)

Pesticide	DMP	DMTP	DMDTP	DEP	DETP	DEDTP
Azinphos methyl	|	|	|			
Chlorethoxyphos				|	|	
Chlorpyrifos				|	|	
Chlorpyrifos methyl	|	|				
Coumaphos				|	|	
Dichlorvos (DDVP)	|					
Diazinon				|	|	
Dicrotophos	|					
Dimethoate	|	|	|			
Disulfoton				|	|	|
Ethion				|	|	|
Fenitrothion	|	|				
Fenthion	|	|				
Isazaphos-methyl	|	|				
Malathion	|	|	|			
Methidathion	|	|	|			
Methyl parathion	|	|				
Naled	|					
Oxydemeton-methyl	|	|				
Parathion				|	|	
Phorate				|	|	|
Phosmet	|	|	|			
Pirimiphos-methyl	|	|				
Sulfotepp				|	|	
Temephos	|	|				
Terbufos				|	|	|
Tetrachlorviphos	|					
Trichlorfon	|					

DMP = dimethylphosphate; DEP = diethylphosphate; DMTP = dimethylthiophosphate; DMDTP =

ED_002061_00044425-00041

dimethyldithiophosphate; DETP = diethylthiophosphate; DEDTP = diethyldithiophosphate.

2.2.4.2.2 Neurological and Other Outcome Measures

With some exceptions, the outcomes assessed in this literature fall into three broad categories: 1) neurobehavioral and/or neurodevelopmental status; 2) birth outcomes; and 3) neurodevelopmental diseases and/or disorders (see Table 2.2-b).

There is a broad body of literature available on the use and interpretation of instruments designed to quantify neurological status that is beyond the scope of this summary. Many instruments were used in this literature to assess infant neurodevelopment and neurobehavior (see Table 2.2-b). Importantly, performance on some of these tests can be influenced by the administrator of the assessment. Also of concern is whether these assessments are sensitive enough to distinguish potentially subtle effects of OP pesticide exposure. Some degree of error in the assessment of neurological outcomes is likely in all of the studies, though the errors were unlikely to be related to exposure status in the well conducted studies.

The assessment of birth outcomes in this literature was primarily conducted by reviewing of medical records or birth certificates; these assessments are likely to have minimal errors, and errors that do arise are almost certainly non-differential with respect to exposure status. In some studies, however, birth outcomes were reported by the mother and in these instances, differential misclassification is possible.

The studies that evaluated specific diagnoses, autism spectrum disorders or developmental delay, for example, relied on existing medical records, with some effort to validate diagnoses in a subset of the investigations.

Studies that relied on standardized, validated instruments to assess neurodevelopment, medical records of birth outcomes, or validated diagnosis of disease states were weighted highly in the judgment of study quality. Those that used standardized instruments that had not been validated in the relevant population or screening assessments were given a moderate weight, while studies relying on selected sections of neurodevelopment assessment tools, maternal report of outcome status, or aggregated (ecological) outcome measures were given the lowest weight.

2.2.4.2.3. Statistical Analysis

Statistical analyses that were appropriate to the study question and study design, supported by adequate sample size, maximized the use of available data, and were well characterized in the report were weighted most highly. Acceptable statistical methods, moderate study power, and analytic choices that resulted in the loss of information or that were not clearly reported were given moderate weight. Reports with only minimal attention paid to the conduct and reporting of the statistical analyses were given the lowest weight.

2.2.4.2.4 Confounding

Risk factors for early life neurodevelopment perturbations that are associated with OP pesticide exposure, but not caused by pesticide exposure, are potential confounders in this literature. Socioeconomic determinants of child development (including, for example, maternal education and access to prenatal and early life health care), quantity and quality of parent-child interactions, and exposure to other environmental toxicants (e.g., lead, PCBs, other pesticides) are difficult to measure and were either not accounted for, or inadequately accounted for in many studies. That said, some studies were relatively homogeneous with respect to these factors and thus limited confounding by design, while others attempted to quantify these factors and adjust for them. Other important potential confounders, such as the child's sex, are easy to identify and adjust for analytically.

2.2.4.2.5 Risk of Bias

The internal validity of the studies reviewed was judged by noting the design strategies and analytic methods used in each study to constrain or eliminate selection bias, information bias, and confounding. Selection bias occurs when the sampling of the population by the investigator yields a study population that is not representative of the exposure and outcome distributions in the population sampled. Put simply, selection bias occurs if selection of the study sample yields a different estimate of the measure of association than that which would have been obtained, had the entire target population been evaluated. Although there are numerous sources of selection bias, there are several mechanisms that may have induced selection bias in the studies reviewed: less than 100% participation rates of eligible individuals due to non-responsiveness or refusal (self-selection bias); loss to follow-up (i.e. failure to retain all study participants initially enrolled in the study); and, in a case-control study, control selection bias arising because the exposure distribution in the control sample does not represent the exposure distribution of the study base (i.e., the population that gave rise to the cases or more formally, the person-time experience of that population).

Information bias (also referred to as observation bias) arises when study participants are incorrectly categorized with respect to their exposure or outcome status, or when errors arise in the measurement of exposure or outcome, in the case of continuously distributed measures. Epidemiologists often distinguish between two mechanisms or types of misclassification – those that are non-differential (or random) and those that are differential (non-random). Non-differential misclassification of exposure (or non-differential exposure measurement error) occurs when the probability or magnitude of error in the classification or measurement of exposure is independent of the outcome status of the study participants. Similarly, non-differential misclassification of outcome (or outcome measurement error) occurs when the probability or magnitude of error in the assignment of outcome status or level is independent of exposure status. In contrast, differential exposure misclassification (or measurement error) occurs when the error in the exposure assignment is not independent of the outcome status.

The mechanisms that cause non-differential misclassification in this literature include errors in the medical records, laboratory errors, sampling of biospecimens for biomarker assays, and errors in recall. The mechanisms that induce differential misclassification include recall bias,

and interviewer/observer bias. Note that mismeasurement of confounders can result in residual confounding of the association of interest, even when adjustment for that confounder has been conducted in the analysis.

Studies in which major sources of potential biases were not likely to be present, or in which potential sources of bias were present but effectively addressed and analyzed to maximize the study validity, and those in which sources of bias were unlikely to influence the magnitude and direction of the risk estimate were given a high weight. Studies where sources of bias were present and acknowledged by the authors but not addressed in the study and yet may influence the magnitude, but not direction of the association estimate received a moderate weight. A low weight was given to studies in which major biases were present and yet were not acknowledged or addressed in the study, such that they cannot be excluded as an alternative explanation for the study finding.

2.2.5 Review of Quality Results

Each of the studies in the 2015 review was judged to be of high, moderate, or low quality in each of five domains of study design and methodology effecting study quality as discussed above in Section 2.1. The results of the quality assessment are presented separately below for each group. The quality categories represent to the total evaluation. In Section 2.2.6, further evaluation of the study design and exposure assessment of the medium and high quality studies. This further evaluation led to additional studies being removed from the final analysis, with these excluded studies not being considered further in the remaining sections of this document.

2.2.5.1 "High" Quality Group

Fifteen articles assigned a high quality rating are shown in Tables 2.2.5.1-1 and 2.2.5.1-2. In general, these were prospective birth cohort studies with moderate to high sample size; exposure assessment was based on an objective biomarker measure, the outcome measurement(s) utilized standardized tests and trained data collectors, appropriate statistical analyses were performed, considering relevant covariates, and risks of bias were minimized to the extent possible. All of the studies from CCCEH, Mt. Sinai, CHAMACOS, and HOME cohorts are assigned a high ranking. Fortenberry *et al.* (2014, Table 2.2.5-1) reported on findings in the ELEMENT study population of 187 mother-child pairs, assessed third trimester maternal urinary TCPy as a biomarker of prenatal chlorpyrifos exposure, and employed a trained and experienced research team to administer a battery of validated ADHD/psychometric assessments (Conner's' Parental Rating Scales-Revised, Conner's' Continuous Performance Test, and the Behavior Assessment System for Children-Parental Rating Scales) that had been translated into Spanish.

ED_002061_00044425-00044

Table 2.2.5.1-1. High Quality Studies from 2015 and 2016 reviews: Summary of Study Design Elements Impacting Study Quality Assignment

Study	Study Location	Cohort Name / Description of Study Population	Study Design	Exposure Assessment	Outcome Assessment	Confounder / Covariate Control	Statistical Analysis	Risk of (other) Bias
Barr *et al*. (2010)	New Brunswick, New Jersey	Mother who underwent elective Cesarean delivery at term and their newborns, Saint Peter's University Hospital	Cross sectional birth cohort, modest sample numbers N=150 Mother-infant pairs	Objective measure, prenatal - parent CPF in maternal and cord serum at birth - exposures do not necessarily precede outcome	Birth outcomes from medical records	Generally appropriate; missing a few, e.g., maternal age, SES, nutrition, pre-natal care, race	Appropriate multivariate analysis	Convenience sample – deemed low probability of selection bias; low potential for exposure or outcome misclassification
Bouchard *et al*. (2010)	U.S. National Population	National Health and Nutrition Examination Survey (NHANES) Age 8-15 years	Cross-sectional, large sample size N=1139	Objective measure, postnatal – single non-OP specific child urinary DAPs	ADHD from DISC-IV diagnosis or medication in children 8-15 years old, standard protocol, trained interviewers	Appropriate: also included blood lead	Appropriate, accounted for NHANES multistage probability sampling	Low probability of selection bias; some potential for non-differential misclassification of exposure
Donauer et al. (2016).	Cincinnati, Ohio, USA	Health Outcomes and Measure of the Environment (HOME)	Prospective Birth Cohort Study – large sample size. N=327 Mother-infant pairs	Metabolite concentrations (total DAP, DM, and DE) of prenatal OP pesticide exposure quantified in two maternal spot urine samples provided at 16 and 26 weeks of gestation	For children, Bayley-II, MDI, and Psychomotor Development Index examinations at 1-3 years of age, and Clinical Evaluation of Language Fundamentals-Preschool, Second Edition, and the Wechsler Preschool and Primary Scale of Intelligence, at ages 4 and 5 were used.	Appropriate. Included household income, maternal intelligence, child's sex, maternal intelligence quotient, and maternal education; house inventory score, maternal age at birth, alcohol consumption during pregnancy, parity, and maternal lead levels	Appropriate. Bivariate and multivariable regression	Lack of frequent urine spot sampling, short half-life of DAP metabolites, and the sporadic exposure to DAP may have led to non-differential exposure misclassification; inability to determine exposure toxicity.
Engel et al. (2015)	Salinas Valley, California; Brown, Butler, Clermont, Hamilton, or Warren, Ohio; South Bronx, New York City; Manhattan, New York City	Pooled analysis of the CHAMACOS (1999-2000), Columbia (2000-2001), Mt. Sinai (1998-2002), and HOME (2003-2006) birth cohorts	Birth Cohort Study – large sample size N= 936 women (377 from CHAMACOS study, 265 from HOME study, 60 from Columbia study, and 234 from Mount Sinai study) Mother-infant pairs	Metabolite concentrations (total DAP, DMP, and DEP) of prenatal OP pesticide exposure quantified in two maternal spot urine samples provided at 13 and 26 weeks and 16 and 26 weeks of gestation (CHAMACOS and HOME studies) and in one maternal spot urine sample at 31 and 32 weeks (Mt. Sinai and Columbia studies) Blood samples from the mother and child (via the umbilical chord) were also collected and genotyped for the *PON1* genotype	For each child, Bayley Scales of Infant Development II (BSID-11) to generate a Mental Development Index (MDI) and a Psychomotor Development Index (PDI) to assess neurodevelopment in early childhood.	Appropriate. Included maternal education, marital status, and age at delivery, alcohol use during pregnancy, race/ethnicity, smoking and drug use during pregnancy, child sex, HOME environment score quartile, breastfeeding at least 3 months after birth, metabolite × center interaction terms, race/ethnicity, smoking and drug use during pregnancy.	Linear models with interaction analysis.	Lack of frequent urine spot sampling, short half-life of DAP metabolites, and the sporadic exposure to DAP may have led to non-differential exposure misclassification; inability to determine exposure toxicity; and hetergoenity of the study populations of the 4 cohorts and exposures may have effected the results
Furlong *et al*. (2014)	New York City, U.S.	Mount Sinai Children's Environmental Health Study	Prospective Cohort N=136 Age 7-9 years	Objective biomarker, prenatal OP pesticide exposure (DAP) quantified in single maternal spot urine sample provided during the 3rd trimester, simple imputation < lowest level of detection (LLOD)	Reciprocal social impairment at age 7-9 years assessed using the Social Responsiveness Scale, completed by mothers. Designed to assess reciprocal social behaviors in evaluating ASDs, used here as general indicator of impaired social responsiveness.	Appropriate. Included maternal and child demographic and SES indicators (age, education) and ETS (not other environmental toxicants).	Appropriate multivariate analysis	Selection bias possible due to considerable loss to follow-up; Residual confounding likely small in magnitude, some potential for non-differential misclassification of exposure possible explanation for null findings).
Fortenberry *et al*. (2014)	Mexico City, Mexico	Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT) study (3 cohorts 1994-1997 (cohort 1), 1997-	Prospective Birth Cohort Study – modest sample size	Objective measure - TCPy concentration was measured in third trimester maternal urine samples. In a subset of women	ADHD - LP - Conner's' Parent Rating Scales-Revised (CPRS-R), Conner's' Continuous Performance Test (CPT), and Behavior Assessment System for Children-Parental Rating Scales	Appropriate. Included continuous maternal IQ, education, socioeconomic status and blood lead one month after delivery, breast feeding (yes/no), child's sex,	Appropriate. Multivariable linear regression	History of maternal and paternal exposure to pesticides not included, use of a single urinary measure to estimate exposure, potential for differential exposure misclassification possible as mothers were

Study	Study Location	Cohort Name / Description of Study Population	Study Design	Exposure Assessment	Outcome Assessment	Confounder / Covariate Control	Statistical Analysis	Risk of (other) Bias
		2000 (cohort 2), and 2001-2005 (cohort 3))	N=187 Mother-infant pairs	randomly selected urinary TCPy in samples collected during all three trimesters of pregnancy was measured.	(BASC-PRS) – These are screening tools, not diagnostic tools. Standardization and quality control checks were conducted by reviews of videotaped evaluations.	continuous age at testing, birth length and head circumference at birth.		likely aware of the neurobehavioral status of their children
Fortenberry *et al.* (2014a)	Mexico City, Mexico	Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT) study	Prospective Birth Cohort Study – large sample size N=591 Mother-infant pairs	Objective measure - blood samples from mother–child pairs were analyzed for PON1. OP exposure was not explicitly assessed.	ADHD - LP - Conner's' Parent Rating Scales-Revised (CPRS-R), Conner's' Continuous Performance Test (CPT), and Behavior Assessment System for Children-Parental Rating Scales (BASC-PRS) - These are screening tools, not diagnostic tools.	Appropriate: Included continuous maternal IQ, education, socioeconomic status and blood lead one month after delivery, breast feeding (yes/no), child's sex, continuous age at testing, birth length and head circumference at birth.	Appropriate. Multivariable linear regression	Potential for differential exposure misclassification possible as mothers were likely aware of the neurobehavioral status of their children.
Harley et al. (2016)	Salinas Valley, California; Brown, Butler, Clermont, Hamilton, or Warren, Ohio; South Bronx, New York City; Manhattan, New York City	Pooled analysis of four CHAMACOS, Columbia, Mt. Sinai, and HOME birth cohorts	Birth Cohort Study – large sample size N= 1,235 women (484 from CHAMACOS study, 328 from HOME study, 82 from Columbia study, and 341 from Mount Sinai study) Mother-infant pairs	Metabolite concentrations (total DAP, DMP, and DEP) of prenatal OP pesticide exposure quantified in two maternal spot urine samples provided at 15.9 and 26.4 weeks of gestation (CHAMACOS and HOME studies) and in one maternal spot urine sample at 31.8 and 33.3 weeks (Mt. Sinai and Columbia studies) Blood samples from the mother and child (via the umbilical chord) were also collected and genotyped for the *PON1* genotype	For each child, head circumference, birth weight, and birth length were obtained from medical records	Appropriate. Included sex, race/ethnicity, cohort, country of origin, marital status, maternal education, smoking during pregnancy, parity, maternal age at delivery, and gestational age.	Appropriate. Multivariable linear regression and mixed-effect models to test the effect modification for sex, maternal education, race/ethnicity, and child *PON1* genotype.	Lack of frequent urine spot sampling, short half-life of DAP metabolites, and the sporadic exposure to DAP may have led to non-differential exposure misclassification; inability to determine exposure toxicity; and hetergoenity of the study populations of the 4 cohorts and exposures may have effected the results
Quirós-Alcalá *et al.* (2011)	Salinas Valley, California, USA	Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS)	Prospective Birth Cohort Study Outcomes assessed at: N=149 (6 months) N=149 (1 year) N=97 (3.5 years) N= 274 (5 years)	Objective biomarker, prenatal OP pesticide exposure (DAP) quantified in two maternal spot urine samples at 14 and 27 weeks gestation (on average), and in single child urine sample provided at time of each outcome assessment	Autonomic nervous system dysregulation at ages 6 months, 1 year, 3.5 years, and 5 years; standard protocol, trained, bilingual research staff, appropriate to age of child. May not be sensitive to resolve subtle effects of OP pesticide exposure.	Appropriate. Included maternal and child demographic and SES indicators (age, education).	Appropriate multivariate analysis	Selection bias possible due to considerable loss to follow-up; Residual confounding likely small in magnitude; some potential for non-differential misclassification of exposure (possible explanation for null findings).
Raanan *et al.* (2015)	Salinas Valley, California	CHAMACOS (Mothers and their children. Children were followed from through age 7)	Prospective Birth Cohort N = 364 children	Diethyl (DE) and dimethyl(DM) phosphate metabolites and other dialkyl phosphate (DAP) metabolites of OP pesticides measured in mother's urine during pregnancy and from children during childhood	Mother's report of child's respiratory symptoms and child's exercise-induced coughing at 5 and 7 years of age	Models were adjusted for child's sex, child's age, maternal smoking during pregnancy, secondhand tobacco smoke exposure during early childhood, season of birth, mean daily fine particulate concentration during first 3 months of life (PM2.5), breastfeeding, mold and cockroaches in home, and home distance from highway in first year.	Generalized estimating equations (GEE) with repeated measures of respiratory symptoms	Risk of misclassification of exposure, due to nonspecificity of DAP metabolite used as biomarker which may reflect exposure to parent compound (pesticide) or preformed DAPs in environment, and also due to potential for daily fluctuations in DAP levels due to high variability in OP pesticide exposures.

Study	Study Location	Cohort Name / Description of Study Population	Study Design	Exposure Assessment	Outcome Assessment	Confounder / Covariate Contro	Statistical Analysis	Risk of (other) Bias
Rauch *et al*. (2012)	Cincinnati, Ohio, USA	Health Outcomes and Measure of the Environment (HOME) Age: newborns	Prospective Birth Cohort Study – large sample size. N=306	Objective biomarker of prenatal OP pesticide exposure (DAP) quantified in two maternal spot urine samples provided at 16 and 26 weeks gestation	Abstracted birth weight from medical records, calculated gestational age from mother's self-reported date of last menstrual period. When gestational age not available, results of an ultrasound was used or a Ballard examination performed just after delivery.	Appropriate. Included income, education, maternal depressive symptoms, maternal IQ, insurance status, area of residence, prenatal care, PON1 genotype, and gestational exposure to alcohol, lead, and tobacco.	Appropriate. multivariable regression	Unable to rule out the possibility that differences in DAP concentrations partially reflect individual variation in metabolism, Recall error
Rauh et al. (2015)	New York City, New York	Columbia Center for Children's Environmental Health (CCCEH) (Children of Black and Dominican mothers, aged 9 – 13.9 years old)	Prospective Birth Cohort Study N = 271	Chlorpyrifos (CPF) concentrations in sample of umbilical cord blood collected at delivery Chlorpyrifos (CPF) concentrations in sample of maternal blood collected within 2 days postpartum	Neurodevelopmental assessment via Archimedes spirals, a drawing exercise, to test for tremor	Models were adjusted for sex, exact age at testing, ethnicity, and medications	Chi-square was to assess crude associations between high CPF exposure and presence of mild to moderate tremor. Binary logistic regression was used to estimate associations between high exposure level and presence of tremor	Risk of misclassification of exposure, particularly due to lack of CPF exposure biomarkers to assess postnatal and childhood exposure. Misclassification may also occur in the outcome assessment due to the use of a clinical scale, based on observer's rating to assess tremor
Stein et al. (2016)	Salinas Valley, California area	CHAMACOS Study (Pregnant women and 7 year-old children)	Prospective longitudinal cohort study N = 329 Mother-infant pairs	Biomarker of prenatal OP pesticide exposure (DAPs) quantified in two maternal spot urine sample collected at 13 and 26 weeks of gestation. Mothers were also interviewed following birth at 6 months, 1, 2, 3.5, 5, and 7 years to collect data on early adversities and assess the home environment using the HOME test	Children's IQ was analyzed using the Wechsler Intelligence Scale for Children	Appropriate. All models were adjusted for maternal IQ score at 6m assessed by Peabody Picture Vocabulary Test. Full-Scale and Verbal Comprehension IQ models were also adjusted for language of neurological assessment. Models in which adversity measures did not include H.O.M.E. subscores were adjusted for long term H.O.M.E. score average; models in which adversity measures did not include parental educational attainment were adjusted for maternal education.	Appropriate. Multivariate linear regression model and three-way interaction model	Selection bias probable; adversity measures calculated via *a priori* hypotheses, instead of incorporating using more advanced statistical methods; lack of frequent maternal urine spot sampling and due to the short half-life of DAP metabolites may have led to exposure misclassification; low concentrations of DAP detected in the urine; lack of study details regarding the urine collection methods
Wolff *et al*. (2007)	New York City, U.S.	Mount Sinai Children's Environmental Health Study	Prospective Cohort – moderate sample size N=404 Mother-infant pairs	Objective biomarker, prenatal OP pesticide exposure (DAP), malathion (MDA) quantified in single maternal spot urine sample provided during the 3rd trimester	Birth outcomes from computerized perinatal database	Appropriate: different covariates for each outcome, e.g., weight: maternal age, race/ethnicity, maternal BMI*pregnancy weight gain, infant sex, gestational age, creatinine	Appropriate PROC GLM, with varying covariates	Selection bias unlikely; some potential for non-differential misclassification of exposure
Yolton et al. (2013)	Cincinnati, Ohio, USA	Health Outcomes and Measure of the Environment (HOME)	Prospective Birth Cohort Study – large sample size. N=350 Mother-infant pairs	Metabolite concentrations (total DAP, DM, and DE) of prenatal OP pesticide exposure quantified in two maternal spot urine samples provided at 16 and 26 weeks of gestation	For children, a neurobehavioral assessment was conducted via the NICU Network Neurobehavorial Scale (NNNS) at ~ 5 weeks of age	Appropriate. Included sex, birthweight, infant weight change from birth to exam, parity, maternal age at delivery, marital status, education, employment during pregnancy, household income, body mass index (BMI) at 16 weeks gestation, weight gain per week during pregnancy, moderate to severe depression (score > 13) measured on the Beck Depression Inventory-II during pregnancy and at 5 weeks post-delivery, reported marijuana and alcohol use during pregnancy, whole blood lead and	Appropriate. Bivariate and multivariable regression	Low concentrations of DAP detected in the urine; lack of frequent urine spot sampling during pregnancy.

						folate during pregnancy, serum cotinine, and reported fruit and vegetable consumption during pregnancy		

Table 2.2.5.1-2 High Quality Studies from 2012/2014 literature search of Children's Environmental Health Epidemiology Studies

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
Whyatt *et al*. (2004) Columbia U. (N=314)	Birth length, Birth weight, head circumference	4 cord plasma chlorpyrifos exposure groups and 4 chlorpyrifos and diazinon exposure groups. Chlorpyrifos only categories, Group 1: levels below LOD (32% of participants); Group 2: lowest 1/3 of detectable levels (20 %); Group 3: middle 1/3 (24%), Group 4: highest 1/3 (25%.) Chlorpyrifos and diazinon together: Group 1: 26%, Group 2: 22 %, Group 3: 26%, Group 4: 26%.	Gestational age, maternal pre-pregnancy weight, maternal net pregnancy weight gain, gender of newborn, parity, race/ethnicity, ETS in home, season, cesarean section	For each log unit increase in cord plasma chlorpyrifos levels, birth weight decreased by 42.6 g (95% CI: –81.8 to –3.8) and birth length decreased by 0.24 cm (95% CI: –0.47 to –0.01). Birth weight averaged 186.3 g less (95% CI: –375.2 to –45.5) among newborns with the highest compared with lowest 26% of exposure levels (p = 0.01).	Associations between birth weight and length and cord plasma chlorpyrifos were statistically significant (p ≤ 0.007) among newborns born before the January 2001 policy change. Among newborns born after January 2001, exposure levels were substantially lower, and no associations with fetal growth outcomes were observed (p > 0.8).	Strengths: prospective nature of the study; direct measurement of chlorpyrifos in cord blood and personal air samples, rather than non-specific markers of organophosphate pesticide exposure; consideration of other pesticides and environmental contaminants as covariates in the multivariate models. Limitations: single exposure sampling period; the authors did not present nor discuss regression diagnostics to assess the degree to which their models met or violated the assumptions implicit in linear models.
Berkowitz *et al*.(2004) Mt. Sinai (N=404)	Birth length, birth weight, head circumference, gestational age	LOD: 11 ug/L (57% <LOD TCPy)	Race/ethnicity, infant sex, and gestational age. The authors also controlled for birth weight or birth length in their assessment of head circumference and pesticide exposure.	Mean levels of birth weight, length, head circumference, and gestational age did not differ between those with urinary pesticide metabolite levels below and above the level of detection. Similarly, no statistically significant associations were observed between reported pesticide	PON1 activity also predictor of smaller head circumference; creatinine corrected	Very well conducted study with numerous strengths and very few weaknesses. The questionnaire-based pesticide exposure questions are subject to imperfect recall. Errors would, on average, attenuate associations between these exposure metrics and fetal development.

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
				exposure and mean indices of fetal growth and gestational age.		Recall-based exposure assessments were fortified by objective measures of pesticides/pesticide metabolites. A metabolite specific for chlorpyrifos (TCPy) was assessed. Statistical analysis was appropriate. Observed mean reductions in the outcome parameters appear to be small in magnitude and may be of little clinical significance. Assessment and control for confounding were appropriate. However, confounding by unmeasured (and mismeasured) risk factors for abnormal growth that are related to pesticide exposure would bias the results in this study. Such factors may be related to socioeconomic status of the study participants, a cofactor which is difficult to define, no less measure in an epidemiologic study. Limited external validity (generalizability) due to the particular study population recruited and the numerous exclusion criteria applied.
Eskenazi *et al*.	Birth length, birth	Total DAPs:	Gestational age, gestational age	Decreases in gestational	Maternal urine collection	Strengths in the study

ED_002061_00044425-00050

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
(2004) CHAMACOS (N=488)	weight, head circumference, Gestation Length, Ponderal index	median 136 nmol/L (range: 10–6,854); DEP: median 22 nmol/L (range: 2–680 nmol/L); TCPy: median 3.3 nmol/L (range: 0.2–56.1nmol/L) (76% >LOD)	squared, maternal age, pregnancy weight gain, week of initiating prenatal care, parity, infant sex, mother's country of birth, body mass index, family income, poverty level, smoking, alcohol, illicit drug use, environmental tobacco smoke, caffeine, history of low birth weight, and history of pre-term delivery.	duration associated with two measures of in utero pesticide exposure: levels of metabolites of dimethyl phosphate pesticide compounds and whole blood ChE.	averaged weeks 14, 26, not creatinine-corrected	design include the longitudinal design, the use of multiple exposure biomarkers, including quantification of non-specific (DAPs), chlorpyrifos-specific (TCPy) metabolites, and other environmental co-exposures. A reasonable set of exclusion criteria were applied.The selection of the CHAMACOS population, which consists mostly of children from low-income families, served to increase the relative statistical efficiency of the study, as this population is at high risk of neurodevelopmental deficits, compared to the general population.The statistical analysis used to assess the associations between the markers of exposure and neurodevelopment were appropriate.Errors in the assignment of exposure in this prospective study will likely have resulted in attenuation of observed associations.
Harley *et al*. (2011) CHAMACOS (N=329)	Birth length, birth weight, head circumference, gestational age	The geometric mean for the DAP concentrations during pregnancy (for the average of the two sampling periods) was 146 nmol/L (95% CI: 133,	Maternal intelligence (Peabody Picture Vocabulary Test (PPVT)), measures of how stimulating the environment is, and known or suspected neurotoxins were measured prenatally. To measure the quality and extent of stimulation available to a child in the home environment,	The authors observed evidence of an association between prenatal exposure to OP pesticides as measured by urinary DAP metabolites in women during pregnancy, is associated with decreased cognitive functioning in	Infants whose PON1 genotype and enzyme activity levels suggested that they might be more susceptible tothe effects of OP pesticide exposure had decreased fetal growth and length of gestation. PON1 may be a contributing	This study has many strengths, the longitudinal design, the measurement of urinary DAP at multiple time points and following children to age seven when tests of cognitive function are reportedly more reliable. The authors were

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
		160); of this, a larger proportion was DMP metabolites (GM = 109 nmol/L; 95% CI =98, 120) than DEPs (GM= 23 nmol/L; 95% CI = 21, 25). Allele frequencies: PON1 192 Q allele= 50%; PON1 -108 T allele= 46%.Mean arylesterase activity: For infants: 33.6 U/mL (SD = 16) For mothers: 136.6 U/mL (SD = 44). Mean paraoxonase activity: For infants: 256.6 U/L (SD = 165); For mothers: 989.0 U/L (SD = 616).	the Infant-Toddler HOME (Home Observation for Measurement of the Environment) inventory was completed at the 6-month, 1, 2, 3.5, 5, and 7 year visits; known or suspected neurotoxicants, polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), p,p'-dichlorodiphenyltrichlorethane (DDT), p,p'-dichlorodiphenyltrichlorethylene (DDE), and lead.	children at age 7.	factor to preterm or low birth weight birth.	able to adjust for or consider many factors related to cognitive function, such as prenatal exposure to other environmental agents, socioeconomic indicators, maternal intelligence and education, and child stimulation. The cohort had a relatively homogenous socioeconomic profile, reducing the potential for uncontrolled confounding.
Engel *et al*. (2007) Mt. Sinai (N=311)	Brazelton Neonatal Behavioral Assessment Scale (BNBAS), primitive reflexes (neurological integrity) measured before hospital discharge.	DEP: 24.7 nmol/L; Total DAP: 82 nmol/L	Maternal age, race, marital status, education, cesarean delivery, delivery anesthesia, infant age at examination, infant gender, infant jaundice, smoking (yes/no), alcohol consumption, caffeine consumption, illicit drug use during pregnancy, and the examiner.	No adverse associations were found for DAPs and any measured behavior. Relative to the first quartile, quartiles 2–4 of total DEPs, DMPs, and DAPs were associated with an increased proportion of abnormal reflexes, although the associations did not increase monotonically and varied in their strength and precision.	Used non-specific biomarker DEP/DAP	This was a well conducted prospective study conducted in a young, predominantly minority population. The study design, analytic approach, and statistical analyses were appropriate. Pesticide metabolites evaluated are not specific for chlorpyrifos. The BNBAS was administered before hospital discharge only on a subset of children in the cohort (n =311/404). Factors related to weekend

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
						delivery (e.g., fewer inductions) would be underrepresented among the tested subjects, and may induce bias, reduce the degree of precision with which associations were estimated, and limit the generalizability of the study findings.The statistical analysis was largely appropriate. Imputing of missing data may affect precision of association estimates and result in attenuated effect estimates as a result of exposure measurement error. Assessment and control for confounding were appropriate. However, confounding by unmeasured (and mis-measured) risk factors for abnormal growth that are related to pesticide exposure would bias the results in this study. Such factors may be related to socioeconomic status of the study participants, a cofactor which is difficult to define, no less measure in an epidemiologic study.
Young *et al*. (2005) CHAMACOS (N=381)	Neurodevelopment, Brazelton Neonatal Behavioral AssessmentScale (BNBAS), abnormal reflexes	DAP (average during pregnancy): median 222nmol/L (range: 7–21,867 nmol/L); DEP (average during pregnancy):	Maternal age, BMI, any smoking/alcohol/drug use during pregnancy, gestational age at which prenatal care was initiated, total number of prenatal care visits, mean pregnancy blood pressure, parity, method of delivery, general anesthesia used during	Among the >3 day old infants, increasing average prenatal urinary metabolite levels were associated with both an increase in number of abnormal reflexes (total DAP: adjusted beta = 0.53, 95% CI = 0.23, 0.82; dimethyls: adjusted beta =	Associations seen pre-natal OP, not post-natal OP exposure, Maternal urine collection averaged weeks 14, 26	Strengths: Longitudinal design, measurement and consideration of many confounders, the prenatal exposure measures were, with some exceptions, the average of two measurements, and thus may better reflect chronic

ED_002061_00044425-00053

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
		median 21 nmol/L (range: 2–680 nmol/L)	delivery, breastfeeding initiated after delivery, poverty level, infant sex, age in days at BNBAS, minutes since last feed at BNBAS, and BNBAS examiner.	0.41, 95% CI = 0.12, 0.69; diethyls: adjusted beta = 0.37, 95% CI = 0.09, 0.64), and the proportion of infants with more than three abnormal reflexes (total DAP: adjusted OR = 4.9, 95% CI = 1.5, 16.1; dimethyls: adjusted OR = 3.2, 95% CI = 1.1, 9.8; diethyls: adjusted OR = 3.4, 95% CI = 1.2, 9.9).		exposure during the pregnancy Weaknesses: Potential for residual confounding, potential for exposure misclassification, potential selection bias (not all children had the outcome measures), study population not generalizable to the whole US.
Rauh *et al*. (2006) Columbia U. (N=254)	Neurodevelopment: The Bayley Scales of Infant Development II (BSID-II), Mental Development Index (MDI) and Psychomotor Development Index (PDI) at 12, 24, and 36 months of age. • Behavior: Child Behavior Checklist (CBCL) at 12, 24, and 36 months. • Quality of the child-care environment: The Home Observation for Measurement of the Environment (HOME)	Exposure levels were categorized as low (≤ 6.17 pg/g) or high (>6.17 pg/g)	Data were collected regarding lead exposure, demographics, education and occupational history, income, active and passive smoking, alcohol and drug use during pregnancy, and residential pesticide use. Final models included prenatal environmental tobacco smoke (ETS) exposure, gender, ethnicity, gestational age at birth, quality of home care-taking environment, maternal education, and maternal IQ.	At the 36 month milestone, the likelihood of highly exposed children developing mental delays were 2.4 times greater (95% CI: 1.12-5.08, p = 0.02) and motor delays were 4.9 greater (95% CI: 1.78-13.72; p = 0.002) than those with lower prenatal exposure. The GLM analysis for PDI scores showed a significant effect of chlorpyrifos exposure over time with an estimated deficit of approximately 7 points by age 36 months (p = 0.01).	The authors summarize three main findings: 1) by age 3, the more highly exposed children demonstrated mental and motor delays; 2) the observed developmental trajectories for PDI and MDI scores confirmed that the adverse impact on cognitive and motor development increased over time; and 3) by age 3, highly exposed children were more likely to demonstrate clinically significant attention problems.	• Only 53% of the children reached the three year milestone with study data collected. It is unclear what percentage of these children did not survive, were lost to follow-up, or too sick to participate. • Reliance on a single exposure level (prenatal/cord blood.) • No control for exposure over the subsequent 3 years • Creation of a dichotomous exposure variable brings limitations due to the amount of within-group variation. • Limitations of the sensitivity and predictive validity of the developmental tests, especially among children less than 3 years of age. • No discussion of whether this 7-point deficit is clinically relevant. • Due to the pervasive, non-specific nature of neurological effects, it is difficult to attribute

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
						causality.
Lovasi *et al*. 2011 Columbia U. (N=266)	Bayley scores (MDI/PDI) 12 months, 24 months, 36 months	N/A	Neighborhood characteristics: The percentage of housing units without complete plumbing, the percentage of vacant housing units, the percentage of residents below the federal poverty line, the percentage of residents older than 25 years of age who completed high school, the percentage of households receiving public assistance, the percentage of housing units with one or more residents per room, racial composition, the percentage of residents born outside the United States, the percentage of Spanish-speaking residents, and the percentage of residents who were linguistically isolated	Neighborhood characteristics did not confound the observed association between chlorpyrifos levels and cognitive development.	Hierarchal regression analysis of potential confounding by SES	Direct measurement of chlorpyrifos.The statistical analyses were generally appropriate. Missing data on covariates were estimated using multiple imputation, and the variance estimates presented appropriately reflect the degree of uncertainty caused by missing covariate data. Robust standard errors were used.The setting of the investigation in a sample drawn from low-income African American and Dominican communities is both a strength (increases the power, restriction of confounders) and a limitation of the study (reduced generalizability).
Engel *et al*. (2011) Mt. Sinai (N=276)	Bayley scores (MDI/PDI) at 12 months, 24 months.	DEP: 24.7 nmol/L; Total DAP: 82 nmol/L (same as Engel 2007)	Maternal age, race/ethnicity, marital status, education, breast-feeding, child sex, alcohol, smoking, or drug use during pregnancy, maternal IQ, a score based on assessment of the home environment (HOME), season of urine collection, language spoken in the home, age at testing, examiner, and urinary creatinine level.	An observed association between prenatal total dialkylphosphate metabolite level and a decrement in mental development at 12 months among blacks and Hispanics.	Used non-specific biomarker DEP/DAP; some evidence of effect modification by PON1 genotype	Limitations include use of non-specific markers of chlorpyrifos pesticide exposure (DAPs), use of only a single (third-trimester) urine sample, and the large proportion of loss to follow-up. Statistical analysis was appropriate. Imputing of missing data may affect precision of association estimates and result in attenuated effect estimates as a result of exposure measurement error, although these are offset by the further

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
						categorization of the exposure levels (at the median). However, binning of exposure levels reduces precision, relative to a continuously distributed measure of exposure. Assessment and control for confounding were appropriate. However, confounding by unmeasured (and mismeasured) risk factors for abnormal growth that are related to pesticide exposure would bias the results in this study. Such factors may be related to socioeconomic status of the study participants, a cofactor which is difficult to define, no less measure in an epidemiologic study.
Eskenazi *et al*. (2007) CHAMACOS (N=372)	Neurodevelopment, Bayley Index (MDI, PDI), Maternal behavior checklist at 6, 12, and 24 months	DEP: geom. Mean in mother 18.1 nmol/L (95% CI = 16.7–19.7); DEP geometric mean in child at 24 months 10.5 nmol/L (95% CI =8.8–12.6); TCPy median 3.54 ug/l	Psychometrician, location of assessment, exact age at assessment, sex, breast-feeding duration (months), HOME score, and household income, parity, maternal PPVT, maternal age, education, depressive symptoms, active/passive smoking exposure during pregnancy, regular alcohol use during pregnancy, marital status, father's presence in home, housing density, maternal work status, ≥ 15 hours out-of-home childcare/week, birth weight, gestational age, abnormal reflexes, PCBs, lead, DDT, β-hexachlorocyclohexane, and hexa-chlorobenzene	DAP metabolite levels during pregnancy, particularly from dimethyl phosphate pesticides, may be negatively associated at 24 months with mental development (MDI) on the Bayley Scales and an increase in risk of maternally reported PDD.	No strong associations identified with DE or TCPy, Maternal urine collection averaged weeks 14, 26	Strengths: Longitudinal design, measurement and consideration of many confounders (including other environmental chemicals); the prenatal exposure measures were, with some exceptions, the average of two measurements, and thus may better reflect chronic exposure during the pregnancy Weaknesses: Potential for residual confounding, potential for exposure misclassification, potential selection bias (not all children had the outcome

ED_002061_00044425-00056

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
						measures), study population not generalizable to the whole US.
Eskenazi *et al*. (2010) CHAMACOS (N=371)	Neurodevelopment, Bayley Index (MDI, PDI), Maternal behavior checklist at 6, 12, and 24 months, PON1 gene and enzyme levels	The geometric mean for the DAP concentrations during pregnancy (for the average of the two sampling periods) was 146 nmol/L (95% CI: 133, 160); of this, a larger proportion was DMP metabolites (GM = 109 nmol/L; 95% CI =98, 120) than DEPs (GM= 23 nmol/L; 95% CI = 21, 25). Allele frequencies: PON1 192 Q allele= 50%; PON1 -108 T allele= 46%.Mean arylesterase activity: For infants: 33.6 U/mL (SD = 16) For mothers: 136.6 U/mL (SD = 44). Mean paraoxonase activity: For infants: 256.6 U/L (SD = 165); For mothers: 989.0 U/L (SD = 616).	Psychometrician, location of assessment, exact age at assessment, sex, breast-feeding duration (months), HOME score, and household income, parity, maternal PPVT, maternal age, education, depressive symptoms, active/passive smoking exposure during pregnancy, regular alcohol use during pregnancy, marital status, father's presence in home, housing density, maternal work status, ≥ 15 hours out-of-home childcare/week, birth weight, gestational age, abnormal reflexes, PCBs, lead, DDT, β-hexachlorocyclohexane, and hexa-chlorobenzene	Decrease MDI (24 months) $PON1_{108TT}$ –5.7 (–9.0 to –2.5) 9=0.01; Decrease PDI (24 months) $PON1_{108TT}$ –2.8 (–5.7 to 0.2) p=0.07; increased odds PDD 2.0 (0.8 to 5.1) p=0.14; no association $PON1_{192}$; no association PON1 activity measured newborn, 2 years, maternal and MDI, PDI, PDD. Evidence of decreasing MDI score by number of $PON1_{108}$ variant alleles: $PON1_{108CC}$ –3.2 (–9.8 to 3.5), CT –3.7 (–8.0 to 0.6), TT –5.5 (–11.1 to 0.1), p-interaction 0.98.	In this study population, evidence PON1 may influence MDI score, but not PDI or PDD risk at two-years. Non-significant evidence of decreasing MDI score by increasing DAP levels across strata of the number of $PON1_{108}$ variant alleles, interaction non-significant. Similar trend with prenatal DEP levels and MDI, PDI by PON108 alleles, but less pronounced. Overall, limited, non-definitive evidence of effect modification by PON1 status in the relation between mental and psychomotor effects and prenatal DAPs.	Strengths: Longitudinal design, measurement and consideration of many confounders (including other environmental chemicals); the prenatal exposure measures were, with some exceptions, the average of two measurements, and thus may better reflect chronic exposure during the pregnancy Weaknesses: Potential for residual confounding, potential for exposure misclassification, potential selection bias (not all children had the outcome measures), study population not generalizable to the whole US. Study may be under-powered to evaluate effect modification by *PON1* status.
Marks *et al*. (2010) CHAMACOS	CBCL; K-CPT; ADHD confidence index;	DAP (geometric mean) pregnancy	Psychometrician, exact age at assessment, sex, maternal	Prenatal DAPs were non-significantly associated	Marked effect modification by gender: 11-fold increase	Strengths: Longitudinal design, measurement and

ED_002061_00044425-00057

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
(N=348)	Hillside behavioral rating scale; composite ADHD indicator	109.0 nmol/L; DEP 17.7 nmol/L	education, depressive symptoms, PPVT (continuous), ≥ 15 hr out-of-home child care/week, breast feeding duration (months), maternal age, parity, marital status, active/passive smoking exposure and regular alcohol use during pregnancy, presence of father in home, maternal work status, and household income	with maternal report of attention problems and ADHD at age 3.5 years, but were significantly related at age 5 years [CBCL attention problems: β = 0.7 points; 95% confidence interval (CI), 0.2-1.2; ADHD: β = 1.3; 95% CI, 0.4-2.1].	ADHD composite indicator in boys, less than 2-fold in girls, however unstable estimates; weak evidence of association DAPs at 3.5, 5 years and attention	consideration of many confounders (including other environmental chemicals); the prenatal exposure measures were, with some exceptions, the average of two measurements, and thus may better reflect chronic exposure during the pregnancy Weaknesses: Potential for residual confounding, potential for exposure misclassification, potential selection bias (not all children had the outcome measures), study population not generalizable to the whole US.
Rauh *et al*. (2011) Columbia U. (N=265)	• Wechsler Scales of Intelligence for Children (WISC-IV) • Child Behavior Checklist (CBCL).	• Chlorpyrifos levels in umbilical cord blood samples, N=256 newborns • If no cord blood (12% of subjects), levels were imputed from mothers' values. • Values for samples with non-detectable chlorpyrifos levels (N=115, 43%) were imputed by using assay-specific limit of detection (LOD) values to impute an approximate	Data were collected regarding lead exposure, demographics, education and occupational history, income, active and passive smoking, alcohol and drug use during pregnancy, and residential pesticide use. Final models included prenatal environmental tobacco smoke (ETS) exposure, gender, ethnicity, gestational age at birth, quality of home care-taking environment, maternal education, and maternal IQ.	Full-Scale IQ: (B) of -0.003, CI = 0.006, 0.001, p= 0.064 Working Memory Index: (B) of -0.006, CI = 0.009, 0.002, p<0.001. The investigators articulated these results as showing that a 1 pg/g increase in chlorpyrifos exposure was associated with a 0.006 point decrease in the log-transformed Working Memory score and a 0.003 point decrease in the log-transformed Full-Scale IQ score. The investigators concluded that for each standard deviation increase in exposure (4.61pg/g) there is a 1.4% reduction in Full-Scale IQ and a 2.8%	For each standard deviation increase in exposure (4.61pg/g) there is a 1.4% reduction in Full-Scale IQ and a 2.8% reduction in Working Memory.	Strengths • Direct assessment of chlorpyrifos levels using maternal serum and cord blood. • Analysis using a continuous CPF level, which, in contrast to dichotomous CPF levels, provides a more meaningful look at potential threshold effects and dose-response trends. • The investigators rigorously evaluated their methods for imputing values for undetectable CPF levels which in the end, were validated. • The authors describe an elegant and

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
		level.		reduction in Working Memory.		methodologically sound statistical analysis, addressing many of the potential shortcomings of their exposure data and covariates. Weaknesses: The use of a single snapshot of prenatal chlorpyrifos exposure may not be an accurate surrogate for full prenatal exposure levels. • There is no control for exposure over the subsequent 7 years which may be critical, especially as the process of neurocognitive development is fluid and rapid during these early childhood years. • Possibility of that an increased awareness of the risks of pesticide exposures could disproportionately affect postnatal exposure behavior.• Complicating this analysis is the pervasive, non-specific nature of neurological effects and the difficulty in attributing causal pathways. • when closely reviewed, the 95% CI for Full Scale IQ for both techniques contain 0 (LASSO: -0.006, 0.001, p=0.064; fully-adjusted: -0.006, 0.001, p=0.048• The authors do not address the clinical relevance of the 1.4% and 2.8% reductions

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
						and how this may impact a child or his/her psychological or educational plans.
Engel *et al*. (2011) Mt. Sinai (N=169)	Wechsler Preschool and Primary Scale of Intelligence, 3rd edition (WPPSI-III) at ages < 7 years; Wechsler-IV Intelligence Scale (verbal comprehension; perceptual reasoning, working memory, processing speed, full scale intelligence) at age 7-9 years	DEP: 24.7 nmol/L; Total DAP: 82 nmol/L (same as Engel 2007)	Maternal age, race/ethnicity, marital status, education, breast-feeding, child sex, alcohol, smoking, or drug use during pregnancy, maternal IQ, a score based on assessment of the home environment (HOME), season of urine collection, language spoken in the home, age at testing, examiner and urinary creatinine level.	At age 6-9 years, non-statistically significant reductions in full scale IQ, perceptual reasoning, verbal comprehension, working memory and processing speed with increasing DAP, more profound with DEP than DMP	Used non-specific biomarker DEP/DAP; some evidence of effect modification by PON1 genotype	Limitations include use of non-specific markers of chlorpyrifos pesticide exposure (DAPs), use of only a single (third-trimester) urine sample, and the large proportion of loss to follow-up. The statistical analysis was largely appropriate. Imputing of missing data may affect precision of association estimates and result in attenuated effect estimates as a result of exposure measurement error, although these are offset by the further categorization of the exposure levels (at the median). Assessment and control for confounding were appropriate. However, confounding by unmeasured (and mis-measured) risk factors for abnormal growth that are related to pesticide exposure would bias the results in this study. Such factors may be related to socioeconomic status of the study participants, a cofactor which is difficult to define, no less measure in an epidemiologic study.

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
Bouchard *et al.* (2011) CHAMACOS (N=329)	Wechsler-IV Intelligence Scale (verbal comprehension; perceptual reasoning, working memory, processing speed, full scale intelligence) measured at age 7 years	Total DAPs (quintiles): Q1 (39 nmol/L); Q2 75 nmol/L; Q3 126 nmol/L; Q4 221 nmol/L; Q5 508 nmol/L. Geometric mean DAP 131 nmol/L	Maternal intelligence, measures of how stimulating the environment is, and known or suspected neurotoxins were measured prenatally. Maternal intelligence was assessed via the Peabody Picture Vocabulary Test (PPVT). To measure the quality and extent of stimulation available to a child in the home environment, the Infant-Toddler HOME (Home Observation for Measurement of the Environment) inventory was completed at the 6-month, 1, 2, 3.5, 5, and 7 year visits; known or suspected neurotoxicants, polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), p,p'-dichlorodiphenyltrichlorethane (DDT), p,p'-dichlorodiphenyltrichlorethylene (DDE), and lead.	The authors observed evidence of an association between prenatal exposures to OP pesticides as measured by urinary DAP metabolites in women during pregnancy, and decreased cognitive functioning in children at age 7.	Prenatal measures taken later half of pregnancy more significantly associated intelligence than early; little evidence post-natal OP exposure associated with intelligence; 7 point reduction in full scale intelligence DAP Q5/Q1 (SS)	Strengths: the longitudinal design, the measurement of urinary DAP at multiple time points and following children to age seven when tests of cognitive function are reportedly more reliable. The authors were able to adjust for or consider many factors related to cognitive function, such as prenatal exposure to other environmental agents, socioeconomic indicators, maternal intelligence and education, and child stimulation. The cohort had a relatively homogenous socioeconomic profile, reducing the potential for uncontrolled confounding.
Whyatt *et al.* (2007) Columbia U. (N=102)	None	Geometric mean, 6.9 ± 17.0 ng/m^3; range < 0.4–171 ng/m^3. Personal air monitor: median 2.8 ng/m^3, mean 6.2 ± 11.1 ng/m^3, range < 0.4–83.4 ng/m^3	N/A	There was little within-home variability and no significant difference in air concentrations within homes over time ($p \geq 0.2$); between-home variability accounted for 88% of the variance in the indoor air levels of propoxur, 92% in chlorpyrifos, 94% in diazinon, and 62% in piperonyl butoxide ($p < 0.001$). Indoor and maternal personal air insecticide levels were highly correlated ($r = 0.7$–0.9, $p < 0.001$).	Indoor and maternal personal air insecticide levels were highly correlated ($r = 0.7$–0.9, $p < 0.001$).	Strengths: study design and exposure assessment techniques, Limitations: only those cohort participants enrolled after 2011 were included in the analysis (most likely due to the lack of serial data from the earlier years.)
Whyatt *et al.*	None	The limit of	N/A	Meconium TCPy	TCPy in maternal urine	Comprehensive exposure

ED_002061_00044425-00061

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
(2009) Columbia U. (N=102)		detection (LOD) of chlorpyrifos in blood samples was 0.5–1 pg/g plasma. The LOD of TCPy in urine samples was 0.26 ng/mL urine. The LOD for TCPy in meconium was 0.2 ng based on a sample weighing 0.5 g. Exposure marker levels below the LOD were given a value of half the level of detection, and were then log10 transformed.		concentrations were positively correlated with chlorpyrifos in maternal and cord blood ($r = 0.25–0.33$, $p < 0.05$) and with TCPy in maternal urine ($r = 0.31$, $p < 0.01$).	samples was not reliable, but the maternal and cord blood chlorpyrifos as well as the TCPy levels in meconium were reliable measures of exposure	assessment including actual blood chlorpyrifos levels, the repeated sampling, and the environmental sampling. Weaknesses: only included participants recruited in the post-cancellation period, use of nonparametric, rank-based statistics is appropriate but the large number of observations below the level of detection receiving equal rank, may be problematic; no dietary assessment
Rauh et al.(2012), (n=40)	Morphological change in the pediatric brain in regions of the brain known to be associated with learning, cognition and social behavior	Tertile 3 (≥4.39 pg/g), compared to Tertiles 0, 1, 2 (<4.39 pg/g, including those not exposed to CPF)	Age, sex	Authors report differences in brain structure (regional cerebral size and thickness) by CPF exposure groups, and the differences (high>low CPF) in regional brain size is likely due to enlargement of underlying white matter. Statistical interaction by gender reported.	Authors concluded that the evidence from the study illustrated changes in brain morphology in association with higher CPF exposure, and that changes observed were in areas of the brain that subserve those learning, cognition and social behavioral, supported by previous observational and experimental literature.	Study supports general hypothesis of CPF influence on brain morphology, but lacks specific hypotheses regarding particular areas of the cerebrum affected; limited and somewhat unbalanced depiction of the available rodent experimental data; statistical methods appropriate, correction for multiple statistical comparisons a strength; MRI image readers blinded to exposure status enhances study validity; lack of information on other validation practices; small sample size, pilot study, low statistical power; external validity

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
						limited; one time measure of pre-natal exposure

2.2.5.2 "Moderate" Quality Group

Fifteen articles were assigned a moderate quality rating, as shown in Table 2.2.5.2-1. In general, these were cross-sectional or prospective cohort studies with small to high sample size; exposure assessment was based on a non-specific biomarker measure or current self-report, the outcome measurement(s) utilized standardized tests or screening tools, appropriate statistical analyses were performed, considering some but maybe not all relevant covariates, and risks of bias were minimized to some extent. For example, Guodong *et al.* (2012) cross-sectionally evaluated the relationship between DAPs concentrations in urine sampled from 301 young children as an objective, non-specific marker of prenatal OP pesticide exposure and Developmental Quotients based on the Gesell Developmental Schedules adapted for a Chinese population.

ED_002061_00044425-00064

Table 2.2.5.2-1. Moderate Quality Studies: Summary of Study Design Elements Impacting Study Quality Assignment

Study	Study Location	Cohort Name / Description of Study Population	Study Design	Exposure Assessment	Outcome Assessment	Confounder / Covariate Control	Statistical Analysis	Risk of (other) Bias
Andersen *et al* (2015)	Denmark	School age children living in Denmark	Prospective Birth Cohort, small sample size N=177 (112 maternal occupational pesticide exposure during pregnancy; 65 without) Age: 6-11 years	Prenatal occupational pesticide exposure ascertained by maternal interview at enrollment	Objective standardized clinical exam; neurophysiological status (heart rate variability); Previously validated, neuropsychological testing with demonstrated sensitivity to environmental pollutants; administered and scored by single neuropsychologist	Appropriate. Self-reported by mother. Included age, maternal demographics and risk factors, SES indicator (broad categories), maternal smoking and alcohol use. Possibility of recall errors (residual confounding). Self-reported (by mother)	Appropriate multivariate analysis. Evaluated numerous hypotheses without adjustment for multiple comparisons. Also constructed structural equation model of interaction between child sex and prenatal pesticide exposure on general intellectual ability (parameterized as a latent variable)	Selection bias possible due to loss to follow-up; Residual confounding likely, small in magnitude; potential for non-differential misclassification of exposure
Guodong *et al*. (2012)	Shanghai, China	2-year old children visiting community hospitals	Cross-sectional, large sample size N=301 Age 23-25 months	Objective biomarker, OP exposure (DAP) quantified in single child spot urine sample, simple imputation <LLOD; OP exposure also assessed via questionnaire administered to mothers after delivery	Developmental Quotients based on Gesell Developmental Schedules, adapted for Chinese population	Appropriate. Assessed via mother report via questionnaire, included child sex, maternal demographics and risk factors, SES indicators (maternal education, household income), maternal smoking and alcohol use; Possibility of recall errors	Appropriate multivariate analysis. Assumed linear relationship between log-transformed DAP level and DQ Scores	Selection bias unlikely; Residual confounding likely, small in magnitude; potential for non-differential misclassification of exposure
Handal *et al*. (2007)	Cayambe-Tabacundo region, Ecuador	EcoSalud Project: infants and young children 3 to 61 months of age in lower-altitude communities A&B dominated largely by cut-flower production and in more traditional rural, higher altitude community C.	Cross-sectional, modest sample size from census N=283 Age 3-61 months - 154 high exposure - 129 low exposure	Objective proxy measure: Community of residence	Ages and Stages Questionnaire (ASQ), adapted into local vernacular, 2 trained testers – considered a screening tool	Appropriate: child health status (anemia, stunting) and other characteristics of the home environment (stimulation by 2 methods)	Appropriate: Multiple linear regressions to evaluate associations between community of residence and delayed development, Pairwise t-tests and chi-square to assess mean difference in ASQ score; Effect size Cohen's d	Selection bias unlikely; Residual confounding likely, small in magnitude; potential for non-differential misclassification of exposure
Handal *et al*. (2008)	Cayambe-Tabacundo region, Ecuador	EcoSalud Project: Children 3 to 23 months of age in lower-altitude communities A&B dominated largely by cut-flower production and in more traditional rural, higher altitude community C.	Cross-sectional, modest sample size N:121 Age 3-23 months	Proxy: Distance home to farm, parental employment, pesticide use on domestic crops & within home, child play activities	Ages and Stages Questionnaire (ASQ) (ages 24-61 months), a screening tool; Visual Motor Integration (VMI) Test (ages 48-61 months); two trained testers.	Appropriate: As above	Appropriate: As above	Selection bias unlikely; Residual confounding likely, small in magnitude; potential for non-differential misclassification of exposure

Table 2.2.5-2. Moderate Quality Studies: Summary of Study Design Elements Impacting Study Quality Assignment (continued)

Study	Study Location	Cohort Name / Description of Study Population	Study Design	Exposure Assessment	Outcome Assessment	Confounder / Covariate Control	Statistical Analysis	Risk of (other) Bias
Handal *et al*.(2007B)	Cayambe-Tabacundo region, Ecuador	EcoSalud Project: Children 24 to 61 months of age in lower-altitude communities designated A & B were dominated largely by cut-flower production than in more traditional rural, higher altitude community C.	Cross-sectional, modest sample size N: ASQ - 142 Age 24-61 months VMI - 57 Age 48-61 months	Proxy: Maternal employment during pregnancy, child plays outdoors, Pesticide use on domestic crops , inside home	Ages and Stages Questionnaire (ASQ) (3-23 months) – screening tool; Reach-and-grasp, UC Berkeley Preferential Looking Test Cards; trained tester	Appropriate: As above	Appropriate: As above; Prehension- Logistic regression models, generalized estimation equations (GEE) to account for between-test dependence	Selection bias unlikely; Residual confounding likely, small in magnitude; potential for non-differential misclassification of exposure
Llop *et al*. (2013)	Spain	INMA (Environment and Childhood) Project	Prospective Birth Cohort, large sample size N=1980 Age 14 months	Maternal self-report of prenatal and postnatal indoor pesticide use (pesticide spray or use of a plug-in device assessed via questionnaire)	Mental and psychomotor development at 14 months assessed using validated instrument (Bayley Scales of Infant Development)	Appropriate. Self-reported by mother. Included maternal demographics and risk factors (Age, BMI) SES indicators (maternal education, occupation), maternal smoking and alcohol use. Also childcare behaviors (breast feeding, number of siblings, day care) Possibility of recall errors (residual confounding)	Appropriate multivariate analysis	Selection bias unlikely; Residual confounding likely, small in magnitude; potential for non-differential misclassification of exposure
Oulhote and Bouchard (2013)	Canadian National Population	Canadian Health Measures Survey (CHMS; cycle 1, 2007–2009);	Cross-sectional Study, large national sample size n = 779 children Age 6-11 years	Child urinary DAP, DMP, DEP collected within 2 weeks of survey questionnaire completion by the parents.	Behavioral problems in children based on the parent version of the Strengths and Difficulties Questionnaire (SDQ) (Goodman 1997) - SDQ is a validated screening questionnaire and accepted by parents.	Appropriate: Included sex, age, race/ethnicity, family income, parental education, blood lead levels, maternal smoking during pregnancy, birth weight, maternal age at child's birth, child's BMI, and fasting status (fasting duration at urine collection > 10 hour/≤ 10 hour)	Appropriate: Logistic Regression	Selection bias unlikely; Study population not mixed, single urine sample
Petit *et al*. (2010)	Brittany, France	Newborn children in the PELAGIE Study.	Prospective Birth Cohort, large sample size N= 3,159 Age: newborns	Ecological, proxy indicator of exposure (proportion of municipality devoted to agricultural activity	Objectively measured birth outcomes assessed using hospital records	Appropriate. Maternal report via questionnaire. Included maternal demographics (age, BMI) and pregnancy risk factors (gestational age, hypertension, diabetes, season of pregnancy) SES indicators (district of residence, maternal education, occupation), maternal smoking and alcohol use. Also childcare behaviors (breast feeding, number of siblings, day care); Possibility of recall errors	Appropriate multivariate analysis. Multiple comparisons were conducted for this evaluation, and the authors did not adjust for multiple testing	Selection bias unlikely; Residual confounding likely, small in magnitude; potential for substantial non-differential misclassification of exposure; Misclassification of outcomes unlikely

Table 2.2.5-2. Moderate Quality Studies: Summary of Study Design Elements Impacting Study Quality Assignment (continued)

Study	Study Location	Cohort Name / Description of Study Population	Study Design	Exposure Assessment	Outcome Assessment	Confounder / Covariate Control	Statistical Analysis	Risk of (other) Bias
Ruckart *et al*. (2004)	Mississippi, USA (29 counties), Ohio (one multi-family facility)	Children who were 6 years or younger in MS and OH when homes sprayed with MP	Retrospective cohort, moderate sample size in 2 states, participating/ nonparticipating similar in sex and age MS: N=365 (147 exposed, 218 unexposed) OH: N=287 (104 exposed, 183 unexposed) Age: 1.9-12.5 years old at testing	Specific OP measures: Household methyl parathion (wipe) by approved labs, OR highest urinary PNP level in household, analyzed by CDC	Variety of neuro measures: PENTB, Parenting Stress Index (PSI), Personality Inventory for Children (PIC), Vineland Adaptive Behavior Scales (VABS)	Appropriate: Income, race, site term, ethnicity, mother's use of chemicals at work, mother health/pregnancy conditions, report that child had lead or mercury poisoning (MS only); Raw scores were child age-adjusted	Appropriate: Linear regression (continuous scores), logistic regression (dichotomous scores)	Selection bias possible due to loss of follow-up; Residual confounding likely; substantial potential for differential exposure misclassification (time between spraying and testing, frequency and duration unknown)
Shelton *et al*. (2014)	California, USA	CHARGE Study (3 year-old children)	Case-control, large sample size N = 970 Age 3 years N=486 (ASD) N=168 (DD) N=316 (Controls)	Proxy indicator of prenatal OP pesticide exposure (residential proximity to agricultural pesticide applications defined using ecological pesticide use data)	Clinical outcomes – validated within the study	Appropriate adjustment for demographics (place of birth, race), SES indicators (paternal education) and vitamin intake during pregnancy	Appropriate multivariate analysis.	Selection bias probable; Residual confounding likely; substantial potential for differential misclassification of exposure. Outcome misclassification unlikely.
Suarez-Lopez *et al*. (2012)	Pedro Moncayo County, Pichincha, Ecuador	Secondary Exposure to Pesticides Among Infants, Children and Adolescents (ESPINA) Study, plus new volunteers	Cross-sectional, moderate sample size, from prior census N=277 Exposed: (n=158) Unexposed: (n=119) Age 4-9 years	Proxy: Cohabitation with flower worker >1yr, Adult questionnaire	Child AChE level using commercial kit	Appropriate: Sex, age, height-for-age, hemoglobin concentration, income, pesticide use within household lot, pesticide use by neighbors, examination date, residence, distance to nearest flower plantation	Appropriate: Multiple linear regression (continuous) and logistic regression (polychotomous variables), adjusted models	Selection bias possible due to loss but deemed low; Residual confounding likely small in magnitude; potential for differential exposure misclassification
Suarez-Lopez *et al*. (2013a)	Pedro Moncayo County, Pichincha, Ecuador	Secondary Exposure to Pesticides Among Infants, Children and Adolescents (ESPINA) Study, plus new volunteers	Cross-sectional, moderate sample size, from prior census N=307 Age 4-9 years	Non-specific measures: Child AChE level using commercial kit; Proxy: Cohabitation with flower worker >1yr, Adult questionnaire	Heart rate (HR), Systolic blood pressure (SBP), Diastolic blood pressure (DBP) by standard methods	Appropriate: Sex, age, height-for-age, hemoglobin concentration, income, pesticide use within household lot, pesticide use by neighbors, examination date, residence, distance to nearest flower plantation	Appropriate: Multiple linear regression (continuous), adjusted.	Selection bias possible due to loss but deemed low; Residual confounding likely small in magnitude; potential for differential exposure misclassification
Suarez-Lopez *et al*. (2013)	Pedro Moncayo County, Pichincha, Ecuador	Secondary Exposure to Pesticides Among Infants, Children and Adolescents (ESPINA) Study, plus new volunteers	Cross-sectional, moderate sample size, from prior census N=271 Age 4-9 years	Non-specific measures: Child AChE level using commercial kit	NEPSY-II test (trained examiners): general assessment battery	Model defined a priori: hemoglobin, age, sex, race, height-for-age z score, household income, maternal education, and flower worker cohabitation status	Logistic models (dichotomous and polychotomous) and linear regression models, adjusted; Effect modification according to sex among significant associations; Some imputed values	Selection bias possible due to loss but deemed low; Residual confounding likely small in magnitude; potential for differential exposure misclassification
Wang *et al*. (2012)	Shanghai, China	Pregnant women and newborn children	Cross-sectional Birth Cohort Study N=187 Age: newborns	Objective biomarker of prenatal OP pesticide exposure (DAP) in single maternal spot urine sample provided at onset of labor. Simple imputation of observations < LLOD. OP pesticide exposure also assessed via questionnaire administered after delivery	Gestational age and pre-term delivery appropriately defined and assessed using medical records	Appropriate. Included maternal anthropomorphic, demographic, and SES indicators (income, occupation), predictors of high-risk pregnancy (pregnancy weight gain, gestational age)	Appropriate multivariate analysis	Selection bias unlikely; Residual confounding likely small in magnitude; potential for differential misclassification of exposure
Zhang *et al*. (2014)	Shenyang, China	Newborn children in a birth cohort study	Prospective Cohort, n=249 mother-infant pairs Age: newborns	Biomarker of prenatal OP pesticide exposure (DAP) quantified in single maternal spot urine sample provided at delivery. Many observations < LLOD	Neonatal neurodevelopment assessed using validated instrument (Neonatal Behavioral Neurological Assessment) by trained examiners	Appropriate. Included maternal demographic and SES indicators (age, education), predictors of high-risk pregnancy (BMI, gestational age) and environmental toxicant exposure (cord blood lead); Results stratified by sex	Appropriate multivariate analysis	Selection bias unlikely; Residual confounding likely small in magnitude; considerable potential for non-differential misclassification of exposure (unlikely to account for non-null findings)

ED_002061_00044425-00067

2.2.5.3 "Low" Quality Group

Seventeen articles were assigned a low quality rating, as shown in Appendix 3. In general, these were small or pilot studies; exposure assessment was based on a proxy measure(s), outcome measurement(s) utilized screening tools or self-report, limited statistical analyses were performed, relevant covariates were not included or discussed, or risks of bias were possible. For example, Acosta-Maldonado *et al.* (2009) conducted a cross-sectional pilot study among 54 women, only nine of whom were considered to have had prenatal exposure to pesticides (defined based on either an exposure history profile or AChE level in blood sampled at the time of admission into the hospital for delivery. The outcome assessed in this study was a standardized but partially subjective assessment of placental maturity (the Placental Maturity Index, PMI). Covariates adjustment of estimated associations between prenatal exposure and PMI in this study was minimal and limited to placental characteristics. Cartier et al (2016) is from the PELAGIE prospective cohort study and used DAP biomarkers as the exposure biomarkers. However, despite these strengths, the urine samples were collected within the home instead of a laboratory or clinic and were returned via the local mail, which could have potentially led to several sampling errors due to inadequate collection and storage methods.

The remaining sections of this document do not discuss further studies identified in the 'low' category. Due to limitations in these studies, they do not provide reliable information evaluating associations between OP exposure and neurodevelopmental outcomes.

2.2.6 Assessment of Epidemiological Studies for Relevance to Analysis

Using the criteria summarized in Section 2.2.4, a total of 47 literature articles were identified in the 2015 literature review and were judged as high, moderate, or low quality. Overall, 15 articles, 15 articles, and 17 articles were judged to be of high, moderate, or low quality, respectively. For the 30 high and moderate quality studies, additional evaluation was conducted as described in this section.

While all of the moderate quality studies had strengths including sample design and outcome assessment, six of these moderate quality studies did not have sufficient exposure assessment methods to determine whether exposure to OPs actually occurred. These studies were conducted on study populations in Spain (Llop *et al.*, 2013), Ecuador (Handal *et al.*, 2007; 2007b; 2008), Denmark (Andersen *et al.*, 2015), and France (Petit *et al.*, 2010). In these studies, participants were considered exposed or unexposed to pesticides based on non-specific exposure measures, such as self-reported occupational exposure, home pesticide spraying, and proportion of municipality devoted to agricultural activity. For all of these proxy exposure assessments, the pesticides used may have included not only OPs, but also pyrethroids, fungicides, and growth regulators. Given the uncertainty about whether OP exposure actually occurred in these studies and whether observed outcomes are associated with OP exposure or with other pesticides, these studies are excluded from further analysis.

The focus of this epidemiological literature review is on the neurodevelopmental outcomes from exposures to low levels of OPs (i.e. below exposures which would result in 10% or more AChE inhibition). Three studies conducted in Ecuador focused on child AChE inhibition and the

potential association of AChE inhibition with other measures including parental occupation (Suarez-Lopez *et al.*, 2012), as well as clinical autonomic nervous system (ANS) outcomes such as blood pressure and heart rate (Suarez-Lopez *et al.*, 2013a), and neurodevelopmental outcomes (Suarez-Lopez *et al.*, 2013). The range of AChE activity levels are lower in the first tertile (range of 1.44 to 2.93 U/mL) compared to the third tertile (range from 3.33 to 4.69 U/mL). Therefore, due to the outcomes assessed and the potentially toxic cholinergic effects that were associated with these outcomes, these studies are not considered to be relevant to this review and are not discussed further here.

In a retrospective cohort study of children exposed to methyl parathion (MP) before age 6 years in Mississippi and Ohio (Ruckart *et al.* 2004), as assessed by household urinary PNP or wipe samples, exposed children performed worse than unexposed children on a few of numerous neurobehavioral development tests conducted. Specifically, participants classified as having had MP exposure had more difficulty with short term memory and attention tasks, and parents reported more behavioral and motor skill problems, relative to unexposed children. However, upon closer inspection of the results across the MS and OH study sites, these neurobehavioral outcomes are not seen consistently. These inconsistencies may be due to differences in how the exposure occurred across the sites, including the fact that MS participants and OH participants were tested 2.5 and 4.5 years after MP spraying in the home. When comparing exposed and unexposed children using general intelligence testing, integrated visual and motor skills testing, and multistep processing, they did not see any differences. The exposure scenario associated with these observations is a critical element in assessing the utility and reliability of this study. Samples were collected in locations from OH and MS where illegal spraying of methyl parathion is known to have occurred during the 1994-1996 time period. Based on the "Revised Organophosphorous Pesticide Cumulative Risk Assessment" (USEPA, 2006), methyl parathion is known to be among the more potent OPs. It is unknown whether study participants were exposed to MP levels that would have induced cholinergic effects. Therefore, given the uncertainty around this illegal use combined with the high potency for cholinergic toxicity, the agency is not emphasizing this study further in the analysis.

In addition, two high quality studies from the CHAMACOS birth cohort (Quirós-Alcalá *et al.* 2011; Ranaan *et al.*, 2015) assessed the association between DAPs and autonomic nervous system (ANS) outcomes and respiratory symptoms, respectively. The ANS outcomes assessed included heart rate and respiratory sinus arrhythmia. Overall, while there was some evidence of ANS dysregulation for infants at 6 months, these results were not consistently observed for the other assessed child (1 year, 3.5 years, and 5 years) and maternal OP exposures. Ranaan et al (2015) reported that total DAPs and DEAP metabolites in urine from the second half of pregnancy were significantly associated with increased odds of respiratory symptoms in children. Ranaan *et al.* l (2015) also reported exercise-induced coughing at 5 and 7 years of age was significantly associated with total DAPs, DEAPs, and DMAPs in children's urine collected between the ages of 6 months and 5 years (AUC). The authors concluded that early-life exposure to OP pesticides was associated with respiratory symptoms consistent with possible asthma in childhood. There is not a body of literature to compare these results against, making it difficult to put them into context. Furthermore, these studies did not focus on

neurodevelopmental outcomes, which is the focus of this analysis. Consequently, Quirós-Alcalá *et al.* (2011) and Raanan *et al.* (2015) are not being evaluated further.

2.2.7 Birth Outcome Epidemiologic Studies

Four identified studies, three high quality and one moderate quality, focused solely on OP exposure and adverse birth outcomes related to fetal growth (Barr *et al.*, 2010; Rauch *et al.*, 2012; Wang *et al.*, 2012; Wolff *et al.*, 2007). Harley et al (2016) is a meta-analysis of data from the HOME, Mt. Sinai, CCCEH, and CHAMACOS cohorts which allowed for a better assessment of race and genetic variability given the larger sample size in the pooled analysis.

The birth outcomes assessed included birth weight, birth length, head circumference at birth, and gestational age. The exposure assessment for these studies was conducted using objective measures or biomarkers such as maternal urinary DAPs (Rauch *et al.*, 2012; Wang *et al.*, 2012; Wolff *et al.*, 2007), maternal urinary malathion (MDA) (Wolff *et al.*, 2007), or maternal and cord blood levels for specific OPs (Barr *et al.*, 2010). The results from these studies are generally inconsistent, with some studies documenting statistically significant associations between OP exposure and birth outcomes whereas others do not. For example, maternal and cord blood serum levels of TCPy were inversely associated in univariate analyses conducted among participants in a prospective cohort study conducted in New Jersey (Barr *et al.* 2010), though the associations did not persist after adjusting for gravidity, maternal pre-pregnancy BMI, infant sex, and gestational age. Chlorpyrifos levels were near the lower limit of detection in this study, though detectable in 98.6% of maternal serum and 62.8% of cord serum samples. Overall, birth length was not associated with third-trimester DAP in the Mount Sinai Cohort (Wolff *et al.* 2007). However, among those with slow-activity paraoxonase-1 (PON1) or PON192, urinary total DMP (but not total DAP or DEP) was statistically significantly associated with shorter birth length ($p= 0.032$). Birth length was also not associated with maternal urinary DAP sampled at delivery in the cross-sectional investigation conducted in Shanghai, China (Wang *et al.* 2012). Similarly, inconsistent results were observed across these studies for birth weight, gestational age, and head circumference at birth.

For the pooled analysis in Harley et al (2016), no evidence of association was observed between OPs exposure via the ∑DAP, ∑DEP, and ∑DMP urinary metabolites and birth outcomes for birth weight, birth length, and head circumference, as all of the 95% CIs encompassed the null value of 0 and all of the p-values were > 0.05 (p-values ranged from 0.46 to 0.98), respectively. For the interaction analysis, no evidence of a statistically significant interaction between the four cohorts and exposure to urinary metabolites (∑DAP, ∑DEP, and ∑DMP) relative to the birth outcomes (birth weight, birth length, and head circumference) were observed since all of the p for interaction values were above > 0.10, respectively. A statistically significant interaction was observed between the PON1-108 genotype (specifically the PON1-108CC gene) and the urinary metabolite ∑DEP relative to pooled birth weight ($p = 0.08$), and between the PON1-108 genotype (specifically the PON1-108CC genes) and the urinary metabolites ∑DAP and ∑DMP relative to pooled birth length (p for interaction = 0.07; $p = 0.02$) among children. With the PON1192 genotype, a statistically significant interaction was observed between the urinary

metabolite ∑DAP relative to pooled head circumference (p for interaction = 0.05). For mothers, a statistically significant interaction was observed between the PON1-108 genotype and the urinary metabolites ∑DAP and ∑DMP relative to pooled head circumference (p for interaction = 0.09 for both), respectively. No additional statistically significant interactions were observed for any other genotypes among children and mothers, and no statistically significant interactions were observed for race, sex or maternal education.

Overall, in this 2015/2016 literature review, inconsistent evidence of OP exposure and association with adverse birth outcomes/fetal growth was observed (Barr *et al.*, 2010; Rauch *et al.*, 2012; Wang *et al.*, 2012; Wolff *et al.*, 2007). This lack of consistency in the literature was also observed for birth outcomes in the recent chlorpyrifos HHRA (USEPA, 2014) which notes that researchers from CCCEH, Mt. Sinai, and CHAMACOS also investigated the possible role of prenatal OP exposure and fetal growth. These results were not consistent across these cohorts. Authors with CCCEH observed evidence of an inverse association, *i.e.*, increasing cord blood chlorpyrifos was associated with decreased measures of birth weight and length, while authors with the Mt. Sinai and CHAMACOS cohorts reported either no association, or evidence of a *positive* relationship, respectively (Berkowitz *et al.*, 2004; Eskenazi *et al.*, 2004; Whyatt *et al.*, 2004). Inconsistent results may be due to differences across study groups in exposure profiles as well as dissimilar methods of prenatal OP exposure assessment (Needham, 2005).

Given the lack of consistency among cohorts for the fetal growth metrics, the proposed link between fetal growth and OP exposure is tenuous. Therefore, consistent with previous evaluations for chlorpyrifos, EPA is focusing the remainder of this document on neurodevelopmental outcomes. Although the agency is not evaluating these birth outcome studies further at this time, the agency will continue to monitor the scientific literature for advances in this line of research.

2.2.8 Neurodevelopment Outcome Epidemiologic Studies

From an initial total of 30 high or moderate quality studies, with the exclusion of six studies for insufficient exposure assessment, three studies with measurable AChE inhibition and potential cholinergic toxicity which are outside the scope of this analysis, one study from an illegal use of a highly potent OP (MP) where cholinergic toxicity cannot be ruled out, five studies with birth outcome as the only assessment, two with outcomes not related to neurodevelopmental outcomes, and one study assessing only PON1 genotype expression and neurobehavioral outcomes (Fortenberry et al, 2014a), a total of twelve studies from the 2015/2016 literature review focusing on neurodevelopmental outcomes remain to be evaluated. Eight are considered high quality (Bouchard *et al.*, 2010; Fortenberry *et al.*, 2014; Furlong *et al.*, 2014; Donauer et al. (2016), Stein et al. (2016), Rauh et al, (2015), Engel et al (2015), and Yolton et al. (2013). Four are considered moderate quality studies (Guodong *et al.*, 2012; Oulhote and Bouchard, 2013; Zhang *et al.*, 2014; Shelton *et al.*, 2014).

Furlong et al (2014) is from the Mt. Sinai cohort study; Stein et al (2016) is from CHAMACOS, and Rauh et al (2015) is from CCCEH--- thus these studies share many of the same strengths and

uncertainties as other studies from these cohorts.

Most of these studies used biomarker measures for their exposure assessment, including child or maternal urinary DAPs (Bouchard *et al.*, 2010; Furlong *et al.*, 2014; Guodong *et al.*, 2012; Oulhote and Bouchard, 2013; Zhang *et al.*, 2014; Yolton et al, 2013; Donauer et al, 2016; Stein et al, 2016, Engel et al, 2015), child urinary DMP and DEPs (Oulhote and Bouchard, 2013; Zhang *et al.*, 2014; Guodong *et al.*, 2012), or maternal TCPy (Fortenberry *et al.*, 2014). Shelton *et al.* (2014) used pesticide use data from the California Department of Pesticide Regulation and geospatial methods to map the specific pesticide use pattern to the participant residence. Engel et al. (2015) reports on the results of a pooled analysis from HOME, CCCEH, CHAMACOS, and Mt. Sinai to evaluate the association between prenatal urinary DAPs and neurodevelopmental outcomes at 24 months only.

The study populations include national in scope (Bouchard *et al.*, 2010; Oulhote and Bouchard, 2013); mainly urban (Fortenberry *et al.*, 2014; Fortenberry *et al.*, 2014a; Furlong *et al.*, 2014; Guodong *et al.*, 2012; Zhang *et al.*, 2014; Rauh et al, 2015, Shelton *et al.*, 2014); suburban (Yolton et al, 2013; Donauer et al, 2016); and agricultural (Stein et al, 2016).

2.2.8.1 CCCEH, CHAMACOS, & Mt. Sinai Cohorts

The CCCEH, Mt. Sinai, and CHAMACOS studies reflect different types of exposed groups in the total population which strengthens the weight of the evidence considerations regarding this stream of information. The CCCEH Mother's and Newborn study and the Mt. Sinai Child Growth and Development study participants were likely exposed to OPs through the diet and through residential use of the pesticide for indoor pest control. In the residential setting, study populations were most likely exposed through indoor residential use of the pesticide during the study time period and additionally exposed to OPs via the oral route through ingesting residues in the diet and from hand-to-mouth contact with in-home surfaces, as well as possible dermal or inhalation exposure through contact with treated areas in the home environment (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). In each of these three US children's health cohorts, the biological measurements in these cohorts were comparable to the general population NHANES.

These cohort studies each enrolled pregnant women during roughly the same time period, measured both environmental exposure to the pesticide during pregnancy and also measured biomarkers representing internal dose during pregnancy and at delivery, and prospectively assessed associations in their newborns and young children through age 7 years. Each study includes several hundred (approximately 100-400) mother-infant pairs; these sample sizes are

sufficient to perform statistically valid analyses. Investigators from each study cohort utilized a similarly strong study design (prospective birth cohort); measured pesticide exposure using several different methods including environmental indicators as well as specific and non-specific biomarkers of OPs; ascertained developmental outcomes using validated assessment tools well-established in both clinical and research settings; and, measured, analyzed, selected and statistically adjusted for potentially confounding variables including socio-economic status and other environmental exposures using reasonable and appropriate methods. Limitations exist as well. These studies utilized a one-time measure (or the average of two measures) of chlorpyrifos or OP exposure to assess prenatal pesticide exposure throughout the gestational period, were unable to assess the influence of mixtures (co-occurring exposures in the relevant biological time window), and reflect a small sample size to fully evaluate the effect of more than one simultaneous exposure on neurodevelopment, *i.e.*, evidence of effect modification.

As noted, two major uncertainties in environmental epidemiology studies are the accurate and reliable measurement of exposure and potential confounding variables such as the influence of mixtures. The researchers with each of the three cohorts have provided supplemental methodological research to address these areas to the extent possible. Across the three children's health cohorts, study authors measured biomarkers of OP exposure. There is uncertainty as to the extent measurement of non-specific metabolites of OP or chlorpyrifos accurately reflects OP exposure; CCCEH and Mt. Sinai studies do not estimate post-natal exposure to chlorpyrifos among child participants, therefore the influence of early life and childhood OP exposure is unaccounted for in these analyses. The CHAMACOS cohort measured urinary levels of DAPs in young children and with the exception of pervasive developmental disorder (PDD) [per 10-fold increase in prenatal and post-natal DAP [prenatal: odds ratio (OR) = 2.3, $p = 0.05$; child DAPs OR = 1.7, $p = 0.04$]. did not observe negative significant associations in relation to neurodevelopment from post-natal exposure (Eskenazi *et al.*, 2007; Marks et al, 2010). The CHAMACOS cohort investigators also measured AChE and butyl ChE as supplemental indicators of OP exposure.

Potential confounding bias is another major uncertainty within environmental epidemiology studies. Confounding variables, exposures that could be related to OP exposure and neurodevelopmental outcomes such as blood lead, may result in an incorrect epidemiological risk estimate. Across these cohort studies, investigators collected relevant information concerning demographic characteristics and other environmental exposures, and were, to the extent possible with the existing information, able to effectively hold constant the influence of these other variables when estimating the association between prenatal chlorpyrifos and adverse neurodevelopmental outcomes. Control of these variables is important to reduce the chances of a false positive study result. Overall, statistical analyses were judged to be appropriate and reasonable (not overly large number of statistical model variables) to the research question by EPA and expert Panel reviews (FIFRA SAP 2008 and 2012).

Researchers with both the Mt. Sinai and CHAMACOS cohorts evaluated neonatal neurological functioning in association with prenatal OP exposure; CCCEH did not conduct these measurements. To measure indices of abnormal neonatal behavior and/or neurological

integrity authors used outcome measures derived from the Brazelton Neonatal Behavioral Assessment Scale (BNBAS), a neurological assessment of 28 behavioral items and 18 primitive reflexes. This tool was administered to infants 2-5 days post-partum by trained neonatologists in the hospital setting using similar environmental conditions. The authors with both study groups observed an increased number of abnormal reflexes in relation to increasing measures of OP exposure (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.

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 chlorpyrifos exposure, using categories informed by results of the previous study on birth characteristics (Whyatt *et al.*, 2004). Authors reported that the difference in MDI scores was "marginally significant" (p = 0.06) between the "high" and "low" exposed groups; the high exposed group scoring an average of 3.3 points lower than the low exposed (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.

CCCEH study authors (Rauh et al., 2015) evaluated the relationship between prenatal chlorpyrifos exposure and motor development/movement among 271 of the cohort participants who had reached the age of approximately 11 years. When comparing children in the upper tertile of exposure (>6.17 pg/g; N=43) to those in the lower tertiles (N=228), they observed statistically significant associations between prenatal chlorpyrifos exposure and mild to moderate tremor in the dominant arm, both arms, either arm, and a marginally statistically significant association in the non-dominant arm. The specific OR calculated associated with these elevated risks of arm tremor are as follows: dominant arm (OR=3.2; 95% CI=l .3-8. l; p=O.O 15); both arms (OR=3.3 ; 95% CI=l .1-9.4; p=0.027); either arm (OR=2.2; 95% CI=l .1-4.6; p=0.028); and non-dominant arm (OR=2. l; 95% CI=0.99-4.3; p=0.055). These associations were observed even after controlling for potential confounding factors such as medication, sex, and ethnicity.

Within the 36 month testing period, the likelihood of highly exposed children developing mental delays were significantly greater (MDI: 2.4 times greater (95% CI: 1.12-5.08, p = 0.02) and PDI: 4.9 times greater (95% CI: 1.78-13.72; p = 0.002)) than those with lower prenatal exposure (Rauh *et al.*, 2006). Within the Mt. Sinai study, authors administered the BSID-II to participating children at 12 and 24 months and observed that prenatal total DAP metabolite

level was associated with a decrement in mental development at 12 months among blacks and Hispanic children; however, these associations either attenuated or were non-existent at the 24-month visit (Engel *et al.*, 2011). In the CHAMACOS 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).

With respect to the findings related to the autism spectrum, from CCCEH, Rauh *et al.* (2006) reported a large odds ratio for PDD (OR=5.39; 95% CI: 1.21-24.11) when comparing high to low chlorpyrifos exposure groups. Among 7-9 years old children in the Mount Sinai Cohort (Furlong *et al.* 2014), there was no overall statistically significant association between maternal third trimester urinary DAP metabolite levels and reciprocal social responsiveness. However, some evidence of modification of the association between prenatal OP pesticide exposure and impaired social responsiveness in early childhood was observed by both race/ethnicity and child sex, with an association between DEAP and poorer social responsiveness observed among black participants and boys. No association was observed among whites or Hispanics, among girls, or for DAP or DMAP biomarker levels. In the CHAMACOS cohort, Eskenazi *et al.* (2010) reported non-significant, but suggestive, increased odds of PDD of 2.0 (0.8 to 5.1; p=0.14), whereas Eskenazi et al (2007) reported a statistically significant association between total DAP exposure and increased odds of PDD.

With respect to attention problems, Rauh *et al.* (2006) also investigated 36-month child behavior checklist (CBCL) (behavioral) scores. Significant differences were observed between the high and low chlorpyrifos exposure groups in the general category of attention-problems (p=0.010), and in the more specific DSM-IV scale for ADHD problems (p = 0.018). The CHAMACOS cohort also investigated attention problems in early childhood using three different assessment tools: maternal report of child behavior at 3.5 and 5 years of age; direct assessment of the child at 3.5 and 5 years; and by a psychometrician's report of the behavior of the child during testing at 5 years. In this study population, higher concentrations of OP metabolites in the urine of pregnant women were associated with increased odds of attention problems and poorer attention scores in their children at age 5 years (Marks et al, 2010).

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. In the Mt. Sinai study, prenatal maternal DEP urinary metabolite concentrations were associated with slight decrements in Full Scale Intelligence Quotient (FSIQ), Perceptual Reasoning, and Working Memory between the ages of 6 and 9 years, and difference in intelligence measures by putative PON1 status were also noted (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.

To determine if early childhood social adversities modify the association between maternal prenatal OP exposure and cognition in children (measured using the WISC-IV results), Stein et al. (2016) showed negative associations between maternal prenatal exposure to DAP metabolites and cognition in children were observed to be stronger among children who reported more adversity, relative to children who experienced less adversity. For example, for boys who experienced a more adverse learning environment, a statistically significant decrease was observed between prenatal DAP concentrations and Full-Scale IQ (β = -13.3; 95% CI: -19.9; -6.7; $p < 0.01$), relative to boys who lived in a less adverse learning environment (β = 4.2; 95% CI: -4.2; 12.5; $p < 0.01$). A similar observation was reported for perceptual reasoning and processing speed (β = -9.8; 95% CI: -17.4; -2.2; $p < 0.01$; β = -12.7; 95% CI: -19.5; -6.0; $p = 0.01$), respectively. For girls who experienced more economic adversity, a stronger effect on the association between prenatal DAP concentrations and Full-Scale IQ was observed, relative to girls who experienced less economic adversity (β = -8.5; 95% CI: -16.7, -0.4 vs. β = -4.7 95% CI: -12.8, 3.4; $p = 0.18$). This was also observed for working memory for girls (β = -5.9; 95% CI: -13.7, -1.8 vs. β = -2.6 95% CI: -10.0, 4.8; $p = 0.05$). In addition, similar findings were observed for girls whose mother's had more maternal adversity (β = -11.5; 95% CI: -18.5, -4.4 vs. β = -1.5 95% CI: -7.9, 4.9; $p = 0.07$).

To ascertain whether observed differences in neurodevelopment after prenatal chlorpyrifos exposure may be explained by differences in brain morphology between exposed groups, investigators compared MRI brain images between high and low chlorpyrifos exposed child study participants (Rauh *et al.*, 2012). Authors determined there were distinct morphological differences in brain areas associated with these neurodevelopmental outcomes. The pilot study included 40 child participants due to strict inclusion and exclusion criteria, and the high cost of performing the imaging studies on each child. EPA convened a Federal Panel of experts to perform a written peer-review of this study.[5] The Federal Panel concurred with the authors' conclusions in general; however, the Federal Panel also noted that significantly greater and more sophisticated MRI imaging studies would be needed to link the morphological changes

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

indicated in this study with specific functional outcomes noted in the CCCEH IQ study. Therefore, while generally supportive of the epidemiologic findings, additional study is needed to make specific links with areas of brain development change.

In sum, across these three children's environmental health studies, authors consistently identified associations with neurodevelopmental outcomes in relation to OP exposure. There is evidence of delays in mental development in infants (24-36 months), attention problems and autism spectrum disorder in early childhood, and intelligence decrements in school age children who were exposed to chlorpyrifos or OPs during gestation. Investigators reported strong measures of statistical association across several of these evaluations (odds ratios 2-4 fold increased in some instances), and observed evidence of exposures-response trends in some instances, *e.g.*, intelligence measures.

EPA has considered the strengths and limitations of these studies, and believes that random or systematic errors in the design, conduct or analysis of these studies were unlikely to fully explain observed positive associations between *in utero* OP exposure and adverse neurodevelopmental effects observed at birth and through childhood (age 7 years). EPA believes these are strong studies which support a conclusion that OPs likely played a role in these outcomes.

2.2.8.2 HOME Cohort

Two studies from the HOME cohort on neurodevelopmental outcomes have been reported (Yolton et al, 2013; Donauer et al, 2016). The HOME study is a prospective cohort study conducted from March 2003 to February 2006, and the study population included pregnant women living in the Cincinnati, Ohio area who successfully delivered live-born infants. The HOME cohort is a more recent US cohort compared to CHAMACOS, Mt. Sinai and CCCEH and represents exposure from post mitigation activities on many OPs which occurred during tolerance reassessment. Thus, the exposure pattern to the mothers in the HOME cohort and the study demographics (i.e. suburban, middle class study population) are likely different relative to studies from CHAMACOS, Mt. Sinai and CCCEH. Moreover, the demographic data reported in the HOME cohort indicate that mothers tended to have a higher socioeconomic status and were more likely to eat and live a healthier lifestyle relative to mothers involved in CHAMACOS, Mt. Sinai and CCCEH. As a result, these life habits were potentially protective towards the OP exposures during pregnancy.

Exposure was assessed twice during pregnancy—at 16 ± 4 weeks and 26 ± 4 weeks of gestation—via spot urine samples that were stored and later laboratory tested. The urine samples were measured for six DAPs.

In the Yolton et al (2013) study, a neurobehavioral assessment, the NICU Network Neurobehavioral Scale (NNNS), was conducted at approximately 5 weeks of age, and each infant was scored based on several categories (or subscales) of the NNNS. A total of 350 mother/ infants were included within this study, and a statistically significant association ($p <$

0.05) between maternal metabolite urinary concentrations (DE and DAP) and the following NNNS subscales for infant performance was observed: attention, lethargy, hypotonia, and autonomic stress. After adjusting the model for covariates, evidence of an association was observed between increased mean urinary DE metabolites and increased attention ($\beta = 0.066$, $p < 0.05$), with no statistically significant covariates. Decreased lethargy and hypotonia were also associated with increased urinary DE metabolites at 16 weeks of gestation ($\beta = -0.069$, $p = 0.04$; $\beta = -0.101$, $p = 0.03$), with black race and birthweight as statistically significant covariates ($\beta = -0.462$, $p = 0.03$; $\beta = -0.044$, $p = 0.01$). For DAP metabolite concentrations, evidence of an association was observed between increased DAP concentrations at 26 weeks of gestation and decreased autonomic stress (β coefficient = -0.010, $p = 0.01$), with birthweight of infants and maternal blood lead levels during gestation as statistically significant covariates ($\beta = -0.003$, $p = 0.02$; $\beta = 0.031$, $p = 0.02$), respectively.

In Donauer et al, 2016, offspring were assessed through neurodevelopmental examinations conducted yearly, at ages 1-5 years. The Bayley-II assessed mental and motor advancement for children ages 1-3 years, and the MDI and PDI were used to analyze cognitive, language, and motor development. For 4 year-old children, language skills were assessed by the Clinical Evaluation of Language Fundamentals-Preschool, and the WPPSI was used to assess intelligence (IQ) including verbal, performance, and full-scale for children 5 years of age. Of the 327 maternal-child cases, evidence of a significant association between maternal levels of urinary metabolites (total DAP and total DM) and verbal IQ of the child from the Wechsler Preschool and Primary Scale of Intelligence test was observed in both the unadjusted and adjusted models at the $p < 0.2$ level (unadjusted model: $\beta = 0.005$, $p = 0.034$, $\beta = 0.003$, $p = 0.144$; adjusted model: $\beta = 0.003$, $p = 0.179$, $\beta = 0.003$, $p = 0.144$), respectively. Although evidence of a positive association was observed for total DAP and total DM metabolites and full-scale IQ (unadjusted model: $\beta = 0.004$, $p = 0.111$; $\beta = 0.004$, $p = 0.124$, when the model was adjusted, no evidence of a positive association was observed (adjusted model: $\beta = 0.001$, $p = 0.494$; $\beta = 0.002$, $p = 0.441$). No evidence of a positive association was observed between performance IQ of the child and total DAP, DM, and DE, respectively. For Bayley-II MDI, evidence of a positive association was observed for maternal exposure to total DM and total DE at the child's 2-year visit (unadjusted model: $\beta = 0.003$, $p = 0.16$; $\beta = 0.003$, $p = 0.16$); however, when the model was adjusted, no evidence of a positive association was observed for total DM and total DE (adjusted model: $\beta = 0.002$, $p = 0.28$; $\beta = 0.002$, $p = 0.37$). No evidence of a positive association was observed between maternal exposure (for total DAP, total DM, and total DE) and the Bayley-II Psychomotor Developmental Index for children aged 1-3 years.

2.2.8.3 Meta-analysis (Engel et al, 2015)

Engel et al. (2015) reports on the results of a pooled analysis from four cohorts (N=936) to evaluate the association between prenatal DAPs and neurodevelopmental outcomes at 24 months only. In addition, researchers in this study assessed the impact on these associations of the specific cohort, race/ethnicity, and the PON I genotype of study participants. Researchers across the four children's health cohorts utilized the BSID-11 to generate MDI and PDI to assess neurodevelopment in early childhood. The four cohorts include CHAMACOS (N=377), HOME

(N=265), Mt. Sinai (N=234), CCCEH (N=60), It is noted that the CCCEH participants included in this analysis are from women enrolled in 2000 to 2001, a time period which is during the phase out of chlorpyrifos in residential settings.

The results of this pooled study are relatively consistent with those seen in the individual cohorts at 24 months. After controlling for race/ethnicity, smoking, and drug use during pregnancy, a statistically significant association was observed in the pooled population between total DAPs exposure and MDI decrements, but not with PDI decrements. Consistent with the results from Eskenazi et al. (2007), the strongest evidence of an association was observed for the CHAMACOS cohort, with statistically significant associations for both total DAPs and total DMAPs exposure and MDI decrements. No significant associations were seen within the Mt. Sinai and CCCEH cohorts, a result which is basically consistent with the previous observations at 24 months in these cohorts (Engel et al., 2011; Rauh et al.,2006). The study authors observed significant heterogeneity from combining the cohorts, especially with regards to race/ethnicity, and noted that impacts on specific subpopulations may be lost when looking at the pooled results.

2.2.8.4 Other Neurodevelopmental Studies

In the first of two Chinese studies focusing on generic neurodevelopmental outcomes, Zhang *et al.* (2014) investigated prenatal exposure to OPs and neurobehavioral development of neonates in a birth cohort study in Shenyang, China. The authors reported that consistent statistically significant associations were observed between all of the quantified urinary biomarkers of prenatal OP pesticide exposure and neonatal neurodevelopment deficits assessed 3 days after birth. A 10-fold increase in total DAPs concentration was associated with an average decrease in Neonatal Behavioral Neurological Assessment (NBNA) summary scores of 1.78 points (95% CI: -2.12 to -1.45). No evidence of departure from linearity of the exposure-response relationship between maternal DAP concentrations and NBNA scores was observed. In the second Chinese study focusing on neurodevelopmental outcomes, Guodong *et al.* (2012) conducted a cross-sectional study, and did not identify any statistically significant associations between the children's urinary DAP metabolite levels and any of the DQ (Developmental quotients) scores. The authors mentioned that their results should be interpreted with caution since OP exposure was quantified in single spot urine sample from children, and should be followed up with a longitudinal study with repeated measurement of exposure levels in urine samples.

Two studies focused on impaired social responsiveness, autism spectrum disorders, or developmental delays (Furlong *et al.*, 2014; Shelton *et al.*, 2014). Among 7-9 years old children in the Mount Sinai Cohort (Furlong *et al.* 2014), there was no overall statistically significant association between maternal third trimester urinary DAP metabolite levels and reciprocal social responsiveness (a measure linked to many neuropsychiatric conditions that involve impaired social functioning (Constantino and Gruber 2005)). However, some evidence of modification of the association between prenatal OP pesticide exposure and impaired social

responsiveness in early childhood was observed by both race/ethnicity and child sex, with an association between DEP and poorer social responsiveness observed among black participants and boys. No association was observed among whites or Hispanics, among girls, or for DAP or DMP biomarker levels.

Shelton *et al.* (2014), investigated autism spectrum disorders and developmental delay (DD) in relation to gestational residential proximity to agricultural pesticide applications utilizing the California population-based Childhood Autism Risks from Genetics and Environment (CHARGE) study. The investigators reported that children with autism spectrum disorder were 60% more likely to have OPs applied near the home [1.25 km distance; adjusted OR (aOR) = 1.60; 95% CI: 1.02–2.51] than mothers of normally developing children. They added that as the buffer distance grew larger, these associations became lesser, indicating an exposure-response effect. The authors also mentioned that each 100-lb (45.4 kg) increase in the amount applied over the course of pregnancy (within 1.5 km of the home) was associated with a 14% higher prevalence of autism spectrum disorder (aOR = 1.14; 95% CI: 1.0, 1.32), but no association was identified with DD.

A total of three studies focused on OP exposure and behavioral, memory, or attention/ADHD outcomes (Oulhote and Bouchard, 2013; Bouchard *et al.*, 2010; Fortenberry *et al.*, 2014). In a national cross-sectional study of Canadian children 2007-2009 data for age 6-11 years (Oulhote and Bouchard, 2013), there were no overall statistically significant associations observed between child urinary DAP, DMP, or DEP metabolite levels and parentally reported behavioral problems. In contrast, Bouchard *et al.* (2010), looking at U.S. children age 8-15 years in the 2000-2004 National Health and Nutrition Examination Survey (NHANES),[6] observed a positive association between attention and behavior problems and DAPs and DMPs, but not DEPs. For example, even after controlling for potential confounders such as sex, age, ethnicity, and creatinine concentration, they found that a 10-fold increase in DMAP concentration was associated with a 55 to 72% increased odds of ADHD.

Fortenberry *et al.* (2014) evaluated the relationship between pesticide exposure and ADHD in school age Mexican children, recruiting 187 mother-child pairs from a prospective birth cohort, ELEMENT (Early Life Exposures in Mexico to Environmental Toxicants). The authors reported that, there were no statistically significant associations between tertiles of maternal third trimester urinary TCPy and measures of attention and hyperactivity in children. However, there was suggestive evidence for increases in the ADHD index in relation to TCPy tertiles among boys (the highest TCPy tertile was associated with an ADHD index score that was 5.55 points higher than children in the lowest tertile; p-value = 0.06).

3.0 Weight of Evidence Analysis: Integration Across Multiple Lines of Evidence

OPP's 2016 "Framework for Incorporating Human Epidemiologic & Incident Data in Risk

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

Assessments for Pesticides" provides the foundation for evaluating multiple lines of scientific evidence (U.S. EPA, 2016c). OPP uses a WOE analysis for evaluating epidemiology and human incident data, such that conclusions are made on the preponderance of the information rather than relying on any one study. OPP uses the modified Bradford Hill criteria like those in the MOA/human relevance framework as a tool for organizing and integrating information from different sources (Hill, 1965; U.S. EPA, 1999, 2005; Sonich-Mullin et al., 2001; Meek et al., 2003; OECD AOP Wiki Users Handbook[7]). It is important to note that the Bradford Hill Criteria are not intended as a check box approach but instead are points to consider when evaluating the totality of evidence. In addition, the availability of a fully elucidated MOA/AOP is a not requirement for using epidemiology studies in human health risk assessment. The Bradford Hill Criteria explicitly considers such concepts as strength, consistency, dose response, temporal concordance and biological plausibility in a weight of evidence analysis; sections 3.1-3.3 below summarize the available evidence based on these principles.

The agency's 2002 guidance on "Determination of the Appropriate FQPA Safety Factor(s) in Tolerance Assessment" contains a discussion of the relative weighing of animal and human data with respect to pre- and post-natal toxicity, dose-response, toxicokinetics, and mode of action. The guidance specifically states that

> "potential for pre- and postnatal toxicity can be determined from human and animal studies. Although human studies are seldom available, human data are the most relevant data for assessing potential health risks. *When sufficient human data are available to judge that an adverse developmental outcome is related to exposure, the degree of concern increases* (p. 33, emphasis added)."

Table 1 of the 2002 FQPA Safety Factor guidance notes that when effects are found in humans related to exposure that these data receive "Increasing Weight". This table lists the following factors as receiving increasing weight in the WOE analysis with respect to pre- and post-natal toxicity:

- Effects found in humans related to exposure
- Same types of effects seen in more than one species
- Effects of a different type with greater potential consequences in young compared to adults
- Persistence or relatively longer recovery of effects in young compared to adults

The agency notes that each of these factors applies to the database of studies for OPs. As described in detail above, epidemiology studies from multiple investigators representing multiple locations and different populations show associations between OP exposure and adverse neurodevelopmental outcomes. Regarding multiple laboratory species in the database of laboratory studies, a range of effects on the developing nervous system has been shown in mouse (e.g., Braquenier et al., 2010; Venerosi et al., 2015), rat (e.g., Carr et al., 2015;

[7] https://aopwiki.org/wiki/index.php/Main_Page#OECD_User_Handbook

Vatanparas et al., 2013), and guinea pig (e.g., Mamczarz et al., 2016). Although very high levels of OP exposure can lead to coma, paralysis, or even death, the 10% AChE point of departure represents a precursor event believed to be protective of this downstream cholinergic neurotoxicity. The 10% AChE PoD is much lower than these exposure levels and thus is believed to be health protective of cholinergic neurotoxicity. In contrast, neurodevelopmental effects such as reduced IQ, autism spectrum disorder, and ADHD are adverse health outcomes of significant public health consequence. Moreover, these types of neurodevelopment effects are likely to persist through life. Further evidence of the long term consequence of such exposure is found in Rauh et al (2015) who have shown even at age 11 children exposed to chlorpyrifos in the home environment were more likely to exhibit mild or mild to moderate arm tremor.

3.1 Dose-Response Relationships & Temporal Concordance

Since the MOA(s)/AOP(s) is/are not established for neurodevelopmental outcomes (USEPA, 2012, 2014), it is not possible to describe the concordance in key events or biological steps leading to neurodevelopmental outcomes. As such, the quantitative linkages between molecular initiating events (MIE)s, intermediate steps, and ultimately the adverse outcome (i.e., neurodevelopmental effects) cannot be determined. (See Appendix 6)

With respect to the timing of exposure, across the epidemiology database of studies the maternal urine, cord blood and other (meconium) measures provide evidence that exposure did occur to the fetus during gestation, but the actual level of such exposure during the critical window(s) of susceptibility is not known. While significant uncertainties remain about the actual exposure levels experienced by mothers and infant participants in the children's health cohorts, it is unlikely that these exposures resulted in AChE inhibition. As part of the CHAMACOS study, Eskenazi *et al.* (2004) measured AChE activity and showed that no differences in AChE activity were observed. The biomarker data from the Columbia University studies are supported by the agency's dose reconstruction analysis using the PBPK-PD model. Following the recommendation of the FIFRA SAP (2012), the agency conducted a dose reconstruction analysis of residential uses available prior to 2000 for pregnant women and young children inside the home (USEPA, 2014). Based on the output from the PBPK-PD model, for the highest exposure considered (i.e., indoor broadcast use of a 1% chlorpyrifos formulation), <1% RBC AChE inhibition in pregnant women would be expected. While uncertainty exists as to actual OP exposure at (unknown) critical windows of exposure, EPA believes it is unlikely individuals in the epidemiology studies experienced RBC AChE inhibition.

Preamble to the Integrated Science Assessments (ISAs) which serve as a scientific foundation for the review of EPA's National Ambient Air Quality Standards (NAAQS) notes that strong evidence for causality can be provided through "natural experiments" when a change in exposure is found to result in a change in occurrence or frequency of health (USEPA, 2015c). Within the Columbia University epidemiology studies, the relationship in time between prenatal chlorpyrifos exposure and adverse neurodevelopmental outcomes is concordant. The

time period under study within the Columbia University (CCCEH) study, spanned the point in time in which pesticide manufacturers voluntarily cancelled the use of chlorpyrifos in the home environment, and researchers were able to show the change in exposure before (high use period) and after (low/no use period) the period of removal of chlorpyrifos products from the residential marketplace. Moreover, prior to the voluntary cancellation there were >80% detectable levels of chlorpyrifos in cord blood but in the time period after the cancellation only 16% of the measured values were greater than the level of detection (LOD); there was only one child born in the time period subsequent to the voluntary cancellation of chlorpyrifos in the residential marketplace for whom the cord blood chlorpyrifos level was in the upper-tertile of pre-cancellation exposure levels. The significantly reduced proportion of measured values greater than the LOD as well as the observation of an absence of an association between prenatal chlorpyrifos exposure among infants born after the voluntary cancellation of chlorpyrifos and neurodevelopmental effects support the hypothesis that chlorpyrifos is related to these outcomes. However, as noted by study authors, EPA and the FIFRA SAP (2012), this could also be due to inadequate sample size to detect a small to modest effect among the group of infants born after the voluntary cancellation. It is notable that epidemiology studies from other research groups have not included analyses across different years of exposure.

3.2 Strength, Consistency & Specificity

In making a weight-of-evidence analysis, it is important to consider the strength of the statistical measures of association between OP exposure and adverse neurodevelopmental outcomes through childhood (epidemiology) and possibly into adulthood (animal studies). It is also important to consider the strength of the integrated qualitative and quantitative evidence, the consistency of the observed associations across epidemiology studies and considering both animal and human data support the conclusion that chlorpyrifos plays a role in adverse neurodevelopmental outcomes.

3.2.1 Strength

As noted in the 2016 epidemiology and incident framework, findings of large, precise risks increases confidence that the association is not likely due to chance, bias, or other factors (USEPA, 2016c). In the case of the CCCEH study, there are a number of notable associations. Regarding infant and toddler neurodevelopment, the CCCEH authors reported statistically significant deficits of 6.5 points on the PDI at 3 years of age when comparing high to low exposure groups (Rauh et al., 2006). Notably these decrements in PDI persist even after adjustment for group and individual level socioeconomic variables (Lovasi et al., 2011). These investigators also observed increased odds of mental delay (OR=2.4; 95% CI: 1.1-5.1) and psychomotor delay (OR=4.9; 95% CI: 1.8-13.7) at age three when comparing high to low exposure groups (Rauh et al., 2006). Rauh et al (2006) also reported extremely large odds ratios for attention disorders (OR=11.26; 95% CI: 1.79-70.99), ADHD (OR=6.50; 95% CI: 1.09-38.69), and PDD (OR=5.39; 95% CI: 1.21-24.11) when comparing high to low chlorpyrifos exposure groups (Rauh et al., 2006). EPA notes that the magnitude of these results are so large that they

are unlikely to be affected by residual confounding although limited sample sizes resulted in imprecise estimates.

From the CHAMACOS cohort, Eskanazi et al (2007) reported that both prenatal and postnatal DAPs were associated with risk of PDD (per 10-fold increase in prenatal DAPs: OR = 2.3, p = 0.05; child DAPs OR = 1.7, p = 0.04). Marks et al (2010) reported prenatal DAPs were associated with scores on the K-CPT ADHD Confidence Index > 70th percentile (OR = 5.1; 95% CI: 1.7–15.7 and with a composite ADHD indicator of the various measures (OR = 3.5; 95% CI: 1.1–10.7). Some outcomes exhibited evidence of effect modification by sex, with associations found only among boys. Children's concurrent total DAP and DMP metabolite levels at 3.5 years and 5 years were unrelated to attention outcomes, and but child DEP concentrations at 5 years were adversely associated with the composite measure of attention (OR = 2.0; 95% CI:1.1–3.6).

In a recent evaluation by Stein et al (2016) from the CHAMACOS cohort, boys who experienced a more adverse learning environment, a statistically significant decrease was observed between prenatal DAP concentrations and Full-Scale IQ (β = -13.3; 95% CI: -19.9; -6.7; p < 0.01), relative to boys who lived in a less adverse learning environment (β = 4.2; 95% CI: -4.2; 12.5; p < 0.01). A similar observation was reported for perceptual reasoning and processing speed (β = -9.8; 95% CI: -17.4; -2.2; p < 0.01; β = -12.7; 95% CI: -19.5; -6.0; p = 0.01), respectively. For girls who experienced more economic adversity, a stronger effect on the association between prenatal DAP concentrations and Full-Scale IQ was observed, relative to girls who experienced less economic adversity (β = -8.5; 95% CI: -16.7, -0.4 vs. β = -4.7 95% CI: -12.8, 3.4; p = 0.18). This was also observed for working memory for girls (β = -5.9; 95% CI: -13.7, -1.8 vs. β = -2.6 95% CI: -10.0, 4.8; p = 0.05).

3.2.2 Consistency

The Preamble to the ISA's note that an inference of causality is strengthened when a pattern of elevated risks is observed across several independent studies. The reproducibility of findings constitutes one of the strongest arguments for causality. Moreover, statistical significance is not the sole criterion by which the presence or absence of an effect is determined (USEPA, 2015c).

Published and submitted laboratory animal studies have been reviewed for OPs. The >30 papers on chlorpyrifos provide evidence of long-lasting neurodevelopmental disorders in rats and mice; however, there was no clear consistency in terms of pattern, timing, or dose response for these effects. The additional toxicological literature and guideline DNT studies with the other OPs provide more evidence for the same conclusions, with again the same caveats and uncertainties. While overall cognitive function and motor activity appeared to be altered the most often, it is apparent that these behaviors were also the most often evaluated.

Across the various epidemiology studies within the scope of this analysis and with adequate quality in design and exposure assessment, it is important to note that these studies used study methods that were highly variable, including different exposure measurement, outcome

ED_002061_00044425-00084

assessment, study design, and geographical location. These differences make it challenging to compare the results across studies. Among the epidemiology studies, two of the cohorts (CCCEH and ELEMENT) have focused on chlorpyrifos whereas the other studies (Mt. Sinai cohort, CHAMACOS cohort, HOME cohort, CHARGE study, Bouchard *et al.*, 2010) have focused on less specific biomarkers (i.e., DAPs) and are not specific to any particular OP. When considered in concert, the epidemiology studies provide consistent findings for some outcomes.

In comparison to the studies from CCCEH, Mt. Sinai, and CHAMACOS, results from the ELEMENT and CHARGE studies provide some supportive evidence for the findings. However, the results from the HOME cohort show different results; namely positive associations between neurodevelopmental outcomes and maternal DAPs (Yolton et al, 2013; Donauer et al, 2016). The HOME study represents a different demographic profile (higher socioeconomic status, suburban, middle class) and different time period (2003-2006) compared to CCCEH, Mt. Sinai, and CHAMACOS which are made up of women with much lower socioeconomic status and who experienced different exposure patterns.

In the two Chinese studies, EPA does not know how the OP exposures from these studies relate to the currently registered use pattern for OPs used in the U.S./North America. In the case of these two Chinese studies (Guodong *et al.,* 2012; Zhang *et al.*, 2014), there may be differences in the study population and outcome measurements that may account for the observed differences in study results, with Zhang *et al.* (2014) documenting statistically significant associations for total DEAPs, total DMAPs, and total DAPs and Guodong *et al.* (2012) observing no association with these exposures. The Zhang *et al.* (2014) study was conducted in Shenyang, with a study population reported as 87% urban and 13% rural, whereas the Guodong *et al.* (2012) study was conducted in Shanghai with a 99% urban and 1% suburban study population. Given the higher percentage of study participants from rural areas, the study participants from Zhang *et al.* (2014) may have had different pattern and magnitude of OP exposures compared to those from Guodong *et al.* (2012). In addition, it is noted that different outcome measurements were made in these studies, with Guodong *et al.* (2012) assessing 23-25 month old children using a developmental quotient score and Zhang *et al.* (2014) assessing 3 day old infants using a Neonatal Behavioral Neurological Assessment. Given the different outcome assessments, exposure potential, study designs (cohort vs. cross-sectional), and ages of the participants in these two studies, it is difficult to draw conclusions on how these study results compare. It is notable that the results from the Zhang *et al.* (2014) study focusing on neonates are consistent with those from other studies which reported statistically significant associations between delayed neurological development measured in newborns and total DEAP, total DMAP, and total DAP exposure (Engel *et al.*, 2007; Young *et al.*, 2005). However, it is noted that neurological development was measured within a few days of birth for Zhang *et al.* (2014) and Engel *et al.* (2007), whereas Young *et al.* (2005) conducted their measurements within two months of birth.

The CHAMACOS and Mt. Sinai cohorts that measured neurological effects at birth (the Brazelton index), observed a putative association with OPs (Engel *et al.*, 2007; Young *et al.*, 2005). Yolton et al (2013) used the NNNS tool at approximately 5 weeks of age in the HOME

cohort and reported evidence of a positive association between increased mean urinary DE metabolites and increased attention and decreased autonomic stress.

Researchers across CCCEH, Mt. Sinai, CHAMACOS, and HOME studies utilized the Bayley Scales of Infant Development II (BSID-II) to generate a MDI and a PDI to assess neurodevelopment in early childhood. In the CCCEH study, Rauh *et al.* (2006) investigated MDI and PDI at 12, 24, and 36 months of age. Children were categorized as having either high (>6.17pg/g) or low (≤6.17pg/g) prenatal chlorpyrifos exposure, using categories informed by results of the previous study on birth characteristics (Whyatt *et al.*, 2004). Authors reported that the difference in MDI scores was "marginally significant" ($p = 0.06$) between the "high" and "low" exposed groups; the high exposed group scoring an average of 3.3 points lower than the low exposed (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.

Within the 36 month testing period, the likelihood of highly exposed children developing mental delays were significantly greater (MDI: 2.4 times greater (95% CI: 1.12-5.08, $p = 0.02$) and PDI: 4.9 times greater (95% CI: 1.78-13.72; $p = 0.002$)) than those with lower prenatal exposure (Rauh *et al.*, 2006). Within the Mt. Sinai study, authors administered the BSID-II to participating children at 12 and 24 months and observed that prenatal total DAP metabolite level was associated with a decrement in mental development at 12 months among blacks and Hispanic children; however, these associations either attenuated or were non-existent at the 24-month visit (Engel *et al.*, 2011). In the CHAMACOS 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). In contrast to the findings of CCCEH, Mt. Sinai, and CHAMACOS, the HOME study (Donauer et al, 2016) reported evidence of a positive association for MDI was observed for maternal exposure to total DM and total DE at the child's 2-year visit; however, when the model was adjusted, no evidence of a positive association was observed for total DM and total DE (adjusted model ($\beta = 0.002$, $p = 0.28$; $\beta = 0.002$, $p = 0.37$). No evidence of an association was observed between maternal exposure (for total DAP, total DM, and total DE) and the Bayley-II Psychomotor Developmental Index for children aged 1-3 years.

With respect to attention problems, Rauh *et al.* (2006) also investigated 36-month child behavior checklist (CBCL) (behavioral) scores. Significant differences were observed between the high and low chlorpyrifos exposure groups in the general category of attention-problems ($p=0.010$), and in the more specific DSM-IV scale for ADHD problems ($p = 0.018$). The CHAMACOS cohort also investigated attention problems in early childhood using three different assessment tools: maternal report of child behavior at 3.5 and 5 years of age; direct assessment of the child at 3.5 and 5 years; and by a psychometrician's report of the behavior of the child during testing at 5 years. In this study population, higher concentrations of OP metabolites in the urine of pregnant women were associated with increased odds of attention problems and poorer attention scores in their children at age 5 years (Marks et al, 2010).

ED_002061_00044425-00086

Of the four new studies focusing on ADHD and OP exposure, three found statistically significant associations, with only Oulhote and Bouchard (2013) finding no association with total DMAP, DEAP, or total DAP exposure. In contrast, Bouchard *et al.* (2010) observed an association with total DMAP and total DAP exposure and ADHD. Fortenberry *et al.* (2014) found suggestive, but not statistically significant, evidence of an association with TCPy and ADHD in boys. Overall, the Fortenberry and Bouchard study results are consistent with that of earlier studies from the CCCEH (Rauh *et al.*, 2006) and CHAMACOS (Eskenazi *et al.*, 2007; Marks *et al.*, 2010). Specifically, statistically significant associations were observed by Rauh *et al.* (2006) with chlorpyrifos exposure and ADHD, Eskenazi *et al.* (2007) with total DMAPs and total DAPs and ADHD, and Marks *et al.* (2010) with total DEAP, DMAP, and total DAP exposure.

It is important to put into context the specific outcome measures used in the assessment of attention and neurobehavioral problems. For example, Bouchard *et al.* (2010) identified statistically significant associations between OP exposure and ADHD/behavioral problems, whereas Oulhote and Bouchard (2013) did not. It is valuable to compare these studies given that they are both cross-sectional studies using large population level datasets with biomarker information, with Oulhote and Bouchard (2013) using a Canadian dataset and Bouchard et al (2010) using a U.S. dataset. Bouchard *et al.* (2010) used criteria for ADHD from DSM-IV, whereas Oulhote and Bouchard (2013) used a "Strengths and Differences Questionnaire (SDQ)," with the SDQ being a more generic assessment of mental health status than the DSM-IV criteria. When Oulhote and Bouchard (2013) compared their results to Bouchard *et al.* (2010), they noted that their outcome measurements may not have been as sensitive and that this may account for the difference in study results.

Across epidemiology studies looking at ADHD/behavioral problems, a suggestive or statistically significant positive association was observed in multiple studies between OP exposure and these neurobehavioral outcomes (Bouchard *et al.*, 2010; Fortenberry *et al.*, 2014; Rauh *et al.*, 2006, Eskenazi *et al.*, 2007). While these studies have differences in the years that the exposure occurred, study design, exposure assessment, and outcome assessment, the commonality in their results is striking.

Several studies have now documented suggestive or positive associations between OP exposure and autism spectrum disorders (Rauh *et al.*, 2006; Shelton *et al.*, 2014; Furlong *et al.*, 2014; Eskenazi *et al.*, 2007; Eskenazi et al, 2010). Specifically, Furlong *et al.* (2014) reported suggestive, but not statistically significant, evidence of an association between total DEAP exposure and reciprocal social responsiveness among black participants and boys. These results are consistent with previous studies conducted on the CCCEH and CHAMACOS cohorts, with these studies also showing statistically significant associations between OP exposure and autism spectrum disorders (Rauh *et al.*, 2006; Eskenazi *et al.*, 2007).[8] Specifically, Eskenazi *et*

[8] The DSM-V defines ASD (autism spectrum disorder) which now encompasses several disorders that were different diagnoses in DSM-IV, including PDD (pervasive developmental disorder, a catch-all where the other

al. (2007) reported a statistically significant association between PDD and total DAP exposure, whereas Rauh *et al.* (2006) showed a significant association between PDD and specifically chlorpyrifos exposure. Both PDD and reciprocal social responsiveness are related to the autism spectrum disorder. Using a different exposure assessment method (geospatial analysis and residential proximity to total OP exposure), Shelton *et al.* (2014) also documented statistically significant associations between total OP exposure and autism spectrum disorders. While these studies vary in the magnitude of the overall strength of association, they have consistently observed a positive association between OP exposure and autism spectrum disorders.

Finally, CCCEH, Mt. Sinai, and CHAMACOS observed an inverse relation between the respective prenatal measures of OPs and intelligence measures at age 7 years. To measure intelligence among school aged children, authors from each of the three children's health cohorts used the WISC-IV. Rauh *et al.* (2011) evaluated the relationship between prenatal chlorpyrifos exposure and neurodevelopment among 265 of the cohort participants who had reached the age of 7 years investigators reported that for each standard deviation increase in exposure (4.61 pg/g) there is a 1.4% reduction in Full-Scale IQ and a 2.8% reduction in Working Memory. In the Mt. Sinai study, prenatal maternal DEP urinary metabolite concentrations were associated with slight decrements in 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. The CHAMACOS results with the WISC-IV were furthered in Stein et al (2016) who evaluated potential impact from adverse learning environment. In contrast, to the findings of CCCEH, Mt. Sinai, and CHAMACOS, the HOME study (Donauer et al, 2016) reported evidence of a significant positive association between maternal levels of urinary metabolites (total DAP and total DM) and verbal IQ of the child from the WPPSI was observed in both the unadjusted and adjusted models. Although evidence of a positive association was observed for total DAP and total DM metabolites and full-scale IQ (unadjusted model: $\beta = 0.004$, $p = 0.111$; $\beta = 0.004$, $p = 0.124$, when the model was adjusted, no evidence of a positive association was observed (adjusted model: $\beta = 0.001$, $p = 0.494$; $\beta = 0.002$, $p = 0.441$).

As stated in the EPA neurotoxicity guidelines[9], direct extrapolation of developmental neurotoxicity results from laboratory animals to humans is limited by the lack of knowledge about underlying toxicological mechanisms and the relevance of these results to humans. EPA notes consistencies across the databases of *in vivo* laboratory animal studies and epidemiology studies, although challenges of making a direct comparison between neurodevelopmental domain inter-species remain. It can be assumed that developmental neurotoxicity effects in

---

categories didn't fit). Depending on when the study was conducted, the authors may use the PDD or ASD criteria and terminology.

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

ED_002061_00044425-00088

animal studies indicate the potential for altered neurobehavioral development in humans, although the specific types of developmental effects seen in experimental animal studies may not be the same as those that may be produced in humans. However, considering the toxicological and epidemiological data in the context of three major neurodevelopmental domains (specifically, cognition, motor control, and social behavior), insights can be gained. Previously reviewed studies of chlorpyrifos in rats and/or mice reported impaired cognition, changes in locomotor activity levels, altered social interaction, and to a lesser extent, changes in neuromotor function (FIFRA SAP 2012; USEPA, 2014). While there are fewer studies for all the other OPs, behavioral effects in the same functional domains were again reported. The most commonly reported outcome in the laboratory animal studies was cognitive dysfunction, and although it was overall consistent there were again differences in cognitive specificity, gender differences, or dose response. Quite a few studies also report changes in motor activity and sensory function in offspring, but there generally fewer laboratory animal studies that assess social interactions for OPs other than chlorpyrifos. It is notable that the laboratory animal studies vary in experimental designs such as species, strain, gender, dosing regimens (age, routes, vehicle), and test parameters (age, protocol). Likewise, observational epidemiology studies vary by population characteristics (race/ethnicity, SES, and pesticide use/exposure profile), co-exposures (mix of chemicals, windows of exposure), and method of exposure and outcome assessment. Given the differences across laboratory animal and epidemiology studies, the qualitative similarity in research findings is striking.

In contrast, quantitatively, there are notable differences between animals and humans. Specifically, in animals, the doses most often used in these studies are sufficient to elicit ≥10% brain and RBC inhibition depending on the study design, age of the animal, and sampling time. In the epidemiology studies, based on the comparisons with biomonitoring data, reported AChE data from CHAMACOS and the results of the chlorpyrifos dose-reconstruction analysis, it is unlikely that RBC AChE would have been inhibited by any meaningful or measurable amount, if any at all, and most likely none in the brain. This key difference in dose response between the experimental toxicology and epidemiology studies poses challenges in interpreting such data. There are a number of possible hypotheses such as: 1) limitations of experimental laboratory studies which have limited statistical power due to relatively small sample sizes; 2) humans display a broader array of behaviors and cognitive abilities than rats, thus limiting the sensitivity of the rat studies; and 3) in the epidemiology studies, the timing of OP application and blood collections are not coupled—thus higher levels of blood OPs were likely missed.

3.2.3 Specificity

In considering the FQPA 10X Safety Factor, the statute indicates that both pre- and post-natal toxicity and exposure be considered. As such, it is appropriate to consider the degree specificity of with regard to lifestage. There are numerous animal studies in the literature which vary in their study design but all involve gestational and/or early postnatal dosing with behavioral evaluation from adolescence to adulthood. The data provide support for the susceptibility of the developing mammalian brain to chlorpyrifos exposure through gestation

and early in life, with adverse outcomes in several neurological domains including cognitive, anxiety and emotion, social interactions, and neuromotor function. The studies have not shown that any specific developmental period is critical overall to the long-term outcomes, since similar effects are observed with different exposure periods. For example, cognitive changes in one laboratory using a radial arm maze were observed following gestational and early, but not late, postnatal exposure (Aldridge *et al.*, 2005; Icenogle *et al.*, 2004; Levin *et al.*, 2002; Levin *et al.*, 2001), whereas other laboratories cognitive deficits in a Morris water maze were reported following both gestational and late-postnatal exposure (Billauer-Haimovitch *et al.*, 2009; Turgeman *et al.*, 2011). Likewise, some changes in anxiety and social behaviors were reported at both gestational and postnatal exposure periods. Overall, these data do not clearly show specific critical periods of exposure but support a conclusion that early life (pre- and post-natal) represent susceptible lifestages.

As discussed above, numerous epidemiological investigations have observed a link between prenatal exposure to chlorpyrifos or OPs (measured as chlorpyrifos, TCPy, or DAPs) and adverse effects on neurodevelopment through age seven years, with additional more limited evidence up through approximately age eleven. As noted previously, for these epidemiology studies chlorpyrifos was only assessed directly in the CCCEH study, with the Mexican cohort study (Fortenberry *et al.*, 2014) assessing the chlorpyrifos metabolite TCPy, and the CHAMACOS, HOME, and Mt. Sinai cohorts focusing on the DAPs.

The majority of epidemiological studies investigated only prenatal exposures. Specifically, with respect to biomarkers representing prenatal exposures, CCCEH, Mt. Sinai, and CHAMACOS each reported evidence of impaired mental and psychomotor development, albeit not consistent by age at time of testing (ranging from 6 month to 36 months across the three cohorts). Statistically significant or suggestive associations between chlorpyrifos or DAPs exposure and attentional problems/ADHD were reported by multiple prospective cohorts (Rauh *et al.*, 2006; Eskenazi *et al.*, 2007; Marks *et al.*, 2010; and Fortenberry *et al.*, 2014) with additional support from a case control study, Bouchard *et al.* (2010). In addition, the three US cohorts and the CHARGE study have reported suggestive or positive associations between OP exposure and autism spectrum disorders (Rauh *et al.*, 2006; Shelton *et al.*, 2014; Eskenazi *et al.*, 2007; Furlong *et al.*, 2014). While the studies from CCCEH, Mt. Sinai, and CHAMACOS studies vary in the magnitude of the overall strength of association, they have consistently observed a positive association between OP exposure and autism spectrum disorder. Several studies have also documented statistically significant association between prenatal DAP exposure and abnormal motor development in neonates (reflexes, Brazelton score or similar measure; Young *et al.*, 2005; Engel *et al.*, 2007; Zhang *et al.*, 2014). Finally, CCCEH, Mt. Sinai and CHAMACOS have reported an inverse relation between the respective prenatal measures of chlorpyrifos or DAPs and intelligence measures at age 7 years (Rauh *et al.*, 2011; Engel *et al.*, 2011; Bouchard *et al.*, 2011).

With regards to effects pre-natal compared to post-natal, a small number of studies have assessed postnatal exposure to DAPs. Postnatal exposure to DAPs has been assessed in the CHAMACOS cohort (Eskenazi *et al.*, 2007; Young *et al.*, 2005; Bouchard *et al.*, 2011) and three

cross-sectional studies (Guodong *et al.*, 2012; Bouchard *et al.*, 2010; Oulhote and Bouchard, 2013). With the exception of Bouchard *et al.* (2010) and findings of PDD increase in post-natal DAP from CHAMACOS (Eskenazi *et al.*, 2007; Marks et al, 2010), no adverse neurodevelopmental associations were found between postnatal urinary metabolite levels and any of the developmental outcomes. Bouchard *et al.* (2010) looked at U.S. children age 8–15 years in the 2000–2004 National Health and Nutrition Examination Survey (NHANES), and observed a positive association between attention and behavior problems and total DAPs and DMAPs, but not DEAPs. Postnatal exposures have not been assessed in the CCCEH and Mt. Sinai studies (Rauh *et al.*, 2011; Engel *et al.*, 2011); as such, there are no studies included in this analysis which directly assessed the potential for postnatal chlorpyrifos exposure and associations with neurodevelopmental effects. However, given that the major source of exposure (residential use) was cancelled partway through the CCCEH study which substantially reduced and largely removed chlorpyrifos from the home environment, this limits the ability of the CCCEH study to inform the impacts of long-term postnatal exposure to chlorpyrifos on neurodevelopment from the current uses of chlorpyrifos.

In sum, given that the extensive experimental laboratory animal database suggests that the post-natal period is a potential susceptible time, the lack of postnatal exposure assessment in the CCCEH and Mt. Sinai studies is a source of uncertainty in the epidemiology database

3.3 Biological Plausability & Coherence

EPA's cancer guidelines (2005) includes guidance which are also applicable to this current evaluation of OPs. In fact, the Guidelines indicate:

"*evaluation of the biological plausibility of the associations observed in epidemiologic studies reflects consideration of both exposure-related factors and toxicological evidence relevant to identification of potential modes of action (MOAs). Similarly, consideration of the coherence of health effects associations reported in the epidemiologic literature reflects broad consideration of information pertaining to the nature of the biological markers evaluated in toxicologic and epidemiologic studies*. [p. 39]."

The Cancer Guidelines further state that "*lack of mechanistic data, however, is not a reason to reject causality* [p. 41]."

At this time, a MOA(s)/AOP(s) has/have not been established for neurodevelopmental outcomes. This growing body of literature does demonstrate, however, that OPs are biologically active on a number of processes that affect the developing brain. Multiple *in vitro* studies on endpoints relevant to the developing brain have identified outcomes in picomole concentrations, including concentrations lower than those that elicit AChE inhibition *in vitro.*

ED_002061_00044425-00091

- 1 pM chlorpyrifos oxon decreased axon length (~50%) in superior cervical ganglion cell cultures (AChE inhibition starting at 1000pM in same cell system): Howard et al., 2005
- 0.03 pM oxon (30 fM) increased in CREB levels (~50%) primary cortical neuron cultures (AChE inhibition starting at 100 pM in same cell system): Schuh et al., 2002
- 10 pM oxon decreased axon length (~40%) in dorsal root ganglion cell cultures (AChE inhibition starting at 100 pM in same cell system): Yang et al., 2008

With regard to coherence, there is a large body of *in vivo* laboratory studies which show long-term behavioral effects from early life exposure, albeit at doses which cause AChE inhibition. EPA considers the results of the toxicological studies relevant to the human population, as qualitatively supported by the results of epidemiology studies. The lack of established MOA/AOP pathway does not undermine or reduce the confidence in the findings of the epidemiology studies. When all the evidence is considered together, there are uncertainties with respect to a number of factors such as exposure assessment, lack of ability to make strong causal linkages, and unknown window(s) of susceptibility. The epidemiology studies reviewed in the 2012/2014 and 2015/2016 literature reviews represent different investigators, locations, points in time, exposure assessment procedures, and outcome measurements. Despite all these uncertainties and differences in study design, multiple investigators have identified associations with neurodevelopmental outcomes such as ADHD/behavioral problems and autism spectrum, in relation to OP exposure. There is evidence of delays in mental development in infants (24-36 months), attention problems and autism spectrum disorder in early childhood, and intelligence decrements in school age children who were exposed to OPs during gestation. Investigators reported strong measures of statistical association across several of these evaluations (odds ratios 2-4 fold increased in some instances), and observed evidence of exposures-response trends in some instances, *e.g.*, intelligence measures. Thus, with respect to biological plausibility and coherence, although uncertainties remain, these uncertainties are diminished in the context of the qualitative similarity between the epidemiology studies.

4.0 10X FQPA Safety Factor for Infants and Children

As section 408(b)(2)(C) of the FFDCA instructs EPA, in making its "reasonable certainty of no harm" finding, that in "the case of threshold effects, an additional tenfold margin of safety for the pesticide chemical residue and other sources of exposure shall be applied for infants and children to take into account potential pre- and postnatal toxicity and completeness of data with respect to exposure and toxicity to infants and children." Section 408 (b)(2)(C) further states that "the Administrator may use a different margin of safety for the pesticide chemical residue only if, on the basis of reliable data, such margin will be safe for infants and children." Given the totality of the evidence, there is sufficient uncertainty in the human dose-response relationship for neurodevelopmental effects which prevents the agency from reducing or removing the statutory 10X FQPA Safety Factor. For the human health risk assessments for the OPs, a value of 10X will be applied. Similarly, a database uncertainty factor of 10X will be retained for occupational risk assessments. The agency will continue to evaluate the

epidemiology studies and pursue approaches for quantitative or semi-quantitative comparisons between doses which elicit AChE inhibition and those which are associated with neurodevelopmental outcomes prior to a revised human health risk assessment. **The FQPA 10X Safety Factor or database uncertainty factor will be retained for OPs for the population subgroups that include infants, children, youths, and women of childbearing age for all exposure scenarios.**

5.0 References

Acosta-Maldonado, Brenda, Sánchez-Ramírez, Blanca, Reza-López, Sandra, and Levario-Carrillo, Margarita. Effects of exposure to pesticides during pregnancy on placental maturity and weight of newborns: A cross-sectional pilot study in women from the Chihuahua State, Mexico. Human and Experimental Toxicology 28(8) 451–459, 2009.

Aldridge, J. E., Levin, E. D., Seidler, F. J., & Slotkin, T. A. (2005). Developmental exposure of rats to chlorpyrifos leads to behavioral alterations in adulthood, involving serotonergic mechanisms and resembling animal models of depression. Environ Health Perspect, 113(5), 527–531.

al-Hachim, G.M., Fink, G.B., 1968a. Effect of DDT or parathion on open-field behavior of offspring from DDT- or parathion-treated mothers. Psychol Rep **22**, 1193-1196.

al-Hachim, G.M., Fink, G.B., 1968b. Effect of DDT or parathion on condition avoidance response of offspring from DDT or parathion-treated mothers. Psychopharmacologia **12**, 424-427.

Andersen, Helle R., Debes, Fróði, Wohlfahrt-Veje, Christine, Murata, Katsuyuki, Grandjean, Philippe. Occupational pesticide exposure in early pregnancy associated with sex-specific neurobehavioral deficits in the children at school age. Neurotoxicology and Teratology 47:1-9, 2015.

Ankley, G. T., Bennett, R. S., Erickson, R. J., Hoff, D. J., Hornung, M. W., Johnson, R. D., *et al.* (2010). Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. *Environ Toxicol Chem, 29*(3), 730-741.

Barr DB, Ananth CV, Yan X, Lashley S, Smulian JC, Ledoux TA, Hore P, Robson MG. Pesticide concentrations in maternal and umbilical cord sera and their relation to birth outcomes in a population of pregnant women and newborns in New Jersey. Sci Total Environ. 2010;408:790–795.

Berkowitz GS, Wetmur JG, Birman-Deych E, Obel J, Lapinski RH, Godbold JH, Holzman IR, Wolff MS. In Utero Pesticide Exposure, Maternal Paraoxonase Activity, and Head Circumference. Environmental Health Perspectives. 2004;112:388–391.

Billauer-Haimovitch, H., Slotkin, T. A., Dotan, S., Langford, R., Pinkas, A., & Yanai, J. (2009). Reversal of chlorpyrifos neurobehavioral teratogenicity in mice by nicotine administration and neural stem cell transplantation. Behav Brain Res, 205(2), 499–504.

Boobis, A. R., Cohen, S. M., Dellarco, V., McGregor, D., Meek, M. E., Vickers, C., *et al.* (2006). IPCS framework for analyzing the relevance of a cancer mode of action for humans. *Crit Rev Toxicol, 36*(10), 781-792.

Boobis, A. R., Doe, J. E., Heinrich-Hirsch, B., Meek, M. E., Munn, S., Ruchirawat, M., *et al.* (2008). IPCS framework for analyzing the relevance of a noncancer mode of action

ED_002061_00044425-00094

for humans. *Crit Rev Toxicol, 38*(2), 87-96.

Bouchard M.F., Bellinger D.C., Wright R.O., Weisskopf M.G., (2010). Attention-Deficit/Hyperactivity Disorder and Urinary Metabolites of Organophosphate Pesticides. PEDIATRICSDOI:10.1542/peds.2009-3058.

Bouchard, M. F., Chevrier, J., Harley, K. G., Kogut, K., Vedar, M., Calderon, N., *et al.* (2011). Prenatal exposure to organophosphate pesticides and IQ in 7-year-old children. Environ Health Perspect, 119(8), 1189-1195.

Braquenier J-B, Quertemont E, Tirelli E, Plumier J-C. Anxiety in adult female mice following perinatal exposure to chlorpyrifos. 2010. Neurotoxicol Teratol. 32:234-239, 2010

Carr RL, Armstrong NH, Buchanan AT, Eells JB, Mohammed AN, Ross MK, Nail CA. Decreased anxiety in juvenile rats following exposure to low levels of chlorpyrifos during development. 2015. Neurotoxicol. 2015, http://dx.doi.org/10.1016/j.neuro.2015.11.016,

Cartier, C., Warembourg, C., Le Maner-Idrissi, G., Lacroix, A., Rouget, F., Monfort, C., ... & Chevrier, C. (2016). Organophosphate Insecticide Metabolites in Prenatal and Childhood Urine Samples and Intelligence Scores at 6 Years of Age: Results from the Mother–Child PELAGIE Cohort (France). Environmental health perspectives, 124(5), 674.

Ceh, K., Jezek, D., Zuzek, M.C., Cebulj-Kadunc, N., Majdic, G., 2012. Effects of organophosphate chlormephos on development and function of testes and brain. Slovenian Veterinary Research **49**, 41-49.

Chen L, Zhao T, Pan C, Ross JH, Krieger RI. (2012) Preformed biomarkers including dialkylphosphates (DAPs) in produce may confound biomonitoring in pesticide exposure and risk assessment. J Agric Food Chem. 2012 Sep 12;60(36):9342-51. doi: 10.1021/jf303116p. Epub 2012 Sep 4.

Clemens, G.R., Hartnagel, R.E., Bare, J.J., Thyssen, J.H., 1990. Teratological, neurochemical, and postnatal neurobehavioral assessment of METASYSTOX-R, an organophosphate pesticide in the rat. Fundam Appl Toxicol **14**, 131-143.

Crowder, L.A., Lanzaro, G.C., Whitson, R.S., 1980. Behavioral effects of methyl parathion and toxaphene exposure in rats. J Environ Sci Health B **15**, 365-378.

Dąbrowski, Sławomir, Hanke, Wojciech, Polańska, Kinga, Makowiec-Dąbrowska, Teresa, and Sobala, Wojciech. Pesticide exposure and birthweight: An epidemiological study in Central Poland. International Journal of Occupational Medicine and Environmental Health 16(1): 31-39, 2003.

de Castro, V.L., Chiorato, S.H., Pinto, N.F., 2001. Relevance of developmental testing of exposure to methamidophos during gestation to its toxicology evaluation. Toxicol Lett **118**, 93-102.

Driver JH, Li Y, Ross JH, Krieger RI. Dialkylphosphates (DAPs) in fruits and vegetables

ED_002061_00044425-00095

may confound biomonitoring in organophosphorus insecticide exposure and risk assessment. J Agric Food Chem. 2008 Nov 26;56(22):10638-45. doi: 10.1021/jf8018084. [1],

Donauer, S., Altaye, M., Xu, Y., Sucharew, H., Succop, P., Calafat, A. M., ... & Yolton, K. (2016). An Observational Study to Evaluate Associations Between Low-Level Gestational Exposure to Organophosphate Pesticides and Cognition During Early Childhood. American Journal of Epidemiology, 184(5), 410-418.

Engel SM, Berkowitz GS, Barr DB, Teitelbaum SL, Siskind J, Meisel SJ, Wetmur JG, Wolff MS. Prenatal Organophosphate Metabolite and Organochlorine Levels and Performance on the Brazelton Neonatal Behavioral Assessment Scale in a Multiethnic Pregnancy Cohort. American Journal of Epidemiology. 2007;165:1397-1404.

Engel SM, Wetmur J, Chen J, Zhu C, Barr DB, Canfield RL, Wolff MS. Prenatal Exposure to Organophosphates, Paraoxonase 1, and Cognitive Development in Childhood. Environmental Health Perspectives. 2011;119:1182-1188.

Engel SM, Bradman A, Wolff MS, Rauh VA, Harley KG, Yang JH, Hoepner LA, Barr DB, Yolton K, Vedar MG, Xu Y, Hornung RW, Wetmur JG, Chen J, Holland NT, Perera FP, Whyatt RM, Lanphear BP, Eskenazi B. 2015. Prenatal organophosphorus pesticide exposure and child neurodevelopment at 24 months: an analysis of four birth cohorts. Environ Health Perspect 124:822-830; http://dx.doi.org/10.1289/ehp.1409474.

Eskenazi B, Harley K, Bradman A, Weltzien E, Jewell NP, Barr DB, Furlong CE, Holland NT. Association of in Utero Organophosphate Pesticide Exposure and Fetal Growth and Length of Gestation in an Agricultural Population. Environmental Health Perspectives. 2004;115:792–798.

Eskenazi B, Marks AR, Bradman A, Harley K, Barr DB, Johnson C, Morga N, Jewell NP. Organophosphate Pesticide Exposure and Neurodevelopment in Young Mexican-American Children. Environmental Health Perspectives. 2007;115:792–798.

Eskenazi, B., Huen, K., Marks, A., Harley, K. G., Bradman, A., Barr, D. B., & Holland, N. (2010). PON1 and neurodevelopment in children from the CHAMACOS study exposed to organophosphate pesticides in utero. Environ Health Perspect, 118(12), 1775-1781.

Fiedler, N., Rohitrattana, J., Siriwong, W., Suttiwan, P., Strickland, P. O., Ryan, P. B., ... & Robson, M. G. (2015). Neurobehavioral effects of exposure to organophosphates and pyrethroid pesticides among Thai children. Neurotoxicology, 48, 90-99.

ED_002061_00044425-00096

FIFRA Scientific Advisory Panel. (2008b). "The Agency's Evaluation of the Toxicity Profile of Chlorpyrifos." Report from the FIFRA Scientific Advisory Panel Meeting of September, 2008. FIFRA Scientific Advisory Panel, Office of Science Coordination and Policy, Office of Prevention, Pesticides and Toxic Substances, U.S. Environmental Protection Agency. Washington, DC. Available: http://www.epa.gov/scipoly/sap/meetings/2008/index.html

FIFRA Scientific Advisory Panel. (2010). February 2 - 4, 2010: Incorporation of Epidemiology and Human Incident Data into Human Risk Assessment.

FIFRA Scientific Advisory Panel. (2012). Transmittal of the Meeting Minutes from the FIFRA Scientific Advisory Panel Meeting of April 10-12, 2012. FIFRA Scientific Advisory Panel, Office of Science Coordination and Policy, Office of Prevention, Pesticides and Toxic Substances, U.S. Environmental Protection Agency. Washington, DC. Available: http://www.epa.gov/scipoly/sap/meetings/2012/041012meeting.html

Fortenberry G.Z., Meeker J.D., Sanchez B.N., *et al.*, (2014). Urinary 3,5,6-tichloro-2-pyridinol (TCPY) in pregnant women from Mexico City: Distribution, temporal variability, and relationship with child attention and hyperactivity. International Journal of Hygiene and Environmental Health 217 (2014) 405-412.

Fortenberry, GZ, Meeker, JD, Sánchez, BN, Barr, DB, Peterson, K, Bellinger, D, Schnaas, L, Solano-González, M, Ettinger, AS, Hernandez-Avila, M, Hu, H, Tellez-Rojoj, MM. Paraoxonase I polymorphisms and attention/hyperactivity in school-age children from Mexico City, Mexico. (2014a.) Environmental Research 132:342–349.

Furlong, Melissa A., Engel, Stephanie M., Boyd Barr, Dana, Wolff, Mary S. Prenatal exposure to organophosphate pesticides and reciprocal social behavior in childhood. 2014. Environment International 70:125-131.

Grandjean, Philippe, Harari, Raul, Barr, Dana B., Debes, Frodi. Pesticide exposure and stunting as independent predictors of neurobehavioral deficits in Ecuadorian school children. 2006. Pediatrics 117(3):e546-e556.

Guodong D., Pei W., Ying T., Jun Z., *et al.*, (2012). Organophosphate Pesticide Exposure and Neurodevelopment in Young Shanghai Children. Environ Sci. Technol. 2012, 46, 2911-2917.

Gupta, R.C., Rech, R.H., Lovell, K.L., Welsch, F., Thornburg, J.E., 1985. Brain cholinergic, behavioral, and morphological development in rats exposed in utero to methylparathion. Toxicol Appl Pharmacol **77**, 405-413.

Handal A.J., Lozoff B., Breilh J., Harlow S.D., (2007). Effect of community of residence on neurobehavioral development in infants and young children in a flower-growing region of Ecuador. Environ Health Perspect. 2007 Jan;115(1): 128-33.

Handal A.J., Lozoff B., Breilh J., Harlow S.D., (2007B). Neurobehavioral Development in Children with Potential Exposure to Pesticides. Epidemiology May 2007; 18: 312-320.

ED_002061_00044425-00097

Handal A.J., Lozoff B., Breilh J., Harlow S.D., (2008). Occupational Exposure to Pesticides During Pregnancy and Neurobehavioral Development of Infants and Toddlers. Epidemiology, Volume 19, number 6, November 2008.

Harari, Raul, Julvez, Jordi, Murata, Katsuyuki, Barr, Dana, Bellinger, David C., Debes, Frodi, and Grandjean, Philippe. Neurobehavioral Deficits and Increased Blood Pressure in School-Age Children Prenatally Exposed to Pesticides. 2010. Environmental Health Perspectives 118(6): 890-896.

Harley KG, Huen K, Schall RA, Holland NT, Bradman A, *et al*. Association of Organophosphate Pesticide Exposure and Paraoxonase with Birth Outcome in Mexican-American Women. PLoS ONE 2011; 6(8)1-10.

Harley KG, Engel SM, Vedar MG, Eskenazi B, Whyatt RM, Lanphear BP, Bradman A, Rauh VA, Yolton K, Hornung RW, Wetmur JG, Chen J, Holland NT, Barr DB, Perera FP, Wolff MS. 2016. Prenatal exposure to organophosphorous pesticides and fetal growth: pooled results from four longitudinal birth cohort studies. Environ Health Perspect 124:1084-1092; http://dx.doi.org/10.1289/ehp.1409362.

Hill AB (1965). The Environment and Disease: Association or Causation? Proc R Soc Med. May 1965; 58(5): 295–300.

Howard, A. S., Bucelli, R., Jett, D. A., Bruun, D., Yang, D., & Lein, P. J. 2005. Chlorpyrifos exerts opposing effects on axonal and dendritic growth in primary neuronal cultures. Toxicol Appl Pharmacol, 207(2), 112-124.

Icenogle, L. M., Christopher, N. C., Blackwelder, W. P., Caldwell, D. P., Qiao, D., Seidler, F. J., et al. (2004). Behavioral alterations in adolescent and adult rats caused by a brief subtoxic exposure to chlorpyrifos during neurulation. Neurotoxicol Teratol, 26(1), 95–101.

Johnson, F.O., Chambers, J.E., Nail, C.A., Givaruangsawat, S., Carr, R.L., 2009. Developmental chlorpyrifos and methyl parathion exposure alters radial-arm maze performance in juvenile and adult rats. Toxicol Sci **109**, 132-142.

Kofman, Ora, Berger, Andrea, Massarwa, Ali, Friedman, Alon, and Abu Jaffar, Abed. Motor Inhibition and Learning Impairments in School-Aged Children Following Exposure to Organophosphate Pesticides in Infancy. Pediatric Research. 60:88-92, 2006.

Koutroulakis, D., Sifakis, S., Tzatzarakis, M.N., Alegakis, A.K., Theodoropoulou, E., Kavvalakis, M.P., Kappou, D., Tsatsakis, A.M. Dialkyl phosphates in amniotic fluid as a biomarker of fetal exposure to organophosphates in Crete, Greece; association with fetal growth. Reproductive Toxicology 46 (2014) 98–105.

Lazarini, C.A., Lima, R.Y., Guedes, A.P., Bernardi, M.M., 2004. Prenatal exposure to dichlorvos: physical and behavioral effects on rat offspring. Neurotoxicol Teratol **26**, 607-614.

Lehotzky, K., Szeberenyi, M.J., Kiss, A., 1989. Behavioral consequences of prenatal

ED_002061_00044425-00098

exposure to the organophosphate insecticide sumithion. Neurotoxicol Teratol **11**, 321-324.

Levin, E. D., Addy, N., Nakajima, A., Christopher, N. C., Seidler, F. J., & Slotkin, T. A. (2001). Persistent behavioral consequences of neonatal chlorpyrifos exposure in rats. Brain Res Dev Brain Res, 130(1), 83–89.

Levin, E. D., Addy, N., Baruah, A., Elias, A., Christopher, N. C., Seidler, F. J., et al. (2002). Prenatal chlorpyrifos exposure in rats causes persistent behavioral alterations. Neurotoxicol Teratol, 24(6), 733–741.

Levin, E.D., Timofeeva, O.A., Yang, L., Petro, A., Ryde, I.T., Wrench, N., Seidler, F.J., Slotkin, T.A., 2009. Early postnatal parathion exposure in rats causes sex-selective cognitive impairment and neurotransmitter defects which emerge in aging. Behav Brain Res **208**, 319-327.

Lima, C.S., Dutra-Tavares, A.C., Nunes, F., Nunes-Freitas, A.L., Ribeiro-Carvalho, A., Filgueiras, C.C., Manhaes, A.C., Meyer, A., Abreu-Villaca, Y., 2013. Methamidophos exposure during the early postnatal period of mice: immediate and late-emergent effects on the cholinergic and serotonergic systems and behavior. Toxicol Sci **134**, 125-139.

Lizardi, Patricia Sanchez, O'Rourke, Mary Kay, and Morris, Richard J. The effects of organophosphate pesticide exposure on Hispanic children's cognitive and behavioral functioning. Journal of Pediatric Psychology 33(1): 91–101, 2008.

Llop S., Julvez J., Marina L.S., Vizcaino E., *et al.*, (2013). Prenatal and postnatal insecticide use and infant neuropsychological development in a multicenter birth cohort study. Environment International 59(2013) 175-182.

Lovasi GS, Quinn JW, Rauh VA, Perera FP, Andrews HF, Garfinkel R, Hoepner L, Whyatt R, Rundle A. Chlorpyrifos Exposure and Urban Residential Environment Characteristics as Determinants of Early Childhood Neurodevelopment. American Journal of Public Health. 2011;101(1):63-70. doi: 10.2105/AJPH.2009.168419. Epub 2010 Mar 18.

Lu, *et al*. Assessing the association between pesticide exposure and cognitive development in rural Costa Rican children living in organic and conventional coffee farms. Int J Adolesc Med Health. 2009; 21(4): 609-621

Mamczarz J, Pescrille JD, Gavrushenko L, Burke RD, Fawcett WP, DeTolla Jr, LJ, Chen H, Pereira EFR, Albuquerque EX. 2016. Spatial learning impairment in prepubertal guinea pigs prenatally exposed to the organophosphorus pesticide chlorpyrifos: Toxicological implications. Neurotoxicol. 56:17-28, 2016

Marks AR, Harley K, Bradman A, Kogut K, Barr DB, Johnson C, *et al*. Organophosphate Pesticide Exposure and Attention in Young Mexican-American Children: The CHAMACOS Study. Environmental Health Perspectives. 2010;118:1768-1774.

Meek ME, Boobis A, Cote I, Dellarco V, Fotakis G, Munn S, Seed J, and Vickers, C. 2014.

ED_002061_00044425-00099

New developments in the evolution and application of the WHO/IPCS framework on mode of action/species concordance analysis. J Appl Toxicol. Jan;34(1):1-18.

Meek ME, Boobis A, Cote I, Dellarco V, Fotakis G, Munn S, Seed J, Vickers C. 2014. New developments in the evolution and application of the WHO/IPCS framework on mode of action/species concordance analysis. *J Appl Toxicol.* 2014 Jan;34(1):1-18.

Moreno-Banda, G., Blanco-Muñoz, J., Lacasaña, M., Rothenberg, S.J., Aguilar-Garduño, C., Gamboa, R., Pérez-Méndez, O. Maternal exposure to floricultural work during pregnancy, PON1 Q192R polymorphisms and the risk of low birth weight. Science of the Total Environment 407: 5478–5485, 2009.

Muñoz-Quezada MT, Lucero BA, Barr DB, Steenland K, Levy K, Ryan PB, Veronica Iglesiase V, Alvaradoe S, Conchaf C, Rojas E, Catalina Vega C. Neurodevelopmental effects in children associated with exposure to organophosphate pesticides: A systematic review. Neurotoxicology 39: 158-168.

Naksen W, Prapamontol T, Mangklabruks A, Chantara S, Thavornyutikarn P, Srinual N, Panuwet P, Ryan PB, Riederer AM, Barr DB. Associations of maternal organophosphate pesticide exposure and PON1 activity with birth outcomes in SAWASDEE birth cohort, Thailand. Environmental Research142: 288–296, 2015.

Nevison. A comparison of temporal trends in United States autism prevalence to trends in suspected environmental factors. Environmental Health. 2014; 13(73): 16 pp

Oulhote, Youssef and Bouchard, Maryse F. (2013) Urinary Metabolites of Organophosphate and Pyrethroid Pesticides and Behavioral Problems in Canadian Children. Environmental Health Perspectives. 121:1378-1384

Petit, Claire, Chevrier, Cécile, Durand, Gaël, Monfort, Christine, Rouget, Florence, Garlantezec, Ronan, Cordier, Sylvaine. Impact on fetal growth of prenatal exposure to pesticides due to agricultural activities: a prospective cohort study in Brittany, France. Environmental Health 9:71-83, 2010.

Quiros-Alcala L., Alkom A.D., Boyce W.T., Lippert S., *et al.*, (2011). Maternal prenatal and child organophosphate pesticide exposures and children's autonomic function. NeuroToxicology 32 (2011) 646-655.

Racke J.D. Degradation of organophosphorous insecticidesin environmental matrices. In: Organophosphates Chemistry, Fate and Effects. Academic Press, Inc., San Diego, CA, 1992, pp 47–72.

Raanan R, Harley KG, Balmes JR, Bradman A, Lipsett M, and Eskenazi B. (2015) Early-life Exposure to Organophosphate Pesticides and Pediatric Respiratory Symptoms in the CHAMACOS Cohort. Environmental Health Perspectives: 123(2), 179-185.

Rauch, Stephen A., Braun, Joe M., Boyd Barr, Dana, Calafat, Antonia M., Khoury, Jane, M., Montesano, Angela, Yolton, Kimberly, and Lanphear, Bruce P. Associations of Prenatal Exposure to Organophosphate Pesticide Metabolites with Gestational Age and Birth Weight. Environmental Health Perspectives. 120:1055–1060 (2012).

ED_002061_00044425-00100

Rauh VA, Garfinkel R, Perera FP, Andrews HF, Hoepner L, Barr DB, Whitehead R, Tang D, Whyatt RW. Impact of Prenatal Chlorpyrifos Exposure on Neurodevelopment in the First 3 Years of Life Among Inner-City Children. Pediatrics. 2006;18(6): 1835-1859.

Rauh V, Arunajadai S, Horton M, Perera F, Hoepner L, Barr DB, *et al*. 2011. Seven-Year Neurodevelopmental Scores and Prenatal Exposure to Chlorpyrifos, a Common Agricultural Pesticide. Environmental Health Perspectives. 2011;119:1196-1201.

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 Natl Acad Sci U S A, 109(20), 7871-7876.

Rauh VA.; Garcia WE.; Whyatt RM.; et al. (2015) Prenatal exposure to the organophosphate pesticide chlorpyrifos and childhood tremor. Neurotoxicology: 51, 80-86.

Roegge, C.S., Timofeeva, O.A., Seidler, F.J., Slotkin, T.A., Levin, E.D., 2007. Developmental diazinon neurotoxicity in rats: later effects on emotional response. Brain Res Bull **75**, 166-172.

Rohlman, Diane S., Arcury, Thomas A., Quandt, Sara A., Lasarev, Michael, Rothlein, Joan, Travers, Rachelle, Tamulinas, Alys, Scherer, Jennifer, Early, Julie, Marín, Antonio, Phillips, Jacki, McCauley, Linda. Neurobehavioral performance in preschool children from agricultural and non-agricultural communities in Oregon and North Carolina. Neurotoxicology 26:895-598, 2005.

Ruckart, *et al*. Long-Term Neurobehavioral Health Effects of Methyl Parathion Exposure in Children in Mississippi and Ohio. Environmental Health Perspectives. 2004; 112(1):46-51.

Samarawickrema, Nirma, Pathmeswaran, Arunasalam, Wickremasinghe, Rajitha, Peiris-John, Roshini, Karunaratna, Madawa, Buckley, Nick, Dawson, Andrew, and De Silva, Janaka. Fetal effects of environmental exposure of pregnant women to organophosphorus compounds in a rural farming community in Sri Lanka. Clinical Toxicology 46:489–495, 2008.

Savitz, David A., Arbuckle, Tye, Kaczor, Diane, and Curtis, Kathryn M. Male pesticide exposure and pregnancy outcome. American Journal of Epidemiology 146(12):1025-1036, 1997.

Schuh RA, Lein PJ, Beckles RA, Jett DA. 2002. Noncholinesterase mechanisms of chlorpyrifos neurotoxicity: altered phosphorylation of Ca2+/cAMP response element binding protein in cultured neurons. Toxicol Appl Pharmacol. 2002 Jul 15;182(2):176-85

Shelton, Janie F., Geraghty, Estella M., Tancredi, Daniel J., Delwiche, Lora D., Schmidt, Rebecca J., Ritz, Beate, Hansen, Robin L., and Hertz-Picciotto, Irva. Neurodevelopmental disorders and prenatal residential proximity to agricultural pesticides: The CHARGE Study. Environmental Health Perspectives 122:1103–1109,

ED_002061_00044425-00101

2014.

Simon TW, Simons SS Jr, Preston RJ, Boobis AR, Cohen SM, Doerrer NG, Fenner-Crisp PA, McMullin TS, McQueen CA, Rowlands JC; RISK21 Dose-Response Subteam. 2014. The use of mode of action information in risk assessment: Quantitative key events/dose-response framework for modeling the dose-response for key events. *Crit Rev Toxicol.* 2014 Aug;44 Suppl 3:17-43.

Slotkin, T.A., Tate, C.A., Ryde, I.T., Levin, E.D., Seidler, F.J. 2006. Organophosphate insecticides target the serotonergic system in developing rat brain regions: Disparate effects of diazinon and parathion at doses spanning the threshold for cholinesterase inhibition. Environ Health Perspec **114**, 1542-1546.

Sochaski, M.A., McManus, B.M., Struve, M.F., Wallace, D.G., Dorman, D.C. 2007. Inhibition and recovery of maternal and foetal cholinesterase enzymes following fenitrothion administration in CD rats. Xenobiotica **37**, 19-29.

Sonich-Mullin C, Fielder R, Wiltse J, et al. 2001. IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. Regul Toxicol Pharmacol. 34:146-152.

Spyker, J.M., Avery, D.L., 1977. Neurobehavioral effects of prenatal exposure to the organophosphate Diazinon in mice. J Toxicol Environ Health **3**, 989-1002.

Stamper, C.R., Balduini, W., Murphy, S.D., Costa, L.G., 1988. Behavioral and biochemical effects of postnatal parathion exposure in the rat. Neurotoxicol Teratol **10**, 261-266.

Stein, L. J., Gunier, R. B., Harley, K., Kogut, K., Bradman, A., & Eskenazi, B. (2016). Early childhood adversity potentiates the adverse association between prenatal organophosphate pesticide exposure and child IQ: The CHAMACOS cohort. NeuroToxicology, 56, 180-187.

Suarez-Lopez J. R., Jacobs D.R., Himes J.H., Alexander B.H., Lazovich D., *et al.*, (2012). Lower acetylcholinesterase activity among children living with flower plantation workers. Environmental Research 114 (2012) 53-59.

Suarez-Lopez J. R., Jacobs D.R., Himes J.H., Alexander B.H., (2013). Aectylcholinesterase activity and neurodevelopment in boys and girls. PEDIATRICS Volume 132, number 6, December 2013.

Suarez-Lopez J. R., Jacobs D.R., Himes J.H., Alexander B.H., (2013a). Acetycholinesterase activity, cohabitation with floricultural workers, and blood pressure in Ecuadorian Children. Environmental Health Perspectives, volume 121, number 5, May 2013.

Timofeeva, O.A., Roegge, C.S., Seidler, F.J., Slotkin, T.A., Levin, E.D., 2008a. Persistent cognitive alterations in rats after early postnatal exposure to low doses of the organophosphate pesticide, diazinon. Neurotoxicol Teratol **30**, 38-45.

Timofeeva, O.A., Sanders, D., Seemann, K., Yang, L., Hermanson, D., Regenbogen, S., Agoos, S., Kallepalli, A., Rastogi, A., Braddy, D., Wells, C., Perraut, C., Seidler, F.J.,

ED_002061_00044425-00102

Slotkin, T.A., Levin, E.D., 2008b. Persistent behavioral alterations in rats neonatally exposed to low doses of the organophosphate pesticide, parathion. Brain Res Bull **77**, 404-411.

Turgeman, G., Pinkas, A., Slotkin, T. A., Tfilin, M., Langford, R., & Yanai, J. (2011). Reversal of chlorpyrifos neurobehavioral teratogenicity in mice by allographic transplantation of adult subventricular zone-derived neural stem cells. J Neurosci Res, 89(8), 1185–1193.

U.S. Environmental Protection Agency. (1999). Guidelines for carcinogen risk assessment. Risk Assessment Forum. SAB review draft. Washington, DC: U.S. Environmental Protection Agency. www.epa.gov/ncea/raf/crasab.htm.

U.S. Environmental Protection Agency. (2002). Determination of The Appropriate FQPA Safety Factor(S) In Tolerance Assessment. Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington, DC, February 28, 2002.

U.S. Environmental Protection Agency. (2005). Guidelines for Carcinogen Risk Assessment. Risk Assessment Forum, U.S. Environmental Protection Agency. Washington, DC. EPA/630/P-03/001F.

U.S. Environmental Protection Agency. (2006). Revised Organophosphorous Pesticide Cumulative Risk Assessment; July 31, 2006. Office of Pesticide Programs, U.S. Environmental Protection Agency. Washington, D.C. Available http://www.epa.gov/pesticides/cumulative/rra-op.

U.S. Environmental Protection Agency. (2008). Draft Science Issue Paper: Chlorpyrifos Hazard and Dose Response Characterization. http://www.epa.gov/scipoly/sap/meetings/2008/091608_mtg.htm

U.S. Environmental Protection Agency. (2010). Draft Framework for Incorporating Human Epidemiologic and Incident Data in Health Risk Assessment, January 7, 2010.

U.S. Environmental Protection Agency. (2011a). Chlorpyrifos: Preliminary Human Health Risk Assessment for Registration Review. June 30, 2011. D388070.

U.S. Environmental Protection Agency. (2011b). Chlorpyrifos: Occupational and Residential Exposure Assessment, EPA Barcode: D388165,. In Office of Pesticide Programs (Ed.). Washington D.C.

U.S. Environmental Protection Agency. (2012). Draft Issue Paper: Scientific Issues Concerning Health Effects of Chlorpyrifos. http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2012-0040-0002

U.S. Environmental Protection Agency. (2014). Chlorpyrifos: Revised Human Health Risk Assessment for Registration Review. December 29, 2014. D424485.

U.S. Environmental Protection Agency. (2015) Literature Review on Neurodevelopment Effects & FQPA Safety Factor Determination for the Organophosphate Pesticides. September 15, 2015.

U.S. Environmental Protection Agency. 2015b. Swe, K. and Holman, E. Summary Reviews from _2015 Epidemiological Literature Review for Organophosphates. September 15, 2015.

U.S. EPA (U.S. Environmental Protection Agency). 2015c. Preamble to the Integrated Science Assessments. National Center for Environmental Assessment, RTP Division, Office of Research and Development, USEPA. https://yosemite.epa.gov/sab/sabproduct.nsf/0/33E1AD305287588F85257D20006BE8CC/$File/ISA_PREAMBLE_FINAL2015.PDF

U.S. Environmental Protection Agency. 2016. Holman, E. and Swe, K. Summary Reviews for Additional Epidemiological Literature Studies from Prospective Birth Cohort Studies. D432184. March 25, 2016

U.S. Environmental Protection Agency. 2016b. Aldridge, A. Summary Reviews for Additional Epidemiological Literature Studies from Prospective Birth Cohort Studies. D432184. December XXX, 2016

U.S. Environmental Protection Agency. 2016c. Framework for Incorporating Human Epidemiologic and Incident Data in Health Risk Assessment, December 28, 2016.

Vatanparast, J., Naseh, M., Baniasadi, M., Haghdoost-Yazdi, H., 2012. 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.

Venerosi A, Tait S, Stecca L, Chiarotti F, DeFelice A, Cometa MF, Volpe MT, Calamandrei G, Ricceri L. Effects of maternal chlorpyrifos diet on social investigation and brain neuroendocrine markers in the offspring – a mouse study. Environ. Health 14:32-42, 2015

Wang, Pei, Tian, Ying, Wang, Xiao-Jin, Gao, Yu, Shi, Rong, Wang, Guo-Quan, Hu, Guo-Hua, Shen, Xiao-Ming. Organophosphate pesticide exposure and perinatal outcomes in Shanghai, China. 4 Environment International 2:100–104, 2012.

Whyatt, R. M., Barr, D. B., Camann, D. E., Kinney, P. L., Barr, J. R., Andrews, H. F., . . . Perera, F. P. (2003). Contemporary-use pesticides in personal air samples during pregnancy and blood samples at delivery among urban minority mothers and newborns. Environ Health Perspect, 111(5), 749-756.

Whyatt RM, Rauh V, Barr DB, Camann DE, Andrews HF, Garfinkel R, Hoepner LA, Diaz D, Dietrich J, Reyes A, Tang D, Kinney PL, Perera FP. Prenatal Insecticide Exposures and Birth Weight and Length among an Urban Minority Cohort. Environmental Health Perspectives. 2004;112:1125–1132.

Whyatt RM, Garfinkel R, Hoepner LA, Holmes D, Borjas M, Williams MK, *et al.* Within- and Between-Home Variability in Indoor-Air Insecticide Levels during Pregnancy among an Inner-City Cohort from New York City. Environmental Health Perspectives 2007;115:383-389.

Whyatt RM, Garfinkel R, Hoepner LA, Andrews H, Holmes D, Williams MK, *et al*. A Biomarker Validation Study of Prenatal Chlorpyrifos Exposure within an Inner-City Cohort during Pregnancy. Environmental Health Perspectives. 2009;117:559-567.

Wickerham, Erin L., Lozoff, Betsy, Shao, Jie, Kaciroti, Niko, Xia, Yankai, Meeker, John D. Reduced birth weight in relation to pesticide mixtures detected in cord blood of full-term infants. Environment International 47:80–85, 2012.

Win-Shwe, T.T., Nakajima, D., Ahmed, S., Fujimaki, H., 2012. Impairment of novel object recognition in adulthood after neonatal exposure to diazinon. Arch Toxicol **87**, 753-762.

Wolff, MS, Engel, S, Berkowitz, G, Teitelbaum, S, Siskind, J, Barr, DB, Wetmur, J. Prenatal Pesticide and PCB Exposures and Birth Outcomes. 2007. Pediatric Research 61(2):243-250.

Yang, D., Howard, A., Bruun, D., Ajua-Alemanj, M., Pickart, C., & Lein, P. J. 2008. Chlorpyrifos and chlorpyrifos-oxon inhibit axonal growth by interfering with the morphogenic activity of acetylcholinesterase. Toxicol Appl Pharmacol, 228(1), 32-41.

Yolton, K., Xu, Y., Sucharew, H., Succop, P., Altaye, M., Popelar, A., ... & Khoury, J. C. (2013). Impact of low-level gestational exposure to organophosphate pesticides on neurobehavior in early infancy: a prospective study. Environmental health, 12(1), 1.

Young JG, Eskenazi B, Gladstone EA, Bradman A, Pedersen L, Johnson C, Barr DB, Furlong CE, Holland NT. Association Between In Utero Organophosphate Pesticide Exposure and Abnormal Reflexes in Neonates. NeuroToxicology. 2005;26:199–209.

Zhang Y., Han S., Liang D., Shi X., Wang F., *et al*., (2014). Prenatal exposure to organophosphate pesticides and neurobehavioral development of neonates: A birth cohort study in Shenyang, China. Plos ONE 9(2):e88491. doi:10.1371/journal.pone.0088491.

Zhang X, Driver JH, Li Y, Ross JH, Krieger RI. (2008) Dialkylphosphates (DAPs) in fruits and vegetables may confound biomonitoring in organophosphorus insecticide exposure and risk assessment. J Agric Food Chem. 2008 Nov 26;56(22):10638-45. doi: 10.1021/jf8018084.

ED_002061_00044425-00105

6.0 Appendices

1. Table of *In Vivo* Developmental Neurotoxicity Studies of OPs.
2. Summary of Guideline DNT Studies Submitted to the Agency for OPs other than Chlorpyrifos.
3. Low Quality Studies: Summary of Study Design Elements Impacting Study Quality Assignment
4. Table of Systematic Review Analysis: Initial Search Research & Initial Exclusion Criteria
5. Table of Systematic Review Analysis: Second Tier Exclusion Criteria
6. Plausible hypotheses on MOA/AOP for neurodevelopmental outcomes (Extracted from Section 4.4.3. RHHRA for chlorpyrifos)

ED_002061_00044425-00106

Appendix 1. Table of *In Vivo* Developmental Neurotoxicity Studies of OPs.

(Effects described are only those measured after weaning. Bold indicates functional domains that were reported to show treatment effects.)

OP	Study	Species & strain	Dose, route, vehicle	Dosing period	ChE inhibition	Domain	Age of testing	Outcomes	NOEL, LOEL	Notes & Study Problems
Chlormephos	Ceh *et al.*, 2012	mouse BALB/c	3.5, 0.35 ug/ml in drinking water of dams ~ 0.6, 0.06 mg/kg/d (@ 5ml/d, 30 g)	7 day premating to weaning	No	**Anxiety & Emotion**	PND70-80	Increased time in closed arms & decreased time in open arms in elevated plus maze, ~0.6 mg/kg/d, M&F	NOEL=0.35 ug/ml in water, ~0.06 mg/kg/d	Litter not unit of statistical analysis No pup allocation described but had to have used some littermates M & F responses appear similar but not statistically compared
Diazinon	Roegge *et al.*, 2008	rat Sprague Dawley	0.5, 2 mg/kg/d sc DMSO	PND1-4	Cites Slotkin *et al.* (2006): 0.5 mg/kg/d produced <10% brain inhibition on PND5, 2 mg/kg/d produced 25-30% brain inhibition 2 hr after dose on PND4, and 10-20% inhibition on PND5	**Anxiety & Emotion**	PND52-56	Decreased time in open arms in elevated plus maze, 2 mg/kg/d, M only	No NOEL LOEL=0.5 mg/kg/d	Pups & dams redistributed daily No effect in F Accepts p<0.1 as significant for interactions No dose-response in feeding or milk preference studies Abstract misstates sex differences
							PND64-67, 78-79	Decreased latency to eat in novelty suppressed but not home-cage feeding, 0.5 & 2 mg/kg/d, M only		
							PND73-74	Decreased chocolate milk preference, 0.5 mg/kg/d only, M only		
							PND86-87	No effect on forced swim test		
Diazinon	Spyker and Avery, 1977	mouse F2 hybrid (NCTR cross bred)	0.18, 9 mg/kg/d in peanut butter	GD1-birth	No	**Sensory**	PND38	Increased errors on visual cliff, 0.18 mg/kg/d only, F only No effect acoustic startle or olfactory responses	No NOEL LOEL=0.18 mg/kg/d	No pup allocation described but had to have used littermates Not clear when both sexes tested and/or compared Statistics not described Maternal weight gain lowered at both doses Weight gain of high dose pups decreased Preweaning testing: decreased contact placing, 0.18 mg/kg/d only No dose-response for some measures Twice as many controls as treated Looks like decreased rotarod endurance PND65, not significant due to high variability
						Neuromotor	PND50, 60, 65,75	No effect swimming ability Increased rod cling endurance, 0.18 & 9 mg/kg/d, sex not specified Decreased inclined plane performance, 0.18 & 9 mg/kg/d, sex not specified		
						Activity	PND75-76	No effect open field		
						Cognition	PND87	No effect errors in Lashley maze		
Diazinon	Timofeeva *et al.*, 2008	rat Sprague Dawley	0.5, 2 mg/kg/d sc DMSO	PND1-4	Cites Slotkin *et al.* (2006): 0.5 mg/kg/d produced <10% brain inhibition on PND5, 2 mg/kg/d produced	Activity	PND28-42	No effect in figure-8 chamber, M&F	No NOEL LOEL=0.5 mg/kg/d	Littermates of those used in Roegge *et al.* 2008 Pups & dams redistributed daily Accepts p<0.1 as significant for interactions
						Sensory	PND77-84	Decreased prepulse inhibition, 0.5 & 2 mg/kg/d, M only		

ED_002061_00044425-00107

					25-30% brain inhibition 2 hr after dose on PND4, and 10-20% inhibition on PND5	**Cognition**	PND28-35, 91-126	No effect on T-maze spontaneous alternation, M&F Increased working memory errors, 0.5 mg/kg/d only, M&F		No mention of sex effects in T-maze No dose-response for some measures
Diazinon	Vatanparast *et al.*, 2013	rat Wistar	1 mg/kg/d sc DMSO	GD15-18 or PND1-4	No	Activity	PND60	Gestational: No effect in open field, M&F Postnatal: No effect in open field, M&F	No NOEL LOEL=1 mg/kg/d	1 M & 1 F per litter but sex not nested within litter in statistics F only affected with gestational exposure, both sexes affected with postnatal, looks like M more affected Large effect sizes Number of dams not mentioned Discrepancy in text on pup sample sizes
						Cognition	PND60-63	Gestational: Decreased latency to cross and increased time spent in dark side on retention trial, no effect acquisition in passive avoidance, F only Postnatal: Decreased latency to cross and increased time spent in dark side on retention trial, no effect acquisition in passive avoidance, M&F		
Diazinon	Win-Shwe *et al.*, 2013	mouse C3H/HeN	0.5, 5 mg/kg/d sc DMSO	PND8-11	No	**Cognition**	PND46-49, 81-84	PND46-49: Decreased novel object exploration and discrimination, 0.5 & 5 mg/kg/d PND81-84: Decreased novel object exploration and discrimination, 5 mg/kg/d only	No NOEL LOEL=0.5 mg/kg/d	M only tested Separate mice at two test times Litter allocation to dose group not described Assumes that ChE inhibition reported by Slotkin in rats would be same as in mice Larger sample size at later age
Dichlorvos	Lazarini *et al.*, 2004	rat Wistar	8 mg/kg po (dilution of technical product); vehicle from formulation	GD6-15	No	**Activity**	PND21, "adult"	PND21: Decreased locomotion open field, M only "Adult": Decreased locomotion and increased immobility, only M tested	No NOEL LOEL=8 mg/kg/d	Data analyzed as litter but sex not nested within litter in statistics No effect on physical and reflex preweaning development Only M tested as after PND21 Adult age not given
						Cognition	"Adult"	Decreased latency to cross on retention trial in passive avoidance		
Fenitrothion (sumithionR, 50% ai)	Lehotzky *et al.*, 1989	rat Lati	5, 10, 15 mg/kg/d po sunflower oil	GD7-15	No	**Neuromotor**	PND26, 36, 104	Decreased latency to fall off rotarod PND26, 104, not 36, 15 mg/kg/d	NOEL=5 mg/kg/d LOEL=10 mg/kg/d	Postnatal mortality at all doses (16-17.5%) No pup allocation described but had to have used some littermates Only M tested Statistics not described Measured startle, righting, contact placing on PND22 but no results given Shorter latency in cognitive task hard to interpret Nonsignificant decrease in activity at PND26
						Activity	PND26, 36, 104	Decreased activity in open field PND104, 15 mg/kg/d		
						Cognition	PND42, 104	Shorter escape latency in conditioned response during acquisition, 10 & 15 mg/kg/d		
						Social behavior	PND62	Increased time in social interaction, 10 & 15 mg/kg/d		

Methamidophos	deCastro *et al.*, 2000	rat Wistar	1 mg/kg/d po water	GD6-15	Pilot in nonpregnant F dosed for 10 d gives 17% plasma inhibition at 1 mg/kg/d	Activity	PND40	No effect in open field, sex no mentioned	NOEL=1 mg/kg/d	Used 2 pups/litter but no mention of sex, apparently used as independent observations No effect on preweaning swimming performance Decreased immobility time PND 14 only Open field measures with really high variability, not reliable
Methamidophos	Lima *et al.*, 2013	mouse Swiss	1 mg/kg/d sc DMSO	PND3-9	Pilot showed for 1 mg/kg/d: ~19% brain inhibition on PND10; ~36%, 46% brain inhibition 1, 4 hr after dosing on PND3; ~53, 61% brain inhibition 1, 4 hr after dosing on PND9; no brain inhibition in PND60 adults	Activity	PND61	No effect in open field, sex not mentioned	No NOEL LOEL=1 mg/kg/d	1 M & 1 F per litter No data for M & F separately or mention of statistical differences Dosing by litter High variability especially with passive avoidance
						Anxiety & Emotion	PND60-61	Increased immobility time in forced swim, sex not mentioned No effect on elevated plus maze, sex not mentioned		
						Cognition	PND63	No effect on passive avoidance, sex not mentioned		
Methyl parathion	Crowder *et al.*, 1980	rat Sprague Dawley	1 mg/kg/d po corn oil	GD7-15	No	**Activity**	PND23, 30, 44, 54, 65	Increased activity in open field, only PND23 and 54, sex not mentioned to criterion	No NOEL LOEL=1 mg/kg/d	Preweaning testing: possibly decreased wire cling time (not analyzed), no effect on righting, startle, placement response Increased postnatal mortality (30%) Littermates used Only 3 litters used Small sample size for maze testing Statistics not mentioned except for maze transfer test, just used t-test Sex not mentioned except for maze transfer test, data not given for M & F Methods & results cursory
						Cognition	>PND68	Slower transfer on 1st, 4th direction change in T-maze learning transfer, sex not mentioned		
Methyl parathion	Gupta *et al.*, 1985	rat Wistar-Furth F mated with F344 M	1 mg/kg/d in peanut butter, 1.5 mg/kg/d po peanut oil	GD6-20	Dams on GD19 show 20, 60% brain inhibition at 1, 1.5 mg/kg/d Pups show brain inhibition up to 50% on PND1, 7, 14, 21, 1 & 1.5 mg/kg/d; on PND28 only 1.5 mg/kg/d	**Cognition**	PND60	No effect on passive avoidance No effect on shuttle box avoidance Slower latency to bar press & increased days to asymptote on operant task (no schedule given), 1 mg/kg/d only, sex not mentioned	No NOEL LOEL=1 mg/kg/d but no effects at 1.5 mg/kg/d	High dose dams had cholinergic signs, increased resorptions Pups moved to foster mothers at 24 hr No effect on preweaning reflexive behaviors Pup allocation not described Statistics barely described M & F apparently tested but data for each not shown or mentioned Methods cursory No dose-response for behavior but there is dose-response for ChE inhibition
						Neuromotor	PND60	No effect on rotarod		
						Activity	PND60	Decreased activity, 1 mg/kg/d only, sex not mentioned		
						Anxiety & Emotion	PND60	Faster cage emergence, 1 mg/kg/d only, sex not mentioned		

						Sensory	PND120	No effect on acoustic startle response		Only 4/dose for operant testing
Methyl parathion	Johnson *et al.*, 2009	rat Sprague Dawley	Incrementing doses: low 0.2 mg/kg/d throughout; mid 0.2, 0.4, 0.6 mg/kg/d every 5-6 d; high 0.3, 0.6, 0.9 mg/kg/d every 5-6 d	PND1-21	Low dose showed 13-15% brain inhibition and high dose showed 63, 20, 18% brain inhibition on PND20, 30, 40; all doses recovered by PND50	**Cognition**	PND29-60	Increased working memory errors, mid and high dose, M only Increased reference memory errors, all doses, M only	No NOEL LOEL=0.2 mg/kg/d	Split-litter dose design Included litter as random effect in statistics No effect on preweaning measures of reflex development No effect in F
Oxydemeton methyl (metasystoxR, 91% ai)	Clemens *et al.*, 1990	rat CD	0.5, 1.5, 4.5 mg/kg/d water	GD6-15	Dams on GD16 show 30, 54, 72% plasma inhibition (RBC similar) & 22, 52, 68% brain inhibition at 0.5, 1.5, 4.5 mg/kg/d Dams on GD20 show 20, 39, 54% brain inhibition at 0.5, 1.5, 4.5 mg/kg/d, 40% RBC inhibition at 4.5 mg/kg/d, and no plasma inhibition Fetuses on GD20 show no brain inhibition	Cognition	PND25, 26, 35	No effect on M-maze	NOEL=4.5 mg/kg/d	High dose dams had tremors 1 M & 1 F per litter No data for M & F separately or mention of statistical differences Statistics barely described No effect on preweaning reflex or sensory tests
						Activity		No effect in open field		
Parathion	Al-Hachim & Fink, 1968	mouse CF1	3 mg/kg/d po Corn oil	3 dosing times: 1st, 2nd, or 3rd trimester	No	Cognition	PND30-37	No effect on conditioned avoidance learning	NOEL=3 mg/kg/d	Very similar experiment as other papers, maybe same study Pup allocation not clear but littermates probably used Inadequate statistics No mention of sex
Parathion	Al-Hachim & Fink, 1968	mouse CF1	3 mg/kg/d po Corn oil	3 dosing times: 1st, 2nd, or 3rd trimester	No	Activity	PND60-66	No effect in open field	NOEL=3 mg/kg/d	Very similar experiment as other papers, maybe same study Pup allocation not clear but littermates probably used Inadequate stats No mention of sex
Parathion	Levin *et al.*, 2010	rat Sprague Dawley	0.1, 0.2 mg/kg/d sc DMSO	PND1-4	Cites Slotkin *et al.* 2006: 0.1 mg/kg/d produced 5-15% brain inhibition on PND5, no data for 0.2 mg/kg/d	**Cognition**	PND420, 510, 570	PND420: Increased working memory errors in radial arm maze, 0.1 mg/kg/d, M only; increased reference memory errors, 0.1 & 0.2 mg/kg/d, M only PND510: Increased working memory errors in radial arm maze, 0.1 & 0.2 mg/kg/d, M only PND570: No effect in radial arm	No NOEL LOEL=0.1 mg/kg/d	Littermates of those used in Timofeeva 2008 Pups & dams redistributed daily 5% mortality high dose No effect in F Accepts p<0.1 as significant for interactions No dose-response for several measures

								maze		
Parathion	Stamper *et al.*, 1988	rat Long Evans	1.3, 1.9 mg/kg/d sc corn oil	PND5-20	35, 68% brn inh PND21; 26, 36% brn in PND28	Activity	PND24	No effect in open field	No NOEL LOEL=1.3 mg/kg/d	Split-litter dose design Pup allocation not clear but littermates probably used High dose produced cholinergic signs, says doses are 33 and 50% of LD50 in PND5 rat Decreased weight gain with both doses Preweaning, increased cliff avoidance latency, no effect righting, negative geotaxis, open field M only tested No post-hoc comparison of groups when significant, but looks like effects in both doses No dose-response in working memory errors
						Neuromotor	PND24	No effect on rotarod		
						Cognition	PND24, PND 35-37	Decreased alternation rate in T-maze, 1.3 & 1.9 mg/kg/d, only M tested Increased working memory errors, 1.3 & 1.9 mg/kg/d, only M tested		
Parathion	Timofeeva *et al.*, 2008	rat Sprague Dawley	0.1. 0.2 mg/kg/d sc DMSO	PND1-4	Cites Slotkin *et al.* 2006: 0.1 mg/kg/d produced 5-15% brain inhibition on PND5, no data for 0.2 mg/kg/d	Activity	PND58-61	No effect in figure-8 chambers	No NOEL LOEL=0.1 mg/kg/d	Pups & dams redistributed daily 5% mortality high dose Accepts p<0.1 as significant for interactions All radial arm maze effects only in low dose
						Cognition	PND35-45 PND112-182	No effect in T-maze spontaneous alternation Decreased working memory errors in radial arm maze, 0.1 mg/kg/d only, M&F		
						Anxiety & Emotion	PND50-53 PND64-72 PND81-94	Increased time in open arms in elevated plus maze, 0.2 mg/kg/d, M&F No effect on novelty suppressed feeding No effect on chocolate milk preference		
						Sensory	PND78-81	Decreased tactile startle, 0.2 mg/kg/d, M&F No effect on prepulse inhibition		

Appendix 2. Summary of Guideline DNT Studies Submitted to the Agency for OPs other than Chlorpyrifos.

(Only changes that were observed after exposure had ended (post weaning, adult) are listed. 'X' indicates no significant changes on tests for each domain.)

Chemicals	Cognition	Motor activity	Acoustic startle	Neuromotor (FOB)	Notes
Acephate	X	X	X	X	
Azinphos-methyl	X	X	X	X	
Coumaphos	X	X	X	X	
Diazinon	Biel maze: increase errors & latency high dose (~33.1 mg/g,/d, dam diet) M, PND24 & PND62; also mid dose (~3.4 mg/kg/d, dam diet) F, PND24	X	X	X	
Dichlorvos[1]	--	--	--	--	
Dicrotophos	X	X	X	X	
Dimethoate	X	X	X	X	
Disulfoton	X	X	X	X	
Ethoprop	M maze: increase trials to criterion high dose (~29.3 mg/kg/d, dam diet) M, PND60	X	X	X	
Fenamiphos	X	X	X	X	
Malathion	X	increased rearing in FOB open field mid dose (50 mg/kg/d to dams & pups), F, significant at PND45 only (maybe also PND60); no change automated motor activity	increased peak amplitude later blocks (perhaps habituation effect) all doses (5, 50, 150 mg/kg/d to dams & pups), F only, PND23; increased peak amplitude without prepulse low dose only, F only, PND60	altered gait mid & high dose (50, 150 mg/kg/d to dams & pups), M & F, PND60 but not earlier	
Methamidophos	~X	X	decreased peak amplitude early blocks mid & high dose (~1.65, 5.2 mg/kg/d dam diet), F only, significant at PND38, looks same but not significant at PND60	X	PA PND24: report says decreased latency but nothing significant & table is questionable; M maze PND60: report says increased trials to criterion (M) and increased (M) or decreased (F) errors, but nothing significant and table is questionable
Methyl parathion	X	X	X	X	

Naled	X	X	increased peak amplitude & decreased latency middle blocks low dose only (0.4 mg/kg/d to dams & pups), F only, PND60; report says decreased amplitude but not significant all blocks high dose (10 mg/kg/d to dams & pups), M only, PND23 & PND60	X	only swimming time in Y maze reported, varied significances, no mention of errors or other performance measures
Phorate	M maze: decrease number reaching criterion with relearning, low & mid dose (0.03 & 0.1 mg/kg/d to dams & pups), M only significant, PND30; not seen in second study with higher dose	X	decreased peak amplitude all blocks high dose (0.1 mg/kg/d to dams & pups), M only, PND60; not seen in second study with higher dose	X	combined two studies; one with low & mid dose, other with high dose; some data didn't agree
Profenofos	X	X	X	X	
Terbufos	X	X	X	X	
Tetrachlorvinphos	X	X	X	X	
Tribufos	X	X	X	X	
Trichlorfon	PA: decreased latency to enter on retention high dose (~205.1 mg/kg/d dam diet), M only, PND29; M maze: increased average errors second trial high dose, F only, PND60	decreased activity middle blocks (maybefaster habituation) mid dose (~76.2 mg/kg/d dam diet) only, F only, PND60	decreased peak amplitude all blocks high dose (~205.1 mg/kg/d dam diet), early blocks mid dose (~76.2 mg/kg/d dam diet), M & F, PND22; decreased amplitude middle blocks (not consistent) all doses (~23.3, 76.2, 205.1 mg/kg/d dam diet), F only, PND38; for M decreased amplitude apparent but not significant high dose PND38 & PND60	X	

[1] high pup mortality in all groups, including control, negated any valid neurotoxicity assessments of dichlorvos

Appendix 3. Low Quality Studies: Summary of Study Design Elements Impacting Study Quality Assignment

Study	Study Location	Cohort Name / Description of Study Population	Study Design	Exposure Assessment	Outcome Assessment	Confounder / Covariate Control	Statistical Analysis	Risk of (other) Bias
Acosta-Maldonado *et al*. (2009)	Chihuahua, Mexico	Singleton pregnancies	Cross-sectional, small pilot study – only 9 women exposed, participant selection and exclusion not detailed N=54 mothers (9 exposed, 45 comparison mothers)	Proxy indicator of exposure - Residence in agricultural community were pesticides had been applied; or home located < 5 km from a pesticide application zone; or cohabitating with worker exposed to pesticides or agricultural labor Also, AChE activity – Objective biomarker of exposure/altered function	Standardized but partially subjective assessment of placental maturity	Minimal. Adjustment for placental characteristics.	Appropriate multivariate analysis; Corrected hypothesis test results for multiple comparisons.	Selection bias unlikely; Residual confounding likely; substantial potential for differential misclassification of exposure.
Dawbrowski *et al*. (2003)	Lodz, Poland	Newborn children among Polish farmers	Case-control, large sample size N=389 Age: newborns	Prenatal pesticide exposure assessed via questionnaire (retrospective self-report). Site visit *after* delivery to evaluate pesticide exposure	Pregnancy outcomes assessed using birth records	Appropriate. Included maternal demographics, predictors of high-risk pregnancy (duration, maternal weight) and environmental toxicant exposure (ETS). No adjustment for SES indicators	Appropriate multivariate analysis.	Selection bias unlikely; Residual confounding likely small in magnitude; considerable potential for non-differential misclassification of exposure (unlikely to account for non-null findings). Minimal misclassification of outcome.
Cartier et al. (2016)	Brittany, France	PELAGIE study (French population; pregnant women and children)	Longitundinal birth cohort n = 231 Mother-infant pairs	Metabolite concentrations (total DAP, DM, and DE) of prenatal OP pesticide exposure quantified in two maternal spot urine samples provided at 16 and 26 weeks of gestation Maternal IQ and the family environment were also assessed using the Wechsler Adult Intelligence Scale and the HOME test	Wechsler Intelligence Scale administered to children to determine IQ	All models were adjusted for HOME score, breastfeeding duration, mothers' IQ, school, maternal education level, psychologist testing the child, and creatinine levels of mother and child	Appropriate: Linear regression and multivariate models	Urine collection methods and storage (urine samples collected at home and mailed back to laboratory); lack of frequent urine spot samling and due to the short half-life of urinary metabolites could have led to exposure misclassification
Fielder et al. (2015)	Bangkok, Thailand area	Thai population (Children aged 6 to 8, who were from two farming regions (aquaculture v. rice)	Cohort, small sample size n = 54 Age 6- 8 years	Child Exposure – Total DEAP, DAP, and DMAP metabolites measured in spot urine samples, collected at three, separate time points (every 6 months) Parental education was assessed via a vocabulary test; the home environment was scored via the HOME test	BARS examination administered to children to determine neurobehavorial performance.	All models were adjusted for age and the HOME scale	Appropriate: Multivariate linear regression model and mix model linear regression to determine interaction	Small sample size (n = 54); errors in exposure classification methods, specifically, urine collection methods and storage (urine samples collected at home); short half-life of urinary metabolites could have led to exposure misclassification
Grandjean *et al*.(2006)	Tabacundo, Ecuador	Healthy 2nd and 3rd grade children	Cross-sectional, small sample size N=72 Age <9 years	Prenatal occupational exposure assessed via questionnaire; Also recent child exposure biomarker assessment (DAP).	Objective anthropometric and other clinical outcomes; Numerous neurobehavioral outcomes evaluated using easy-to-administer screening instruments; Age appropriate. May be insensitive to subtle effects of OP pesticide exposure effects.	Appropriate. Homogenous population limited confounding by design; Included child demographics (age, sex, weight); SES indicators (maternal race, housing, running water, sewage), diet (meals/day), environmental toxicants (maternal alcohol and smoking) and medical history.	Appropriate multivariate analysis; Numerous hypotheses evaluated without correction for multiple comparisons.	Selection bias unlikely; Residual confounding likely small in magnitude; considerable potential for non-differential misclassification of exposure (likely to account for null findings). Potential misclassification of outcome.

Study	Study Location	Cohort Name / Description of Study Population	Study Design	Exposure Assessment	Outcome Assessment	Confounder / Covariate Control	Statistical Analysis	Risk of (other) Bias
Harari *et al.* (2010)	Tabacundo, Ecuador (Andean plateau north of Quito)	6- to 8-year-old children in the two lowest grades (called second and third) of one of two schools	Cross-sectional, small sample size n = 81 Age 6- 8 years	Child Current Exposure - DMP metabolites measured in a spot urine samples. A blood sample was analyzed for AChE. Maternal Exposure – Interview by skilled interviewers	Blood pressure and neurophysiologic measures - the instruments used for neurophsychologic measures were validated to avoid cross-cultural influences.	Appropriate: child's sex, age, BMI, number of daily meals (only in current exposure), stunting, hematocrit, school grade, having repeated one grade, maternal education level, family living in a traditional house, drinking water supply, and paternal education and employment	Appropriate: Standard parametric tests and logistic regression	Errors in exposure classification status since it was based on the maternal self-report, mothers likely being aware of the neurobehavioral status of their children, exposure assessment based on a spot urine sample
Kofman *et al.* (2006)	Israel (Negev region)	Bedouin population (Children aged 6 to 12 at the time of the study, who were victims of poisoning before age three)	Retrospective cohort study, small sample size N = 52 9-Exposed to OP; 17-Exposed to Kerosene/paint thinner 26-Controls Aged 6-12 years	OP poisoning was confirmed by low serum butyrylcholinesterase activity based on hospital records	Neuropsychological evaluation and structured interview of parents. Errors in assessment minimized as psychologists were qualified individuals, language and cultural differences taken into consideration, each child tested on same day and in same place as matched control.	Age, sex, background (cultural and demographic)	Difference in means	Errors is outcome classification likely since psychologists who administered the tests knew which children were exposed, small sample size
Koutroulakis *et al.* (2014)	Crete, Greece	Women with singleton pregnancies, permanent residents for at least two years, referral for amniocentesis to the Fetal-Maternal Unit, Department of Obstetrics and Gynecology, University Hospital of Heraklion	Prospective Cohort Study – large sample size, ethical issues n = 415 Age: newborns	Objective. DAP measurement in a single amniotic fluid samples collected at either 16th or 20th weeks of gestation – Novel biomarker. Questionnaire was also used.	Birth weight and head circumference. Unclear how the outcome information was obtained.	Neonatal sex, maternal age, agricultural activities, and gestational age at amniocentesis – Rationale for confounder selection not provided	Appropriate: multiple linear regression	Comparing exposure measurements in AF against known biomarker (e.g., OP metabolite levels in urine) for validation of AF not conducted. Smoking status of participants before/during pregnancy, high risk pregnancies, gestational diabetes, PON1 enzyme activity in the fetuses not considered.

Appendix 3. Low Quality Studies: Summary of Study Design Elements Impacting Study Quality Assignment (continued)

Study	Study Location	Cohort Name / Description of Study Population	Study Design	Exposure Assessment	Outcome Assessment	Confounder / Covariate Control	Statistical Analysis	Risk of (other) Bias
Lizardi *et al*. (2008)	Yuma County, Arizona, USA	Children's Pesticide Survey (CPS)	Cross-sectional, small sample size N=48 Age 7 years	Objective Biomarker of prenatal OP pesticide exposure (DAP) quantified in single child spot urine sample provided at the time of the cognitive assessment.	Cognitive assessment using battery of test instruments considered valid and reliable in similar populations. Spanish translation as necessary.	None	Correlation coefficients (type unspecified). Statistically significant confounders were not robust due to influential outliers	Selection bias unlikely; Residual confounding likely; considerable potential for non-differential misclassification of exposure Potential misclassification of outcome.
Lu *et al*. (2009)	Cota Brus, Costa Rica	4-10 yr. old children whose parents worked in organic coffee farm (La Amistad) and conventional coffee farms (Las Mellizas)	Cross-Sectional (pilot), low sample numbers N=35: 17 Organic farm 18 Conventional farms Age 4-10 years old	Good measure (urinary PNP, IMPY, TCPy), but no major differences between exposure groups	Good measure (CBARS) but different SES and demographic characteristics for exposure groups	Limited number (group, age, sex, handedness, grade)	Appropriate: Linear mixed effects for significant test outcomes from paired t-test analyses	Somewhat high - Convenience sample with different recruitment methods; exposure misclassification
Moreno-Banda *et al*. (2009)	Mexico (Villa Guerrero, Coatepec de Harinas, Tenancingo (Mexico); Cuernavaca, Cuautla, Jiutepec, Temixco, (Morelos)	Newborn children of floricultural workers and families	Cross-sectional, large sample size N=328 Age: newborns	Proxy indicator of prenatal occupational OP pesticide exposure (self-reported floricultural occupation)	Objectively measured birth outcomes assessed using birth certificate (partial). Self-reported by mother if birth certificate unavailable.	Appropriate, though minimal – history of adverse reproductive outcomes, infant sex, maternal smoking and alcohol use during pregnancy.	Appropriate multivariate analysis	Selection bias likely; Residual confounding likely small in magnitude; considerable potential for non-differential misclassification of exposure (likely accounts for null findings). Potential outcome misclassification (self-report in subset of participants).
Nevison (2014)	U.S. National population	Children with birth years 1970–2005 in 1) California Department of Developmental Services (CDDS) reports 2) Individuals with Disabilities Education Act (IDEA) reports	Ecological (time trend) N=NP (national database)	Non-specific, proxy OP exposure measure (lbs/yr)	Two large reporting DBs: CA Department of Developmental Services (CDDS), US Individuals with Disabilities Education Act (IDEA)	Limited: differences in autism definitions, changes in diagnostic criteria	Appropriate: ratio of age-resolved snapshot; tracking trend slopes; correlation coefficient between temporal trend and composite autism prevalence curve	Selection bias unlikely; do not know individual exposure

Appendix 3. Low Quality Studies: Summary of Study Design Elements Impacting Study Quality Assignment (continued)

Study	Study Location	Cohort Name / Description of Study Population	Study Design	Exposure Assessment	Outcome Assessment	Confounder / Covariate Control	Statistical Analysis	Risk of (other) Bias
Rohlman *et al*. (2005)	Oregon and North Carolina, USA	Latino children of immigrant parents living in Oregon or North Carolina	Cross-Sectional, small sample size N= 78 Age 48-71 months	Proxy indicator of chronic OP pesticide exposure - Residence in highly agricultural communities	Battery of neurocognitive development; Screening tools; Some instruments likely not appropriate for use in study population - Not all participants able to complete all evaluations. Poor administration. Tests administered twice; only performance on 2nd evaluation considered in analysis.	Self-reported covariate information collected via questionnaire. Adjustment for age, SES indicator (maternal education). No environmental toxicants.	One-sided hypothesis tests. Numerous hypotheses evaluated without correction for multiple comparisons.	Selection bias unlikely; Residual confounding likely; substantial potential for differential misclassification of exposure and outcome. Inadequate presentation of study results. Seemingly post-hoc evaluation of effect modification.
Samarawickrema *et al*. (2008)	Southern Sri Lanka	Pregnant women delivering at Embilipitiya Base Hospital	Cross-sectional Birth Cohort, small sample size N=41 end of two pesticide spraying seasons; N=25 at beginning of spraying season	Proxy indicator of prenatal OP pesticide exposure (delivery during pesticide spray season); Objective pesticide biomarkers assessed (OP pesticide residues), but detected in only two subject's specimens – not evaluated	Objective biomarkers of early biological effect outcomes - Maternal and fetal butyrylcholinesterase (BuChE) activity; antioxidant status; fetal oxidative stress; fetal DNA fragmentation	No adjustment for potential confounders (though comparison groups were considered to be relatively homogeneous).	Largely appropriate. Assumptions of some statistical tests likely violated.	Selection bias unlikely; Residual confounding likely; substantial potential for differential misclassification of exposure; Seemingly post-hoc evaluation of effect modification.
Savitz *et al*. (1997)	Ontario, Canada	Newborn children in the Ontario Farm Family Health Study;	Retrospective Birth Cohort, large sample size N= 1,898 couples; 3,984 Age: newborns	Proxy indicator of pre-conception paternal para-occupational OP pesticide exposure (self-reported male farm activities in 3-month period prior to conception.	Objectively measured birth outcomes assessed by maternal self-report	Appropriate. Included family/child demographics (sex, weight, maternal age, ethnicity); SES indicators (maternal and paternal education and occupation, per capita income) race, housing, running water, sewage), diet (meals/day), pregnancy risks (maternal caffeine, alcohol and smoking) and medical history.	Inappropriate multivariate regression analysis. Likely misspecification of true variance; No adjustment for multiple comparisons.	Selection bias probable; Residual confounding likely; substantial potential for differential misclassification of exposure and outcome.
Wickerham *et al*. (2012)	Zhejiang Province, China	Newborn children delivered at the Fuyang Maternal and Children's hospital	Cross-sectional, small pilot study n=116 Age: Full term infants	Objective biomarker of pesticide exposure (pesticide residues in cord blood) – parameterized as number of pesticide residues detected. Methods unlikely suitable for detecting low levels.	Birth weight assessed using birth records and maternal report	Appropriate. Assessed using questionnaire (maternal self-report) and medical records.	Appropriate multivariate analysis.	Selection bias unlikely; Residual confounding likely; substantial potential for differential misclassification of exposure. Some outcome misclassification unlikely .
Naksen et al (2015)	Fang district, Chiang Mai province, Thailand	Pregnant women delivering at Fang Hospital	Prospective Cohort, small pilot study n=52 Age: newborns	Objective biomarkers of pesticide exposure (DAPs). Also AChE, BChE, and PON1 genotype expression measurement. Maternal blood and urinary samples taken, plus cord blood. Questionnaire to assess other exposures and covariates.	Birth outcomes (Body weight and length, and head circumference) abstracted from medical records.	Appropriate. Assessed using questionnaire (maternal self-report) and medical records.	Some errors in statistical analysis were identified; e.g., for gestation age, log total DEAP at 32 weeks of pregnancy, a 0.7 beta was reported, but the confidence interval is reported as (-0.1, -1.4)]. No adjustment for multiple comparisons.	Inadequate presentation of study results. Selection bias possible due to loss to follow-up; Residual confounding likely, small in magnitude; potential for non-differential misclassification of exposure.

Appendix 4. Table of Systematic Review Analysis: Initial Search Research & Initial Exclusion Criteria.

Initial search yielded 299 studies. Removing duplicates (56), there were 243 articles, and 79 were determined to be epidemiological investigations of potential relevance. The 164 studies excluded from the analysis comprised 57 exposure only studies; 51 review articles; 33 reports of acute OP intoxication; 20 studies in non-human systems; and 3 were otherwise not relevant).

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
1	Abdallah, M. A. E., & Covaci, A. (2014). Organophosphate flame retardants in indoor dust from Egypt: implications for human exposure. [Journal article]. *Environmental Science & Technology, 48*, 4782-4789.	EXPO
2	Acosta-Maldonado, B., Sanchez-Ramirez, B., Reza-Lopez, S., & Levario-Carrillo, M. (2009). Effects of exposure to pesticides during pregnancy on placental maturity and weight of newborns: a cross-sectional pilot study in women from the Chihuahua State, Mexico. *Hum Exp Toxicol, 28*(8), 451-459.	EPI
3	Ali, P., Anwer, A., Bashir, B., Jabeen, R., Haroon, H., & Makki, K. (2012). Clinical pattern and outcome of organophosphorus poisoning. [Article]. *Journal of the Liaquat University of Medical and Health Sciences, 11*, 15-18.	ACR
4	Alizadeh, A. M., Hassanian-Moghaddam, H., Shadnia, S., Zamani, N., & Mehrpour, O. (2014). Simplified acute physiology score II/Acute physiology and chronic health evaluation ii and prediction of the mortality and later development of complications in poisoned patients admitted to intensive care unit. [Article]. *Basic and Clinical Pharmacology and Toxicology, 115*, 297-300.	ACR
5	Andersen, H. R., Debes, F., Wohlfahrt-Veje, C., Murata, K., & Grandjean, P. (2015). Occupational pesticide exposure in early pregnancy associated with sex-specific neurobehavioral deficits in the children at school age. *Neurotoxicology and Teratology, 47*, 1-9.	EPI
6	Attfield, K. R., Hughes, M. D., Spengler, J. D., & Lu, C. (2014). Within- and between-child variation in repeated urinary pesticide metabolite measurements over a 1-year period. [Article]. *Environmental Health Perspectives, 122*, 201-206.	EXPO

ED_002061_00044425-00118

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
7	Attfield, K. R., Hughes, M. D., Spengler, J. D., & Lu, C. S. (2014). Within- and between-child variation in repeated urinary pesticide metabolite measurements over a 1-year period. [Journal article]. *Environmental Health Perspectives, 122*, 201-206.	DUPLICATE
8	Babina, K., Dollard, M., Pilotto, L., & Edwards, J. W. (2012). Environmental exposure to organophosphorus and pyrethroid pesticides in South Australian preschool children: A cross sectional study. [Article]. *Environment International, 48*, 109-120.	DUPLICATE
9	Babina, K., Dollard, M., Pilotto, L., & Edwards, J. W. (2012). Environmental exposure to organophosphorus and pyrethroid pesticides in South Australian preschool children: A cross sectional study. [Article]. *Environment International, 48*, 109-120.	EXPO
10	Balakumar, K., Misha, K., & Milind, K. (2013). Increased fetal endocardial echogenicity mimicking endocardial fibroelastosis following maternal organophosphorus poisoning and its complete regression in utero. [Article]. *Indian Journal of Radiology and Imaging, 23*, 262-265.	ACR
11	Baltazar, M. T., Dinis-Oliveira, R. J., de Lourdes Bastos, M., Tsatsakis, A. M., Duarte, J. A., & Carvalho, F. (2014). Pesticides exposure as etiological factors of Parkinson's disease and other neurodegenerative diseases—A mechanistic approach. *Toxicology Letters, 230*, 85-103.	REV
12	Barr, D. B., Ananth, C. V., Yan, X., Lashley, S., Smulian, J. C., Ledoux, T. A., et al. (2010). Pesticide concentrations in maternal and umbilical cord sera and their relation to birth outcomes in a population of pregnant women and newborns in New Jersey. *Sci Total Environ, 408*(4), 790-795.	EPI
13	Béchaux, C., Zetlaoui, M. l., Tressou, J., Leblanc, J.-C., Héraud, F., & Crépet, A. l. (2013). Identification of pesticide mixtures and connection between combined exposure and diet. [Article]. *Food and Chemical Toxicology, 59*, 191-198.	EXPO
14	Bedi, J. S., Gill, J. P. S., Aulakh, R. S., Kaur, P., Sharma, A., & Pooni, P. A. (2013). Pesticide residues in human breast milk: Risk assessment for infants from Punjab, India. *Science of The Total Environment, 463–464*, 720-726.	EXPO

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
15	Bellinger, D. C. (2012a). A strategy for comparing the contributions of environmental chemicals and other risk factors to neurodevelopment of children. [Article]. *Environmental Health Perspectives, 120*, 501-507.	REV
16	Bellinger, D. C. (2012b). Comparing the population neurodevelopmental burdens associated with children's exposures to environmental chemicals and other risk factors. [Article]. *NeuroToxicology, 33*, 641-643.	REV
17	Berkowitz, G. S., Wetmur, J. G., Birman-Deych, E., Obel, J., Lapinski, R. H., Godbold, J. H., et al. (2004). In utero pesticide exposure, maternal paraoxonase activity, and head circumference. *Environ Health Perspect, 112*(3), 388-391.	EPI
18	Berton, T., Mayhoub, F., Chardon, K., Duca, R.-C., Lestremau, F., Bach, V., et al. (2014a). Development of an analytical strategy based on LC-MS/MS for the measurement of different classes of pesticides and theirs metabolites in meconium: Application and characterisation of foetal exposure in France. *Environmental Research, 132*, 311-320.	EXPO
19	Berton, T., Mayhoub, F., Chardon, K., Duca, R.-C., Lestremau, F., Bach, V., et al. (2014a). Development of an analytical strategy based on LC–MS/MS for the measurement of different classes of pesticides and theirs metabolites in meconium: Application and characterisation of foetal exposure in France. *Environmental Research, 132*, 311-320.	DUPLICATE
20	Berton, T., Mayhoub, F., Chardon, K., Duca, R.-C., Lestremau, F., Bach, V., et al. (2014b). Development of an analytical strategy based on LC–MS/MS for the measurement of different classes of pesticides and theirs metabolites in meconium: Application and characterisation of foetal exposure in France. *Environmental Research, 132*, 311-320.	DUPLICATE
21	Berton, T., Mayhoub, F., Chardon, K., Duca, R.-C., Lestremau, F., Bach, V., et al. (2014b). Development of an analytical strategy based on LC—MS/MS for the measurement of different classes of pesticides and theirs metabolites in meconium: Application and characterisation of foetal exposure in France. [article]. *Environmental research (New York, N.Y. : Print), 132*, 311-320.	DUPLICATE

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
22	Bettegowda, C. (2012). Pesticides perturb prenatal brain. [Note]. *Science Translational Medicine, 4.*	REV
23	Betts, K. S. (2013). Lasting impacts: Pre- and postnatal PBDE exposures linked to IQ deficits. [Note]. *Environmental Health Perspectives, 121*.	REV
24	Bhagavath, P., Monteiro, F. N. P., & Gnanadev, N. C. (2012). Epidemiology of intentional self poisoning. [Article]. *Medico-Legal Update, 12*, 52-54.	ACR
25	Bhaskar, R., & Mohanty, B. (2014). Pesticides in mixture disrupt metabolic regulation: In silico and in vivo analysis of cumulative toxicity of mancozeb and imidacloprid on body weight of mice. *General and Comparative Endocrinology, 205*, 226-234.	TOX
26	Bhatnagar, S., Das, U. M., & Bhatnagar, G. (2012). Comparison of oral midazolam with oral tramadol, triclofos and zolpidem in the sedation of pediatric dental patients: An in vivo study. [Article]. *Journal of Indian Society of Pedodontics and Preventive Dentistry, 30*, 109-114.	OTH
27	Biello, D. (2012). Bad for bugs and brains? A common pesticide may interfere with a child's brain development. [Article]. *Scientific American, 307*, 22.	REV
28	Bouchard, M. F., Bellinger, D. C., Wright, R. O., & Weisskopf, M. G. (2010). Attention-deficit/hyperactivity disorder and urinary metabolites of organophosphate pesticides. [Research Support, N.I.H., Extramural]. *Pediatrics, 125*(6), e1270-1277.	EPI
29	Bouchard, M. F., Chevrier, J., Harley, K. G., Kogut, K., Vedar, M., Calderon, N., et al. (2011). Prenatal exposure to organophosphate pesticides and IQ in 7-year-old children. *Environ Health Perspect, 119*(8), 1189-1195.	EPI

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
30	Bradman, A., Kogut, K., Eisen, E. A., Jewell, N. P., Quirós-Alcalá, L., Castorina, R., et al. (2013). Variability of organophosphorous pesticide metabolite levels in spot and 24-hr urine samples collected from young children during 1 week. [Article]. *Environmental Health Perspectives, 121*, 118-124.	EXPO
31	Brown, J. S., Jr. (2013). *Psychiatric Effects of Organic Chemical Exposure*: BLACKWELL SCIENCE PUBL, OSNEY MEAD, OXFORD OX2 0EL, UK.	REV
32	Bulgaroni, V., Lombardo, P., Rivero-Osimani, V., Vera, B., Dulgerian, L., Cerban, F., et al. (2013). Environmental pesticide exposure modulates cytokines, arginase and ornithine decarboxylase expression in human placenta. [Article]. *Reproductive Toxicology, 39*, 23-32.	TOX
33	Burdorf, A., Brand, T., Jaddoe, V. W., Hofman, A., Mackenbach, J. P., & Steegers, E. A. (2011). The effects of work-related maternal risk factors on time to pregnancy, preterm birth and birth weight: the Generation R Study. *Occup Environ Med, 68*(3), 197-204.	EPI
34	Burns, C. J., McIntosh, L. J., Mink, P. J., Jurek, A. M., & Li, A. A. (2013). Pesticide exposure and neurodevelopmental outcomes: Review of the epidemiologic and animal studies. [Article]. *Journal of Toxicology and Environmental Health - Part B: Critical Reviews, 16*, 127-183.	REV
35	Butt, C. M., Congleton, J., Hoffman, K., Fang, M. L., & Stapleton, H. M. (2014). Metabolites of organophosphate flame retardants and 2-ethylhexyl tetrabromobenzoate in urine from paired mothers and toddlers. [Journal article]. *Environmental Science & Technology, 48*, 10432-10438.	EXPO
36	Butt, C. M., Congleton, J., Hoffman, K., Fang, M., & Stapleton, H. M. (2014). Metabolites of organophosphate flame retardants and 2-ethylhexyl tetrabromobenzoate in urine from paired mothers and toddlers. *Environ Sci Technol, 48*, 10432-10438.	DUPLICATE
37	Camann, D. E., Schultz, S. T., Yau, A. Y., Heilbrun, L. P., Zuniga, M. M., Palmer, R. F., et al. (2013). Acetaminophen, pesticide, and diethylhexyl phthalate metabolites, anandamide, and fatty acids in deciduous molars: Potential biomarkers of perinatal exposure. [Article]. *Journal of Exposure Science and Environmental Epidemiology, 23*, 190-196.	EXPO

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
38	Cappiello, A., Famiglini, G., Palma, P., Termopoli, V., Lavezzi, A. M., & Matturri, L. (2014). Determination of selected endocrine disrupting compounds in human fetal and newborn tissues by GC-MS. [Article]. *Analytical and Bioanalytical Chemistry, 406*, 2779-2788.	EXPO
39	Carmichael, S. L., Yang, W., Roberts, E. M., Kegley, S. E., Wolff, C., Guo, L., et al. (2013). Hypospadias and residential proximity to pesticide applications. [Article]. *Pediatrics, 132*, e1216-e1226.	EPI
40	Carmichael, S. L., Yang, W., Roberts, E., Kegley, S. E., Padula, A. M., English, P. B., et al. (2014). Residential agricultural pesticide exposures and risk of selected congenital heart defects among offspring in the San Joaquin Valley of California. [Article]. *Environmental Research, 135*, 133-138.	EPI
41	Carpenter, D. O. (2013). *Intellectual Developmental Disability Syndromes and Organic Chemicals*: BLACKWELL SCIENCE PUBL, OSNEY MEAD, OXFORD OX2 0EL, UK.	REV
42	Castorina, R., Bradman, A., McKone, T. E., Barr, D. B., Harnly, M. E., & Eskenazi, B. (2003). Cumulative organophosphate pesticide exposure and risk assessment among pregnant women living in an agricultural community: a case study from the CHAMACOS cohort. *Environ Health Perspect, 111*(13), 1640-1648.	EXPO
43	Cecchi, A., Rovedatti, M. G., Sabino, G., & Magnarelli, G. G. (2012a). Environmental exposure to organophosphate pesticides: Assessment of endocrine disruption and hepatotoxicity in pregnant women. [Article]. *Ecotoxicology and Environmental Safety, 80*, 280-287.	EPI
44	Cecchi, A., Rovedatti, M. G., Sabino, G., & Magnarelli, G. G. (2012b). Environmental exposure to organophosphate pesticides: Assessment of endocrine disruption and hepatotoxicity in pregnant women. *Ecotoxicology and Environmental Safety, 80*, 280-287.	DUPLICATE
45	Cequier, E., Ionas, A. C., Covaci, A., Marcé, R. M., Becher, G., & Thomsen, C. (2014). Occurrence of a broad range of legacy and emerging flame retardants in indoor environments in Norway. [Journal article]. *Environmental Science & Technology, 48*, 6827-6835.	EXPO

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
46	Cequier, E., Marcé, R. M., Becher, G., & Thomsen, C. (2014). A high-throughput method for determination of metabolites of organophosphate flame retardants in urine by ultra performance liquid chromatography-high resolution mass spectrometry. [Article]. *Analytica Chimica Acta, 845*, 98-104.	EXPO
47	Chen, W.-Q., Zhang, Y.-Z., Yuan, L., Li, Y.-F., & Li, J. (2014). Neurobehavioral evaluation of adolescent male rats following repeated exposure to chlorpyrifos. [Article]. *Neuroscience Letters, 570*, 76-80.	TOX
48	Chen, Y., Garcia, G. E., Huang, W., & Constantini, S. (2014). The involvement of secondary neuronal damage in the development of neuropsychiatric disorders following brain insults. [Article]. *Frontiers in Neurology, 5 MAR*.	DUPLICATE
49	Chen, Y., Garcia, G. E., Huang, W., & Constantini, S. (2014). The involvement of secondary neuronal damage in the development of neuropsychiatric disorders following brain insults. [Article]. *Frontiers in Neurology, 5 MAR*.	REV
50	Cole, T. B., Li, W.-F., Co, A. L., Hay, A. M., MacDonald, J. W., Bammler, T. K., et al. (2014). Repeated Gestational Exposure of Mice to Chlorpyrifos Oxon Is Associated with Paraoxonase 1 (PON1) Modulated Effects in Maternal and Fetal Tissues. [Article]. *Toxicological Sciences, 141*, 409-422.	TOX
51	Crane, A. L., Abdel Rasoul, G., Ismail, A. A., Hendy, O., Bonner, M. R., Lasarev, M. R., et al. (2013). Longitudinal assessment of chlorpyrifos exposure and effect biomarkers in adolescent Egyptian agricultural workers. [Article]. *Journal of Exposure Science and Environmental Epidemiology, 23*, 356-362.	EXPO
52	Dabrowski, S., Hanke, W., Polanska, K., Makowiec-Dabrowska, T., & Sobala, W. (2003). Pesticide exposure and birthweight: an epidemiological study in Central Poland. *Int J Occup Med Environ Health, 16*(1), 31-39.	EPI
53	Dahlgren, J. G., Takhar, H. S., Ruffalo, C. A., & Zwass, M. (2004). Health effects of diazinon on a family. *J Toxicol Clin Toxicol, 42*(5), 579-591.	ACR

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
54	Das, S., Chatterjee, K., Sarkar, N., Aich, B., & Dolui, S. (2013). Cholinergic crisis, intermediate syndrome and delayed polyneuropathy following malathion poisoning. [Article]. *Journal of Pediatric Intensive Care, 2*, 137-141.	ACR
55	De Cock, M., Maas, Y. G. H., & De Bor, M. V. (2012). Does perinatal exposure to endocrine disruptors induce autism spectrum and attention deficit hyperactivity disorders? Review. [article]. *Acta paediatrica (Oslo), 101*, 811-818.	REV
56	de Joode, B. V., Barraza, D., Ruepert, C., Mora, A. M., Cordoba, L., Oberg, M., et al. (2012). Indigenous children living nearby plantations with chlorpyrifos-treated bags have elevated 3,5,6-trichloro-2-pyridinol (TCPy) urinary concentrations. [Article]. *Environmental Research, 117*, 17-26.	EXPO
57	Detweiler, M. B. (2014). Organophosphate intermediate syndrome with neurological complications of extrapyramidal symptoms in clinical practice. [Article]. *Journal of Neurosciences in Rural Practice, 5*, 298-301.	REV
58	Deziel, N. C., Ward, M. H., Bell, E. M., Whitehead, T. P., Gunier, R. B., Friesen, M. C., et al. (2013). Temporal variability of pesticide concentrations in homes and implications for attenuation bias in epidemiologic studies. [Article]. *Environmental Health Perspectives, 121*, 565-571.	EXPO
59	Dhakal, A. K., Shrestha, D., Shakya, A., Shah, S. C., & Shakya, H. (2014). Clinical profile of acute poisoning in children at a teaching hospital in lalitpur. [Article]. *Journal of Nepal Paediatric Society, 34*, 100-103.	ACR
60	Ding, G. D., Wang, P., Tian, Y., Zhang, J., Gao, Y., Wang, X. J., et al. (2012). Organophosphate Pesticide Exposure and Neurodevelopment in Young Shanghai Children. [Article]. *Environmental Science & Technology, 46*(5), 2911-2917.	EPI
61	Ding, G., & Tian, Y. (2014). Organophosphate pesticide exposure and child health in China. [Article]. *Environmental Science and Pollution Research, 21*, 759-760.	REV

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
62	Ding, G., Han, S., Wang, P., Gao, Y., Shi, R., Wang, G., et al. (2012). Increased levels of 8-hydroxy-2′-deoxyguanosine are attributable to organophosphate pesticide exposure among young children. [Article]. *Environmental Pollution, 167*, 110-114.	EXPO
63	Dulaurent, S., Gaulier, J. M., Blanc-Lapierre, A., Imbert, L., & Lachâtre, G. (2013). Urinary determination of 2-isopropyl-4-methyl-6-hydroxypyrimidine in case of non fatal poisoning with diazinon. [Article]. *Forensic Science International, 228*, e20-e24.	ACR
64	Engel, S. M., Berkowitz, G. S., Barr, D. B., Teitelbaum, S. L., Siskind, J., Meisel, S. J., et al. (2007). Prenatal organophosphate metabolite and organochlorine levels and performance on the Brazelton Neonatal Behavioral Assessment Scale in a multiethnic pregnancy cohort. *Am J Epidemiol, 165*(12), 1397-1404.	EPI
65	Engel, S. M., Wetmur, J., Chen, J., Zhu, C., Barr, D. B., Canfield, R. L., et al. (2011). Prenatal exposure to organophosphates, paraoxonase 1, and cognitive development in childhood. *Environ Health Perspect, 119*(8), 1182-1188.	EPI
66	Eskenazi, B., Chevrier, J., Rauch, S. A., Kogut, K., Harley, K. G., Johnson, C., et al. (2013). In Utero and Childhood Polybrominated Diphenyl Ether (PBDE) Exposures and Neurodevelopment in the CHAMACOS Study. [Article]. *Environmental Health Perspectives, 121*(2), 257-262.	EPI
67	Eskenazi, B., Chevrier, J., Rauch, S. A., Kogut, K., Harley, K. G., Johnson, C., et al. (2013). In utero and childhood polybrominated diphenyl ether (PBDE) exposures and neurodevelopment in the CHAMACOS study. [Note]. *Environmental Health Perspectives, 121*, 257-262.	DUPLICATE
68	Eskenazi, B., Harley, K., Bradman, A., Weltzien, E., Jewell, N. P., Barr, D. B., et al. (2004). Association of in utero organophosphate pesticide exposure and fetal growth and length of gestation in an agricultural population. *Environ Health Perspect, 112*(10), 1116-1124.	EPI
69	Eskenazi, B., Huen, K., Marks, A., Harley, K. G., Bradman, A., Barr, D. B., et al. (2010). PON1 and neurodevelopment in children from the CHAMACOS study exposed to organophosphate pesticides in utero. *Environ Health Perspect, 118*(12), 1775-1781.	EPI

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
70	Eskenazi, B., Kogut, K., Huen, K., Harley, K. G., Bouchard, M., Bradman, A., et al. (2014a). Organophosphate pesticide exposure, PON1, and neurodevelopment in school-age children from the CHAMACOS study. *Environmental Research, 134*, 149-157.	EPI
71	Eskenazi, B., Kogut, K., Huen, K., Harley, K. G., Bouchard, M., Bradman, A., et al. (2014b). Organophosphate pesticide exposure, PON1, and neurodevelopment in school-age children from the CHAMACOS study. [Article]. *Environmental Research, 134*, 149-157.	DUPLICATE
72	Eskenazi, B., Marks, A. R., Bradman, A., Harley, K., Barr, D. B., Johnson, C., et al. (2007). Organophosphate pesticide exposure and neurodevelopment in young Mexican-American children. *Environ Health Perspect, 115*(5), 792-798.	EPI
73	Eskenazi, B., Rosas, L. G., Marks, A. R., Bradman, A., Harley, K., Holland, N., et al. (2008). Pesticide toxicity and the developing brain. *Basic Clin Pharmacol Toxicol, 102*(2), 228-236.	REV
74	Estevan, C., Fuster, E., Del Río, E., Pamies, D., Vilanova, E., & Sogorb, M. A. (2014). Organophosphorus pesticide chlorpyrifos and its metabolites alter the expression of biomarker genes of differentiation in D3 mouse embryonic stem cells in a comparable way to other model neurodevelopmental toxicants. [Article]. *Chemical Research in Toxicology, 27*, 1487-1495.	TOX
75	Fenske, R. A., Lu, C., Negrete, M., & Galvin, K. (2013). Breaking the take home pesticide exposure pathway for agricultural families: Workplace predictors of residential contamination. [Article]. *American Journal of Industrial Medicine, 56*, 1063-1071.	EXPO
76	Ferreira, J. D., Couto, A. C. z., Pombo-de-Oliveira, M. S., & Koifman, S. (2013). In utero pesticide exposure and leukemia in Brazilian children < 2 years of age. [Note]. *Environmental Health Perspectives, 121*, 269-275.	EPI
77	Feyzioğlu, B. r., Özdemir, M., Kutlu, N. O., Baykan, M., & Baysal, B. (2014). Pulmonary infection caused by chryseobacterium indologenes in a patient with a diagnosis of organophosphate poisoning. [Article]. *Anatolian Journal of Clinical Investigation, 8*, 137-139.	ACR

ED_002061_00044425-00127

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
78	Fortenberry, G. Z., Meeker, J. D., Sanchez, B. N., Barr, D. B., Panuwet, P., Bellinger, D., et al. (2014). Urinary 3,5,6-trichloro-2-pyridinol (TCPY) in pregnant women from Mexico City: Distribution, temporal variability, and relationship with child attention and hyperactivity. [article]. *International journal of hygiene and environmental health, 217*, 405-412.	DUPLICATE
79	Fortenberry, G. Z., Meeker, J. D., Sanchez, B. N., Barr, D. B., Panuwet, P., Bellinger, D., et al. (2014). Urinary 3,5,6-trichloro-2-pyridinol (TCPY) in pregnant women from Mexico City: distribution, temporal variability, and relationship with child attention and hyperactivity. *Int J Hyg Environ Health, 217*(2-3), 405-412.	EPI
80	Fortenberry, G. Z., Meeker, J. D., Sánchez, B. N., Barr, D. B., Panuwet, P., Bellinger, D., et al. (2014). Urinary 3,5,6-trichloro-2-pyridinol (TCPY) in pregnant women from Mexico City: Distribution, temporal variability, and relationship with child attention and hyperactivity. *International Journal of Hygiene and Environmental Health, 217*, 405-412.	DUPLICATE
81	Fortenberry, G. Z., Meeker, J. D., Sánchez, B. N., Barr, D. B., Panuwet, P., Bellinger, D., et al. (2014). Urinary 3,5,6-trichloro-2-pyridinol (TCPY) in pregnant women from Mexico City: distribution, temporal variability, and relationship with child attention and hyperactivity. [Journal article]. *International Journal of Hygiene and Environmental Health, 217*, 405-412.	DUPLICATE
82	Fortenberry, G. Z., Meeker, J. D., Sánchez, B. N., Bellinger, D., Peterson, K., Schnaas, L., et al. (2014). Paraoxonase I polymorphisms and attention/hyperactivity in school-age children from Mexico City, Mexico. [Article]. *Environmental Research, 132*, 342-349.	EPI
83	Fortenberry, G. Z., Meeker, J. D., Sanchez, B. N., Bellinger, D., Peterson, K., Schnaas, L., et al. (2014). Paraoxonase I polymorphisms and attention/hyperactivity in school-age children from Mexico City, Mexico. *Environ Res, 132*, 342-349.	DUPLICATE
84	Fortenberry, G. Z., Meeker, J. D., Sanchez, B. N., Bellinger, D., Peterson, K., Schnaas, L., et al. (2014). Paraoxonase I polymorphisms and attention/hyperactivity in school-age children from Mexico City, Mexico. *Environmental Research, 132*, 342-349.	DUPLICATE
85	Fortenberry, G. Z., Meeker, J. D., Sánchez, B. N., Bellinger, D., Peterson, K., Schnaas, L., et al. (2014). Paraoxonase I polymorphisms and attention/hyperactivity in school-age children from Mexico City, Mexico. *Environmental Research, 132*, 342-349.	DUPLICATE

ED_002061_00044425-00128

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
86	Fortenberry, G. Z., Meeker, J. D., Sánchez, B. N., Bellinger, D., Peterson, K., Schnaas, L., et al. (2014). Paraoxonase I polymorphisms and attention/hyperactivity in school-age children from Mexico City, Mexico. *Environmental Research, 132*, 342-349.	DUPLICATE
87	Fromme, H., Lahrz, T., Kraft, M., Fembacher, L., Mach, C., Dietrich, S., et al. (2014). Organophosphate flame retardants and plasticizers in the air and dust in German daycare centers and human biomonitoring in visiting children (LUPE 3). [Article]. *Environment International, 71*, 158-163.	EXPO
88	Furlong, M. A., Engel, S. M., Barr, D. B., & Wolff, M. S. (2014). Prenatal exposure to organophosphate pesticides and reciprocal social behavior in childhood. *Environ Int, 70*, 125-131.	EPI
89	Furlong, M. A., Engel, S. M., Barr, D. B., & Wolff, M. S. (2014a). Prenatal exposure to organophosphate pesticides and reciprocal social behavior in childhood. *Environment International, 70*, 125-131.	DUPLICATE
90	Furlong, M. A., Engel, S. M., Barr, D. B., & Wolff, M. S. (2014b). Prenatal exposure to organophosphate pesticides and reciprocal social behavior in childhood. [article]. *Environment international, 70*, 125-131.	DUPLICATE
91	Gonza´lez-Alzaga, B., Lacasan, tild, a, M., Aguilar, G., o, C., et al. (2014). A systematic review of neurodevelopmental effects of prenatal and postnatal organophosphate pesticide exposure. [Article]. *Toxicology Letters, 230*, 104-121.	REV
92	Gonzalez, V., Huen, K., Venkat, S., Pratt, K., Xiang, P., Harley, K. G., et al. (2012). Cholinesterase and paraoxonase (PON1) enzyme activities in MexicanAmerican mothers and children from an agricultural community. [Article]. *Journal of Exposure Science and Environmental Epidemiology, 22*, 641-648.	EXPO
93	Gonzalez-Alzaga, B., Lacasana, M., Aguilar-Garduno, C., Rodriguez-Barranco, M., Ballester, F., Rebagliato, M., et al. (2013). A systematic review of neurodevelopmental effects of prenatal and postnatal organophosphate pesticide exposure. *Toxicol Lett, 230*, 104-121.	DUPLICATE

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
94	González-Alzaga, B., Lacasaña, M., Aguilar-Garduño, C., Rodríguez-Barranco, M., Ballester, F., Rebagliato, M., et al. (2014). A systematic review of neurodevelopmental effects of prenatal and postnatal organophosphate pesticide exposure. *Toxicology Letters, 230*, 104-121.	DUPLICATE
95	Goodman, J. E., Prueitt, R. L., & Rhomberg, L. R. (2013). INCORPORATING LOW-DOSE EPIDEMIOLOGY DATA IN A CHLORPYRIFOS RISK ASSESSMENT. [Article]. *Dose-Response, 11*(2), 207-219.	REV
96	Goodman, J. E., Prueitt, R. L., & Rhomberg, L. R. (2013). Incorporating low-dose epidemiology data in a chlorpyrifos risk assessment. [Article]. *Dose-Response, 11*, 207-219.	DUPLICATE
97	Gowda, V. K. N., Bannigidad, N. B., Kumar, P., Srikanteswara, P.-K., Shivananda, Govindraj, et al. (2014). Predictors of non-traumatic coma in a pediatric cohort from a South Indian tertiary care center: Results of a multivariate analysis. [Article]. *Journal of Pediatric Neurology, 12*, 35-43.	ACR
98	Grandjean, P., Harari, R., Barr, D. B., & Debes, F. (2006). Pesticide exposure and stunting as independent predictors of neurobehavioral deficits in Ecuadorian school children. [Research Support, Non-U.S. Gov't]. *Pediatrics, 117*(3), e546-556.	EPI
99	Guha, N., Ward, M. H., Gunier, R., Colt, J. S., Suzanne Lea, C., Buffer, P. A., et al. (2013). Characterization of residential pesticide use and chemical formulations through self-report and household inventory: The northern California childhood leukemia study. [Note]. *Environmental Health Perspectives, 121*, 276-282.	EXPO
100	Guiñazú, N., Rena, V., Genti-Raimondi, S., Rivero, V., & Magnarelli, G. (2012). Effects of the organophosphate insecticides phosmet and chlorpyrifos on trophoblast JEG-3 cell death, proliferation and inflammatory molecule production. [Article]. *Toxicology in Vitro, 26*, 406-413.	TOX
101	Guodong, D., & Yixiao, B. A. O. (2014). Revisiting pesticide exposure and children's health: Focus on China. [article]. *Science of the total environment, 472*, 289-295.	REV

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
102	Guodong, D., Pei, W., Ying, T., Jun, Z., Yu, G., Xiaojin, W., et al. (2012). Organophosphate pesticide exposure and neurodevelopment in young Shanghai children. [Article]. *Environmental Science and Technology, 46*, 2911-2917.	DUPLICATE
103	Guodong, D., Pei, W., Ying, T., Jun, Z., Yu, G., Xiaojin, W., et al. (2012). Organophosphate pesticide exposure and neurodevelopment in young Shanghai children. *Environ Sci Technol*.	EPI
104	Handal, A. J., Harlow, S. D., Breilh, J., & Lozoff, B. (2008). Occupational exposure to pesticides during pregnancy and neurobehavioral development of infants and toddlers. *Epidemiology, 19*(6), 851-859.	EPI
105	Handal, A. J., Lozoff, B., Breilh, J., & Harlow, S. D. (2007a). Effect of community of residence on neurobehavioral development in infants and young children in a flower-growing region of Ecuador. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Environ Health Perspect, 115*(1), 128-133.	EPI
106	Handal, A. J., Lozoff, B., Breilh, J., & Harlow, S. D. (2007b). Neurobehavioral development in children with potential exposure to pesticides. *Epidemiology, 18*(3), 312-320.	EPI
107	Harari, R., Julvez, J., Murata, K., Barr, D., Bellinger, D. C., Debes, F., et al. (2010). Neurobehavioral deficits and increased blood pressure in school-age children prenatally exposed to pesticides. *Environ Health Perspect, 118*(6), 890-896.	EPI
108	Harley, K. G., Huen, K., Schall, R. A., Holland, N. T., Bradman, A., Barr, D. B., et al. (2011). Association of organophosphate pesticide exposure and paraoxonase with birth outcome in Mexican-American women. *PLoS One, 6*(8), e23923.	EPI
109	Hoffman, K., Daniels, J. L., & Stapleton, H. M. (2014). Urinary metabolites of organophosphate flame retardants and their variability in pregnant women. [Article]. *Environment International, 63*, 169-172.	EXPO

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
110	Hoffman, K., Daniels, J. L., & Stapleton, H. M. (2014). Urinary metabolites of organophosphate flame retardants and their variability in pregnant women. [Journal article]. *Environment International, 63*, 169-172.	DUPLICATE
111	Hore, P., Robson, M., Freeman, N., Zhang, J., Wartenberg, D., Ozkaynak, H., et al. (2005). Chlorpyrifos accumulation patterns for child-accessible surfaces and objects and urinary metabolite excretion by children for 2 weeks after crack-and-crevice application. *Environ Health Perspect, 113*(2), 211-219.	EXPO
112	Hore, P., Zartarian, V., Xue, J., Oezkaynak, H., Wang, S.-W., Yang, Y.-C., et al. (2006). Childrens residential exposure to chlorpyrifos: Application of CPPAES field measurements of chlorpyrifos and TCPy within MENTOR/SHEDS-Pesticides model. *Science of the Total Environment, 366*(2-3), 525-537.	EXPO
113	Horton, M. K., Kahn, L. G., Perera, F., Barr, D. B., & Rauh, V. (2012). Does the home environment and the sex of the child modify the adverse effects of prenatal exposure to chlorpyrifos on child working memory? *Neurotoxicol Teratol, 34*(5), 534-541.	EPI
114	Horton, M. K., Kahn, L. G., Perera, F., Barr, D. B., & Rauh, V. (2012). Does the home environment and the sex of the child modify the adverse effects of prenatal exposure to chlorpyrifos on child working memory? *Neurotoxicology and Teratology, 34*, 534-541.	DUPLICATE
115	Horton, M. K., Kahn, L. G., Perera, F., Boyd Barr, D., & Rauh, V. (2012). Does the home environment and the sex of the child modify the adverse effects of prenatal exposure to chlorpyrifos on child working memory? [article]. *Neurotoxicology and teratology, 34*, 534-541.	DUPLICATE
116	How, V., Hashim, Z., Ismail, P., Said, S. M., Dzolkhifli, O., & Shamsul Bahri, M. T. (2014). Exploring cancer development in adulthood: cholinesterase depression and genotoxic effect from chronic exposure to organophosphate pesticides among rural farm children. [Journal article]. *Journal of Agromedicine, 19*, 35-43.	EPI
117	Huen, K., Bradman, A., Harley, K., Yousefi, P., Barr, D. B., Eskenazi, B., et al. (2012). Organophosphate pesticide levels in blood and urine of women and newborns living in an agricultural community. [Article]. *Environmental Research, 117*, 8-16.	EXPO

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
118	Huen, K., Bradman, A., Harley, K., Yousefi, P., Boyd Barr, D., Eskenazi, B., et al. (2012). Organophosphate pesticide levels in blood and urine of women and newborns living in an agricultural community. [Article]. *Environmental Research, 117*, 8-16.	DUPLICATE
119	Huen, K., Harley, K., Beckman, K., Eskenazi, B., & Holland, N. (2013). Associations of PON1 and Genetic Ancestry with Obesity in Early Childhood. [Article]. *PLoS ONE, 8*.	EPI
120	Iftikhar, B., Siddiqui, S., & Rehman, S. (2014). Assessment of the dietary transfer of pesticides to dairy milk and its effect on human health. [Journal article]. *African Journal of Biotechnology, 13*, 476-485.	EXPO
121	Jafarzadeh, M., Nasri Nasrabadi, Z., Sheikhazadi, A., Abbaspour, A., Vasigh, S., Yousefinejad, V., et al. (2013). Is there a role for progesterone in the management of acute organophosphate poisoning during pregnancy? [Article]. *Medical Hypotheses, 80*, 804-805.	REV
122	Jamkhande, P. G., Chintawar, K. D., & Chandak, P. G. (2014a). Teratogenicity: a mechanism based short review on common teratogenic agents. *Asian Pacific Journal of Tropical Disease, 4*, 421-432.	REV
123	Jamkhande, P. G., Chintawar, K. D., & Chandak, P. G. (2014b). Teratogenicity: A mechanism based short review on common teratogenic agents. [Article]. *Asian Pacific Journal of Tropical Disease, 4*, 421-432.	DUPLICATE
124	Jones, K., Everard, M., & Harding, A. H. (2014). Investigation of gastrointestinal effects of organophosphate and carbamate pesticide residues on young children. [Journal article]. *International Journal of Hygiene and Environmental Health, 217*, 392-398.	EPI
125	Kajta, M., & Wojtowicz, A. K. (2013). Impact of endocrine-disrupting chemicals on neural development and the onset of neurological disorders. [Literature Review]. *Pharmacological Reports, 65*, 1632-1639.	REV

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
126	Karki, R. K., & Risal, A. (2012). Study of poisoning cases in a tertiary care hospital. [Article]. *Kathmandu University Medical Journal, 10*, 70-73.	ACR
127	Kauffman, R., Marcus, M., Wold, J. L., & Lu, C. (2009). Pesticide exposure and neurodevelopment in migrant farmworker children of South Georgia. *International Journal of Child and Adolescent Health. Special Issue: Break the cycle of adverse health and developmental disparities for children, 2*(3), 303-312.	REV
128	Kezios, K. L., Liu, X. H., Cirillo, P. M., Cohn, B. A., Kalantzi, O. I., Wang, Y. Z., et al. (2013). Dichlorodiphenyltrichloroethane (DDT), DDT metabolites and pregnancy outcomes. [Article]. *Reproductive Toxicology, 35*, 156-164.	EPI
129	Khan, H. A. A., & Waseem, A. (2014). The effect of temperature on the toxicity of insecticides against Musca domestica L.: implications for the effective management of diarrhea. [Journal article]. *PLoS ONE, 9*, e95636.	TOX
130	Khan, K., Ismail, A. A., Abdel Rasoul, G., Bonner, M. R., Lasarev, M. R., Hendy, O., et al. (2014). Longitudinal assessment of chlorpyrifos exposure and self-reported neurological symptoms in adolescent pesticide applicators. [Article]. *BMJ Open, 4*.	DUPLICATE
131	Khan, K., Ismail, A. A., Abdel Rasoul, G., Bonner, M. R., Lasarev, M. R., Hendy, O., et al. (2014). Longitudinal assessment of chlorpyrifos exposure and self-reported neurological symptoms in adolescent pesticide applicators. [Article]. *BMJ Open, 4*.	EPI
132	Kicinski, M., Vrijens, J., Vermier, G., Hond, E. D., Schoeters, G., Nelen, V., et al. (2015). Neurobehavioral function and low-level metal exposure in adolescents. *International Journal of Hygiene and Environmental Health, 218*, 139-146.	EPI
133	Kim, H.-H., Lim, Y.-W., Yang, J.-Y., Shin, D.-C., Ham, H.-S., Choi, B.-S., et al. (2013a). Health risk assessment of exposure to chlorpyrifos and dichlorvos in children at childcare facilities. [Article]. *Science of the Total Environment, 444*, 441-450.	EXPO

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
134	Kim, H.-H., Lim, Y.-W., Yang, J.-Y., Shin, D.-C., Ham, H.-S., Choi, B.-S., et al. (2013b). Health risk assessment of exposure to chlorpyrifos and dichlorvos in children at childcare facilities. *Science of The Total Environment, 444*, 441-450.	DUPLICATE
135	Kofman, O., Berger, A., Massarwa, A., Friedman, A., & Jaffar, A. A. (2006). Motor inhibition and learning impairments in school-aged children following exposure to organophosphate pesticides in infancy. *Pediatr Res, 60*(1), 88-92.	EPI
136	Koirala, D. P., Rao, K. S., Malla, K. K., & Malla, T. (2013). A study of clinical features, management and outcome of organophosphate and carbamate poisoning in children. [Article]. *Journal of Nepal Paediatric Society, 33*, 85-90.	ACR
137	Koureas, M., Tsakalof, A., Tsatsakis, A., & Hadjichristodoulou, C. (2012). Systematic review of biomonitoring studies to determine the association between exposure to organophosphorus and pyrethroid insecticides and human health outcomes. [article]. *Toxicology letters, 210*, 155-168.	REV
138	Koutroulakis, D., Sifakis, S., Tzatzarakis, M. N., Alegakis, A. K., Theodoropoulou, E., Kavvalakis, M. P., et al. (2014). Dialkyl phosphates in amniotic fluid as a biomarker of fetal exposure to organophosphates in Crete, Greece; association with fetal growth. [Article]. *Reproductive Toxicology, 46*, 98-105.	EPI
139	Kr, M. Z., Öztürk, G. I., Gürler, M., Karaarslan, B., Erden, G. n. I., Karapirli, M., et al. (2013). Pesticide poisoning cases in Ankara and nearby cities in Turkey: An 11-year retrospective analysis. [Article]. *Journal of Forensic and Legal Medicine, 20*, 274-277.	ACR
140	Krishnamurthy, L., Rohini, K., & Shashikala, P. (2012). Socio-demographic profile of organophosphorous poisoning in a tertiary care hospital. [Article]. *Indian Journal of Public Health Research and Development, 3*, 169-171.	ACR
141	Kristensen, P., Irgens, L. M., Andersen, A., Bye, A. S., & Sundheim, L. (1997). Gestational age, birth weight, and perinatal death among births to Norwegian farmers, 1967-1991. *Am J Epidemiol, 146*(4), 329-338.	EPI

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
142	Kumar, M., Vignan Kumar, G., Babu, P., Kumar, S., Subrahmanyam, B., Veeraprasad, M., et al. (2014). A retrospective analysis of acute organophosphorus poisoning cases admitted to the tertiary care teaching hospital in South India. [Article]. *Annals of African Medicine, 13*, 71-75.	ACR
143	Kumar, M., Vignan Kumar, G., Babu, P., Kumar, S., Subrahmanyam, B., Veeraprasad, M., et al. (2014). A retrospective analysis of acute organophosphorus poisoning cases admitted to the tertiary care teaching hospital in South India. [Article]. *Annals of African Medicine, 13*, 71-75.	DUPLICATE
144	Kumar, N., Gupta, A. K., Najotra, D. K., Digra, S. K., Gupta, S., Gupta, P. K., et al. (2013). Incidence and pattern of acute poisoning among children aged 1 month to 18 years: An experience from Jammu. [Article]. *Indian Journal of Forensic Medicine and Toxicology, 7*, 135-138.	ACR
145	Lee, D., Jung, K., Choi, Y., & Cheon, Y. (2014). Body mass index as a prognostic factor in organophosphate-poisoned patients. [Journal article]. *American Journal of Emergency Medicine, 32*, 693-696.	ACR
146	Lee, J., & Freeman, J. L. (2014). Zebrafish as a model for investigating developmental lead (Pb) neurotoxicity as a risk factor in adult neurodegenerative disease: A mini-review. *Neurotoxicology, 43*, 57-64.	TOX
147	Lee, W. J., Ko, Y., & Cha, E. S. (2014). Acute pesticide poisoning among children in south korea: Findings from national health insurance claims data, 2006-2009. [Article]. *Journal of Tropical Pediatrics, 60*, 4-9.	ACR
148	Li, A. A., Levine, T. E., Burns, C. J., & Kent Anger, W. (2012). Integration of epidemiology and animal neurotoxicity data for risk assessment. [Article]. *NeuroToxicology, 33*, 823-832.	REV
149	Li, A. A., Lowe, K. A., McIntosh, L. J., & Mink, P. J. (2012). EVALUATION OF EPIDEMIOLOGY AND ANIMAL DATA FOR RISK ASSESSMENT: CHLORPYRIFOS DEVELOPMENTAL NEUROBEHAVIORAL OUTCOMES. [Literature Review]. *Journal of Toxicology and Environmental Health Part B Critical Reviews, 15*, 109-184.	DUPLICATE

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
150	Li, A. A., Lowe, K. A., McIntosh, L. J., & Mink, P. J. (2012). Evaluation of epidemiology and animal data for risk assessment: chlorpyrifos developmental neurobehavioral outcomes. *J Toxicol Environ Health B Crit Rev, 15*(2), 109-184.	REV
151	Li, J., Yu, N., Zhang, B., Jin, L., Li, M., Hu, M., et al. (2014). Occurrence of organophosphate flame retardants in drinking water from China. [Article]. *Water Research, 54*, 53-61.	DUPLICATE
152	Li, J., Yu, N., Zhang, B., Jin, L., Li, M., Hu, M., et al. (2014). Occurrence of organophosphate flame retardants in drinking water from China. [Article]. *Water Research, 54*, 53-61.	EXPO
153	Liu, P., Wu, C., Chang, X., Qi, X., Zheng, M., & Zhou, Z. (2014). Assessment of chlorpyrifos exposure and absorbed daily doses among infants living in an agricultural area of the Province of Jiangsu, China. [Journal article]. *International Archives of Occupational and Environmental Health, 87*, 753-762.	EXPO
154	Lizardi, P. S., O'Rourke, M. K., & Morris, R. J. (2008). The effects of organophosphate pesticide exposure on Hispanic children's cognitive and behavioral functioning. *J Pediatr Psychol, 33*(1), 91-101.	EPI
155	Llop, S., Julvez, J., Fernandez-Somoano, A., Santa Marina, L., Vizcaino, E., Iñiguez, C., et al. (2013). Prenatal and postnatal insecticide use and infant neuropsychological development in a multicenter birth cohort study. *Environment International, 59*, 175-182.	EPI
156	London, L., Beseler, C., Bouchard, M. F., Bellinger, D. C., Colosio, C., Grandjean, P., et al. (2012). Neurobehavioral and neurodevelopmental effects of pesticide exposures. [Article]. *NeuroToxicology, 33*, 887-896.	REV
157	Lopez-Carrillo, L., & Cebrian, M. E. (2013). *Cognitive Function*: BLACKWELL SCIENCE PUBL, OSNEY MEAD, OXFORD OX2 0EL, UK.	REV

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
158	Lovasi, G. S., Quinn, J. W., Rauh, V. A., Perera, F. P., Andrews, H. F., Garfinkel, R., et al. (2011). Chlorpyrifos exposure and urban residential environment characteristics as determinants of early childhood neurodevelopment. *Am J Public Health, 101*(1), 63-70.	EPI
159	Lozowicka, B. (2015). Health risk for children and adults consuming apples with pesticide residue. [Journal article]. *Science of the Total Environment, 502*, 184-198.	REV
160	Lu, C., & Andres, L. (2012). Reconstructing organophosphorus pesticide doses using the reversed dosimetry approach in a simple physiologically-based pharmacokinetic model. [Article]. *Journal of Toxicology, 2012*.	EXPO
161	Lu, C., Essig, C., Root, C., Rohlman, D. S., McDonald, T., & Sulzbacher, S. (2009). Assessing the association between pesticide exposure and cognitive development in rural Costa Rican children living in organic and conventional coffee farms. *Int J Adolesc Med Health, 21*(4), 609-621.	EPI
162	Marks, A. R., Harley, K., Bradman, A., Kogut, K., Barr, D. B., Johnson, C., et al. (2010). Organophosphate pesticide exposure and attention in young Mexican-American children: the CHAMACOS study. *Environ Health Perspect, 118*(12), 1768-1774.	EPI
163	Marri, M. Z., Mirza, F. H., & Hasan, Q. (2012). Pattern, incidence and mortality rate of acute poisonings at Karachi- one year study. [Article]. *Medical Forum Monthly, 23*, 6-10.	ACR
164	Martin, I. G., Thottempudi, S. R. K., & Frattarelli, D. A. C. (2013). Changes to Prescription Drug Pediatric Labeling: Awareness by Practicing Pediatricians. [Article]. *Drug Information Journal, 47*, 214-218.	OTH
165	Mascarelli, A. (2013). Growing up with pesticides. [Note]. *Science, 341*, 740-741.	REV

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
166	McGinnis, W. R., Audhya, T., & Edelson, S. M. (2013). Proposed toxic and hypoxic impairment of a brainstem locus in autism. [Article]. *International Journal of Environmental Research and Public Health, 10*, 6955-7000.	REV
167	Memon, A., Shaikh, J. M., Kazi, S. A. F., & Kazi, A. (2012). Changing trends in deliberate self poisoning at Hyderabad. [Article]. *Journal of the Liaquat University of Medical and Health Sciences, 11*, 124-126.	ACR
168	Meyer-Baron, M., Kim, E. A., Nuwayhid, I., Ichihara, G., & Kang, S.-K. (2012). Occupational exposure to neurotoxic substances in Asian countries - Challenges and approaches. [Article]. *NeuroToxicology, 33*, 853-861.	REV
169	Michalakis, M., Tzatzarakis, M. N., Kovatsi, L., Alegakis, A. K., Tsakalof, A. K., Heretis, I., et al. (2014). Hypospadias in offspring is associated with chronic exposure of parents to organophosphate and organochlorine pesticides. [Article]. *Toxicology Letters, 230*, 139-145.	EPI
170	Mink, P. J., Kimmel, C. A., & Li, A. A. (2012). POTENTIAL EFFECTS OF CHLORPYRIFOS ON FETAL GROWTH OUTCOMES: IMPLICATIONS FOR RISK ASSESSMENT. [Article]. *Journal of Toxicology and Environmental Health Part B Critical Reviews, 15*, 281-316.	REV
171	Mink, P. J., Kimmel, C. A., & Li, A. A. (2012). Potential effects of chlorpyrifos on fetal growth outcomes: implications for risk assessment. *J Toxicol Environ Health B Crit Rev, 15*(4), 281-316.	DUPLICATE
172	Moreno-Banda, G., Blanco-Munoz, J., Lacasana, M., Rothenberg, S. J., Aguilar-Garduno, C., Gamboa, R., et al. (2009). Maternal exposure to floricultural work during pregnancy, PON1 Q192R polymorphisms and the risk of low birth weight. *Sci Total Environ, 407*(21), 5478-5485.	EPI
173	Morgan, M. K., & Jones, P. A. (2013). Dietary predictors of young children's exposure to current-use pesticides using urinary biomonitoring. [Article]. *Food and Chemical Toxicology, 62*, 131-141.	EXPO

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
174	Morgan, M. K., Wilson, N. K., & Chuang, J. C. (2014). Exposures of 129 preschool children to organochlorines, organophosphates, pyrethroids, and acid herbicides at their homes and daycares in North Carolina. [Article]. *International Journal of Environmental Research and Public Health, 11*, 3743-3764.	EXPO
175	Morgan, M. K., Wilson, N. K., & Chuang, J. C. (2014). Exposures of 129 preschool children to organochlorines, organophosphates, pyrethroids, and acid herbicides at their homes and daycares in North Carolina. [Journal article]. *International Journal of Environmental Research and Public Health, 11*, 3743-3764.	DUPLICATE
176	Mosaddegh, M. H., Emami, F., & Asghari, G. (2014). Evaluation of residual diazinon and chlorpiryfos in children herbal medicines by headspace-SPME and GC-FID. [Article]. *Iranian Journal of Pharmaceutical Research, 13*, 541-549.	EXPO
177	Munoz-Quezada, M. T., Lucero, B. A., Barr, D. B., Steenland, K., Levy, K., Ryan, B., et al. (2013). Neurodevelopmental effects in children associated with exposure to organophosphate pesticides: A systematic review. *Neurotoxicology, 39*, 158-168.	REV
178	Munoz-Quezada, M. T., Lucero, B. A., Barr, D. B., Steenland, K., Levy, K., Ryan, P. B., et al. (2013). Neurodevelopmental effects in children associated with exposure to organophosphate pesticides: A systematic review. [article]. *Neurotoxicology (Park Forest South), 39*, 158-168.	DUPLICATE
179	Naseh, M., & Vatanparast, J. (2014). Enhanced expression of hypothalamic nitric oxide synthase in rats developmentally exposed to organophosphates. [Journal article]. *Brain Research, 1579*, 10-19.	TOX
180	Neal, R. E., Chen, J., Jagadapillai, R., Jang, H., Abomoelak, B., Brock, G., et al. (2014). Developmental cigarette smoke exposure: Hippocampus proteome and metabolome profiles in low birth weight pups. *Toxicology, 317*, 40-49.	TOX
181	Nevison, C. D. (2014). A comparison of temporal trends in United States autism prevalence to trends in suspected environmental factors. [Article]. *Environmental Health: A Global Access Science Source, 13*.	EPI

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
182	Oates, L., Cohen, M., Braun, L., Schembri, A., & Taskova, R. (2014). Reduction in urinary organophosphate pesticide metabolites in adults after a week-long organic diet. [Journal article]. *Environmental Research, 132*, 105-111.	EXPO
183	Ostrea Jr, E. M., Reyes, A., Villanueva-Uy, E., Pacifico, R., Benitez, B., Ramos, E., et al. (2012). Fetal exposure to propoxur and abnormal child neurodevelopment at 2 years of age. [Article]. *NeuroToxicology, 33*, 669-675.	DUPLICATE
184	Ostrea, E. M., Bielawski, D. M., Posecion, N. C., Corrion, M., Villanueva-Uy, E., Bernardo, R. C., et al. (2009). Combined analysis of prenatal (maternal hair and blood) and neonatal (infant hair, cord blood and meconium) matrices to detect fetal exposure to environmental pesticides. *Environ Res, 109*(1), 116-122.	EXPO
185	Ostrea, E. M., Bielawski, D. M., Posecion, N. C., Corrion, M., Villanueva-Uy, E., Jin, Y., et al. (2008). A comparison of infant hair, cord blood and meconium analysis to detect fetal exposure to environmental pesticides. [Evaluation Studies Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S.]. *Environ Res, 106*(2), 277-283.	EXPO
186	Ostrea, E. M., Morales, V., Ngoumgna, E., Prescilla, R., Tan, E., Hernandez, E., et al. (2002). Prevalence of fetal exposure to environmental toxins as determined by meconium analysis. *NeuroToxicology, 23*(3), 329-339.	EXPO
187	Ostrea, E. M., Reyes, A., Villanueva-Uy, E., Pacifico, R., Benitez, B., Ramos, E., et al. (2011). Fetal exposure to propoxur and abnormal child neurodevelopment at 2 years of age. *NeuroToxicology*.	EPI
188	Ostrea, E. M., Villanueva-Uy, E., Bielawski, D. M., Posecion, N. C., Corrion, M. L., Jin, Y., et al. (2006). Maternal hair--an appropriate matrix for detecting maternal exposure to pesticides during pregnancy. *Environ Res, 101*(3), 312-322.	EXPO

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
189	Oulhote, Y., & Bouchard, M. F. (2013). Urinary metabolites of organophosphate and pyrethroid pesticides and behavioral problems in Canadian children. [Article]. *Environmental Health Perspectives, 121*, 1378-1384.	EPI
190	Padmanabha, T. S., Gumma, K., & Kulkarni, G. P. (2014). Study of profile of organophosphorus poisoning cases in a tertiary care hospital, North Karnataka, Bidar, India. [Article]. *International Journal of Pharma and Bio Sciences, 5*, P332-P339.	ACR
191	Papoutsis, I., Mendonis, M., Nikolaou, P., Athanaselis, S., Pistos, C., Maravelias, C., et al. (2012). Development and Validation of a Simple GC-MS Method for the Simultaneous Determination of 11 Anticholinesterase Pesticides in Blood-Clinical and Forensic Toxicology Applications. [Article]. *Journal of Forensic Sciences, 57*, 806-812.	EXPO
192	Patel, A. B., Dewan, A., & Kaji, B. C. (2012). Monocrotophos poisoning through contaminated millet flour. [Article]. *Arhiv za Higijenu Rada i Toksikologiju, 63*, 377-383.	ACR
193	Perry, L., Adams, R. D., Bennett, A. R., Lupton, D. J., Jackson, G., Good, A. M., et al. (2014). National toxicovigilance for pesticide exposures resulting in health care contact-An example from the UK's National Poisons Information Service. [Article]. *Clinical Toxicology, 52*, 549-555.	ACR
194	Peshin, S. S., Srivastava, A., Halder, N., & Gupta, Y. K. (2014). Pesticide poisoning trend analysis of 13 years: A retrospective study based on telephone calls at the National Poisons Information Centre, All India Institute of Medical Sciences, New Delhi. [Article]. *Journal of Forensic and Legal Medicine, 22*, 57-61.	ACR
195	Petit, C., Chevrier, C., Durand, G., Monfort, C., Rouget, F., Garlantezec, R., et al. (2010). Impact on fetal growth of prenatal exposure to pesticides due to agricultural activities: A prospective cohort study in Brittany, France. [Research Support, Non-U.S. Gov't]. *Environ Health, 9*, 71.	EPI
196	Phillips, M. L. (2006). Registering skepticism: Does the EPA'S pesticide review protect children? *Environmental Health Perspectives, 114*(10), A592-A595.	REV

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
197	Pillai, H. K., Fang, M. L., Beglov, D., Kozakov, D., Vajda, S., Stapleton, H. M., et al. (2014). Ligand binding and activation of PPARγ by Firemaster® 550: effects on adipogenesis and osteogenesis in vitro. [Journal article]. *Environmental Health Perspectives, 122*, 1225-1232.	TOX
198	Polanska, K., Jurewicz, J., & Hanke, W. (2013). REVIEW OF CURRENT EVIDENCE ON THE IMPACT OF PESTICIDES, POLYCHLORINATED BIPHENYLS AND SELECTED METALS ON ATTENTION DEFICIT/HYPERACTIVITY DISORDER IN CHILDREN. [Literature Review]. *International Journal of Occupational Medicine and Environmental Health, 26*, 16-38.	REV
199	Potera, C. (2012). Newly discovered mechanism for cchlorpyrifos eeffects on neurodevelopment. [Article]. *Environmental Health Perspectives, 120*, A270.	REV
200	Prapamontol, T., Sutan, K., Laoyang, S., Hongsibsong, S., Lee, G., Yano, Y., et al. (2014). Cross validation of gas chromatography-flame photometric detection and gas chromatography—mass spectrometry methods for measuring dialkylphosphate metabolites of organophosphate pesticides in human urine. [article]. *International journal of hygiene and environmental health, 217*, 554-566.	EXPO
201	Prapamontol, T., Sutan, K., Laoyang, S., Hongsibsong, S., Lee, G., Yano, Y., et al. (2014a). Cross validation of gas chromatography-flame photometric detection and gas chromatography-mass spectrometry methods for measuring dialkylphosphate metabolites of organophosphate pesticides in human urine. *International Journal of Hygiene and Environmental Health, 217*(4-5), 554-566.	DUPLICATE
202	Prapamontol, T., Sutan, K., Laoyang, S., Hongsibsong, S., Lee, G., Yano, Y., et al. (2014b). Cross validation of gas chromatography-flame photometric detection and gas chromatography–mass spectrometry methods for measuring dialkylphosphate metabolites of organophosphate pesticides in human urine. *International Journal of Hygiene and Environmental Health, 217*, 554-566.	DUPLICATE
203	Quiros-Alcala, L., Alkon, A. D., Boyce, W. T., Lippert, S., Davis, N. V., Bradman, A., et al. (2011). Maternal prenatal and child organophosphate pesticide exposures and children's autonomic function. *Neurotoxicology, 32*(5), 646-655.	EPI

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
204	Quirós-Alcalá, L., Bradman, A., Smith, K., Weerasekera, G., Odetokun, M., Barr, D. B., et al. (2012). Organophosphorous pesticide breakdown products in house dust and children's urine. [Article]. *Journal of Exposure Science and Environmental Epidemiology, 22*, 559-568.	EXPO
205	Radwan, N. M., & El-Nabarawy, N. A. (2014). The role of muscle injury in organophosphate poisoning and development of intermediate syndrome. [Article]. *International Journal of Pharmaceutical Sciences Review and Research, 28*, 61-66.	ACR
206	Rajanandh, M. G., Santhosh, S., & Ramasamy, C. (2013). Prospective analysis of poisoning cases in a super specialty hospital in India. [Article]. *Journal of Pharmacology and Toxicology, 8*, 60-66.	ACR
207	Rajasekharan, C., Renjith, S. W., & Jayapal, T. (2012). Opsoclonus and lingual myoclonus due to organophosphate poisoning: images in clinical medicine. [Article]. *BMJ case reports, 2012*.	ACR
208	Ram, P., Kanchan, T., & Unnikrishnan, B. (2014). Pattern of acute poisonings in children below 15 years - A study from Mangalore, South India. [Article]. *Journal of Forensic and Legal Medicine, 25*, 26-29.	ACR
209	Ramanath, K. V., & Naveen Kumar, H. D. (2012). Study the assessment of poisoning cases in a rural tertiary care teaching hospital by a clinical pharmacist. [Article]. *Asian Journal of Pharmaceutical and Clinical Research, 5*, 138-141.	ACR
210	Rauch, S. A., Braun, J. M., Barr, D. B., Calafat, A. M., Khoury, J., Montesano, M. A., et al. (2012). Associations of prenatal exposure to organophosphate pesticide metabolites with gestational age and birth weight. [Article]. *Environmental Health Perspectives, 120*, 1055-1060.	EPI
211	Rauh, V. A., Garfinkel, R., Perera, F. P., Andrews, H. F., Hoepner, L., Barr, D. B., et al. (2006). Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children. *Pediatrics, 118*(6), e1845-1859.	EPI

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
212	Rauh, V. A., Perera, F. P., Horton, M. K., Whyatt, R. M., Bansal, R., Hao, X. J., et al. (2012). Brain anomalies in children exposed prenatally to a common organophosphate pesticide. [Article]. *Proceedings of the National Academy of Sciences of the United States of America, 109*(20), 7871-7876.	DUPLICATE
213	Rauh, V. A., Perera, F. P., Horton, M. K., Whyatt, R. M., Bansal, R., Hao, X., et al. (2012). Brain anomalies in children exposed prenatally to a common organophosphate pesticide. [Article]. *Proceedings of the National Academy of Sciences of the United States of America, 109*, 7871-7876.	DUPLICATE
214	Rauh, V. A., Perera, F. P., Horton, M. K., Whyatt, R. M., Bansal, R., Hao, X., et al. (2012). Brain anomalies in children exposed prenatally to a common organophosphate pesticide. *Proc Natl Acad Sci U S A, 109*(20), 7871-7876.	EPI
215	Rauh, V., Arunajadai, S., Horton, M., Perera, F., Hoepner, L., Barr, D. B., et al. (2011). Seven-year neurodevelopmental scores and prenatal exposure to chlorpyrifos, a common agricultural pesticide. *Environ Health Perspect, 119*(8), 1196-1201.	EPI
216	Redmond, E., Egeston, C., & Bralley, J. A. (2012). Low level prenatal exposure to organophosphate pesticides significantly lowers IQ in children. *Townsend Letter*(342), 58-61.	REV
217	Ridano, M. E., Racca, A. C., Flores-Martin, J., Camolotto, S. A., De Potas, G. M., Genti-Raimondi, S., et al. (2012). Chlorpyrifos modifies the expression of genes involved in human placental function. [article]. *Reproductive toxicology (Elmsford, NY), 33*, 331-338.	TOX
218	Ridano, M. E., Racca, A. C., Flores-Martín, J., Camolotto, S. A., de Potas, G. M., Genti-Raimondi, S., et al. (2012). Chlorpyrifos modifies the expression of genes involved in human placental function. *Reproductive Toxicology, 33*, 331-338.	DUPLICATE
219	Roberts, J. R., & Karr, C. J. (2012). Pesticide Exposure in Children. [Article]. *Pediatrics, 130*, E1765-E1788.	REV

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
220	Roca, M., Leon, N., Pastor, A., & Yusà, V. (2014). Comprehensive analytical strategy for biomonitoring of pesticides in urine by liquid chromatography-orbitrap high resolution mass spectrometry. [Article]. *Journal of Chromatography A, 1374*, 66-76.	EXPO
221	Roca, M., Miralles-Marco, A., Ferré, J., Pérez, R., & Yusà, V. (2014). Biomonitoring exposure assessment to contemporary pesticides in a school children population of Spain. [Article]. *Environmental Research, 131*, 77-85.	DUPLICATE
222	Roca, M., Miralles-Marco, A., Ferré, J., Pérez, R., & Yusà, V. (2014). Biomonitoring exposure assessment to contemporary pesticides in a school children population of Spain. [Article]. *Environmental Research, 131*, 77-85.	EXPO
223	Rodriguez, T., Van Wendel De Joode, B., Lindh, C. H., Rojas, M., Lundberg, I., & Wesseling, C. (2012). Assessment of long-term and recent pesticide exposure among rural school children in Nicaragua. [article]. *Occupational and environmental medicine (London), 69*, 119-125.	DUPLICATE
224	Rodriguez, T., van Wendel de Joode, B., Lindh, C. H., Rojas, M., Lundberg, I., & Wesseling, C. (2012). Assessment of long-term and recent pesticide exposure among rural school children in Nicaragua. *Occup Environ Med, 69*(2), 119-125.	EXPO
225	Rogers, T. D., McKimm, E., Dickson, P. E., Goldowitz, D., Blaha, C. D., & Mittleman, G. (2013). Is autism a disease of the cerebellum?: An integration of clinical and pre-clinical research. [Article]. *Frontiers in Systems Neuroscience.*	REV
226	Rohitrattana, J., Siriwong, W., Tunsaringkarn, T., Panuwet, P., Ryan, P. B., Barr, D. B., et al. (2014). Organophosphate pesticide exposure in school-aged children living in rice and aquacultural farming regions of Thailand. [Journal article]. *Journal of Agromedicine, 19*, 406-416.	EXPO
227	Rohlman, D. S., Arcury, T. A., Quandt, S. A., Lasarev, M., Rothlein, J., Travers, R., et al. (2005). Neurobehavioral performance in preschool children from agricultural and non-agricultural communities in Oregon and North Carolina. *NeuroToxicology, 26*(4 Spec. Iss.), 589-598.	EPI

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
228	Rohlman, D. S., Ismail, A. A., Abdel-Rasoul, G., Lasarev, M., Hendy, O., & Olson, J. R. (2014). Characterizing exposures and neurobehavioral performance in Egyptian adolescent pesticide applicators. [Article]. *Metabolic Brain Disease, 29*, 845-855.	EPI
229	Rohlman, D. S., Lasarev, M., Anger, W. K., Scherer, J., Stupfel, J., & McCauley, L. (2007a). Neurobehavioral performance of adult and adolescent agricultural workers. *Neurotoxicology, 28*(2), 374-380.	EPI
230	Rohlman, D. S., Lasarev, M., Anger, W. K., Scherer, J., Stupfel, J., & McCauley, L. (2007b). Neurobehavioral performance of adult and adolescent agricultural workers. *Neurotoxicology, 28*(2), 374-380.	DUPLICATE
231	Rojas, M., Agreda, O., & Infante, S. (2008). A preliminary statistical study of whether pesticide use could be related to birth defects in a rural area of Venezuela. *Rev Salud Publica (Bogota), 10*(1), 85-93.	EPI
232	Ross, M. K., Borazjani, A., Mangum, L. C., Wang, R., & Crow, J. A. (2014). Effects of toxicologically relevant xenobiotics and the lipid-derived electrophile 4-hydroxynonenal on macrophage cholesterol efflux: Silencing carboxylesterase 1 has paradoxical effects on cholesterol uptake and efflux. [Article]. *Chemical Research in Toxicology, 27*, 1743-1756.	TOX
233	Ross, S. M., McManus, I. C., Harrison, V., & Mason, O. (2013). Neurobehavioral problems following low-level exposure to organophosphate pesticides: a systematic and meta-analytic review. [Literature Review]. *Critical Reviews in Toxicology, 43*, 21-44.	REV
234	Roth, N., & Wilks, M. F. (2014). Neurodevelopmental and neurobehavioural effects of polybrominated and perfluorinated chemicals: A systematic review of the epidemiological literature using a quality assessment scheme. *Toxicology Letters, 230*, 271-281.	REV
235	Ruckart, P. Z., Kakolewski, K., Bove, F. J., & Kaye, W. E. (2004). Long-term neurobehavioral health effects of methyl parathion exposure in children in Mississippi and Ohio. [Comparative Study]. *Environ Health Perspect, 112*(1), 46-51.	EPI

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
236	Samarawickrema, N., Pathmeswaran, A., Wickremasinghe, R., Peiris-John, R., Karunaratna, M., Buckley, N., et al. (2008). Fetal effects of environmental exposure of pregnant women to organophosphorus compounds in a rural farming community in Sri Lanka. *Clin Toxicol (Phila), 46*(6), 489-495.	EPI
237	Santhosh, C. S., Kumar, S., & Nawaz, B. (2012). Profile of poisoning cases autopsied at district government hospital, Davangere. [Article]. *Indian Journal of Forensic Medicine and Toxicology, 6*, 104-106.	ACR
238	Savitz, D. A., Arbuckle, T., Kaczor, D., & Curtis, K. M. (1997). Male pesticide exposure and pregnancy outcome. *Am J Epidemiol, 146*(12), 1025-1036.	EPI
239	Schug, T. T., Barouki, R., Gluckman, P. D., Grandjean, P., Hanson, M., & Heindel, J. J. (2013). PPTOX III: Environmental stressors in the developmental origins of disease-Evidence and mechanisms. [Article]. *Toxicological Sciences, 131*, 343-350.	REV
240	Selvam, V., Panneer Selvam, G., & Vijayanath, V. (2012). Study of death incidence by insecticide poisoning in Salem. [Article]. *International Journal of Medical Toxicology and Forensic Medicine, 2*, 20-26.	ACR
241	Senarathna, L., Jayamanna, S. F., Kelly, P. J., Buckley, N. A., Dibley, M. J., & Dawson, A. H. (2012). Changing epidemiologic patterns of deliberate self poisoning in a rural district of Sri Lanka. [Article]. *BMC public health, 12*, 593.	ACR
242	Sexton, K., Salinas, J. J., McDonald, T. J., Gowen, R. M. Z., Miller, R. P., McCormick, J. B., et al. (2013). Biomarkers of maternal and fetal exposure to organochlorine pesticides measured in pregnant hispanic women from brownsville, texas. [Article]. *International Journal of Environmental Research and Public Health, 10*, 237-248.	EXPO
243	Shelton, J. F., Geraghty, E. M., Tancredi, D. J., Delwiche, L. D., Schmidt, R. J., Ritz, B., et al. (2014). Neurodevelopmental disorders and prenatal residential proximity to agricultural pesticides: The charge study. [Article]. *Environmental Health Perspectives, 122*, 1103-1109.	EPI

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
244	Shelton, J. F., Geraghty, E. M., Tancredi, D. J., Delwiche, L. D., Schmidt, R. J., Ritz, B., et al. (2014). Neurodevelopmental Disorders and Prenatal Residential Proximity to Agricultural Pesticides: The CHARGE Study. *Environmental Health Perspectives, 122*(10), 1103-1109.	DUPLICATE
245	Shomar, B., Al-Saad, K., & Nriagu, J. (2014). Mishandling and exposure of farm workers in Qatar to organophosphate pesticides. *Environmental Research, 133*, 312-320.	EXPO
246	Simescu, M., Igna, C. P., Nicolaescu, E., Ion, I., Ion, A. C., Caragheorgheopol, A., et al. (2014). MULTIPLE PESTICIDES EXPOSURE OF GREENHOUSE WORKERS AND THYROID PARAMETERS. [article]. *International journal of sustainable development and planning (Print), 9*, 15-28.	EPI
247	Simescu, M., Igna, C. P., Nicolaescu, E., Ion, I., Ion, A. C., Caragheorgheopol, A., et al. (2014). Multiple pesticides exposure of greenhouse workers and thyroid parameters. [Journal article]. *International Journal of Sustainable Development and Planning, 9*, 15-28.	DUPLICATE
248	Singh, A. K., Kashyap, M. P., Jahan, S., Kumar, V., Tripathi, V. K., Siddiqui, M. A., et al. (2012). Expression and inducibility of cytochrome P450s (CYP1A1, 2B6, 2E1, 3A4) in human cord blood CD34+ stem cell-derived differentiating neuronal cells. [Article]. *Toxicological Sciences, 129*, 392-410.	TOX
249	Slomski, A. (2012). Chronic mental health issues in children now loom larger than physical problems. [Short Survey]. *JAMA - Journal of the American Medical Association, 308*, 223-225.	REV
250	Slotkin, T. A., Card, J., & Seidler, F. J. (2013). Adverse benzoapyrene effects on neurodifferentiation are altered by other neurotoxicant coexposures: Interactions with dexamethasone, chlorpyrifos, or nicotine in PC12 cells. [Article]. *Environmental Health Perspectives, 121*, 825-831.	TOX
251	Slotkin, T. A., Card, J., & Seidler, F. J. (2014). Prenatal dexamethasone, as used in preterm labor, worsens the impact of postnatal chlorpyrifos exposure on serotonergic pathways. [Journal article]. *Brain Research Bulletin, 100*, 44-54.	TOX

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
252	Smith, J. N., Hinderliter, P. M., Timchalk, C., Bartels, M. J., & Poet, T. S. (2014). A human life-stage physiologically based pharmacokinetic and pharmacodynamic model for chlorpyrifos: Development and validation. [Article]. *Regulatory Toxicology and Pharmacology, 69*, 580-597.	TOX
253	Smith, J. N., Hinderliter, P. M., Timchalk, C., Bartels, M. J., & Poet, T. S. (2014). A human life-stage physiologically based pharmacokinetic and pharmacodynamic model for chlorpyrifos: Development and validation. *Regulatory Toxicology and Pharmacology, 69*(3), 580-597.	DUPLICATE
254	Snijder, C. A., Heederik, D., Pierik, F. H., Hofman, A., Jaddoe, V. W., Koch, H. M., et al. (2013). Fetal growth and prenatal exposure to bisphenol A: The generation R study. [Article]. *Environmental Health Perspectives, 121*, 393-396.	EPI
255	Stapleton, H. M., Misenheimer, J., Hoffman, K., & Webster, T. F. (2014). Flame retardant associations between children's handwipes and house dust. [Article]. *Chemosphere, 116*, 54-60.	EXPO
256	Suarez-Lopez, J. R., Himes, J. H., Jacobs Jr, D. R., Alexander, B. H., & Gunnar, M. R. (2013). Acetylcholinesterase activity and neurodevelopment in boys and girls. [Article]. *Pediatrics, 132*, e1649-e1658.	EPI
257	Suarez-Lopez, J. R., Jacobs Jr, D. R., Himes, J. H., & Alexander, B. H. (2013). Acetylcholinesterase activity, cohabitation with floricultural workers, and blood pressure in Ecuadorian children. [Article]. *Environmental Health Perspectives, 121*, 619-624.	EPI
258	Suarez-Lopez, J. R., Jacobs, D. R., Himes, J. H., Alexander, B. H., Lazovich, D., & Gunnar, M. (2012). Lower acetylcholinesterase activity among children living with flower plantation workers. [Article]. *Environmental Research, 114*, 53-59.	EPI
259	Tajima, S., Araki, A., Kawai, T., Tsuboi, T., Ait Bamai, Y., Yoshioka, E., et al. (2014). Detection and intake assessment of organophosphate flame retardants in house dust in Japanese dwellings. [Article]. *Science of the Total Environment, 478*, 190-199.	EXPO

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
260	Tajima, S., Araki, A., Kawai, T., Tsuboi, T., Bamai, Y. A., Yoshioka, E., et al. (2014). Detection and intake assessment of organophosphate flame retardants in house dust in Japanese dwellings. [Journal article]. *Science of the Total Environment, 478*, 190-199.	DUPLICATE
261	Tan, J., Loganath, A., Chong, Y. S., & Obbard, J. P. (2009). Exposure to persistent organic pollutants in utero and related maternal characteristics on birth outcomes: A multivariate data analysis approach. *Chemosphere, 74*(3), 428-433.	EPI
262	Taxvig, C., Rosenmai, A. K., & Vinggaard, A. M. (2014). Polyfluorinated alkyl phosphate ester surfactants - current knowledge and knowledge gaps. [Article]. *Basic and Clinical Pharmacology and Toxicology, 115*, 41-44.	REV
263	Thompson, B., Griffith, W. C., Barr, D. B., Coronado, G. D., Vigoren, E. M., & Faustman, E. M. (2014). Variability in the take-home pathway: farmworkers and non-farmworkers and their children. [Journal article]. *Journal of Exposure Science and Environmental Epidemiology, 24*, 522-531.	EXPO
264	Torres-Sanchez, L., Rothenberg, S. J., Schnaas, L., Cebrian, M. E., Osorio, E., Del Carmen Hernandez, M., et al. (2007). In utero p,p'-DDE exposure and infant neurodevelopment: A perinatal cohort in Mexico. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Environ Health Perspect, 115*(3), 435-439.	EPI
265	Torres-Sanchez, L., Schnaas, L., Cebrian, M. E., Hernandez Mdel, C., Valencia, E. O., Garcia Hernandez, R. M., et al. (2009). Prenatal dichlorodiphenyldichloroethylene (DDE) exposure and neurodevelopment: a follow-up from 12 to 30 months of age. [Research Support, Non-U.S. Gov't]. *NeuroToxicology, 30*(6), 1162-1165.	EPI
266	Trairatvorakul, C., & Detsomboonrat, P. (2012). Success rates of a mixture of ciprofloxacin, metronidazole, and minocycline antibiotics used in the non-instrumentation endodontic treatment of mandibular primary molars with carious pulpal involvement. [Article]. *International Journal of Paediatric Dentistry, 22*, 217-227.	OTH
267	Trunnelle, K. J., Bennett, D. H., Tulve, N. S., Clifton, M. S., Davis, M. D., Calafat, A. M., et al. (2014). Urinary pyrethroid and chlorpyrifos metabolite concentrations in northern California families and their relationship to indoor residential insecticide levels, part of the Study of Use of Products and Exposure Related Behavior (SUPERB). [Article]. *Environmental Science and*	DUPLICATE

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
	Technology, 48, 1931-1939.	
268	Trunnelle, K. J., Bennett, D. H., Tulve, N. S., Clifton, M. S., Davis, M. D., Calafat, A. M., et al. (2014). Urinary pyrethroid and chlorpyrifos metabolite concentrations in northern California families and their relationship to indoor residential insecticide levels, part of the Study of Use of Products and Exposure Related Behavior (SUPERB). [Journal article]. *Environmental Science & Technology, 48*, 1931-1939.	DUPLICATE
269	Trunnelle, K. J., Bennett, D. H., Tulve, N. S., Clifton, M. S., Davis, M. D., Calafat, A. M., et al. (2014). Urinary Pyrethroid and Chlorpyrifos Metabolite Concentrations in Northern California Families and Their Relationship to Indoor Residential Insecticide Levels, Part of the Study of Use of Products and Exposure Related Behavior (SUPERB). [Article]. *Environmental Science & Technology, 48*(3), 1931-1939.	EXPO
270	Ueyama, J., Saito, I., Takaishi, A., Nomura, H., Inoue, M., Osaka, A., et al. (2014). A revised method for determination of dialkylphosphate levels in human urine by solid-phase extraction and liquid chromatography with tandem mass spectrometry: application to human urine samples from Japanese children. [Journal article]. *Environmental Health and Preventive Medicine, 19*, 405-413.	EXPO
271	van Balen, E. C., Wolansky, M. J., & Kosatsky, T. (2012). Increasing use of pyrethroids in Canadian households: Should we be concerned? [Note]. *Canadian Journal of Public Health, 103*, 404-407.	REV
272	Van Dyke, M., Martyny, J. W., & Serrano, K. A. (2014). Methamphetamine Residue Dermal Transfer Efficiencies from Household Surfaces. [Article]. *Journal of Occupational and Environmental Hygiene, 11*(4), 249-258.	EXPO
273	Van Thriel, C., Westerink, R. H. S., Beste, C., Bale, A. S., Lein, P. J., & Leist, M. (2012). Translating neurobehavioural endpoints of developmental neurotoxicity tests into in vitro assays and readouts. [Article]. *NeuroToxicology, 33*, 911-924.	REV

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
274	Van Wendel De Joode, B., Barraza, D., Ruepert, C., Mora, A. M., Cordoba, L., ÖBerg, M., et al. (2012). Indigenous children living nearby plantations with chlorpyrifos-treated bags have elevated 3,5,6-trichloro-2-pyridinol (TCPy) urinary concentrations. [article]. *Environmental research (New York, N.Y. : Print), 117*, 17-26.	DUPLICATE
275	Veale, D. J. H., Wium, C. A., & Müller, G. J. (2013). Toxicovigilance I: A survey of acute poisonings in South Africa based on Tygerberg Poison Information centre data. [Article]. *South African Medical Journal, 103*, 293-297.	ACR
276	Venerosi, A., Ricceri, L., Tait, S., & Calamandrei, G. (2012). Sex dimorphic behaviors as markers of neuroendocrine disruption by environmental chemicals: The case of chlorpyrifos. [Article]. *NeuroToxicology, 33*, 1420-1426.	TOX
277	Vera, B., Cruz, S. S., & Magnarelli, G. (2012). Plasma cholinesterase and carboxylesterase activities and nuclear and mitochondrial lipid composition of human placenta associated with maternal exposure to pesticides. [Article]. *Reproductive Toxicology, 34*(3), 402-407.	TOX
278	Vera, B., Santa Cruz, S., & Magnarelli, G. (2012). Plasma cholinesterase and carboxylesterase activities and nuclear and mitochondrial lipid composition of human placenta associated with maternal exposure to pesticides. [article]. *Reproductive toxicology (Elmsford, NY), 34*, 402-407.	DUPLICATE
279	Vogt, R., Bennett, D., Cassady, D., Frost, J., Ritz, B., & Hertz-Picciotto, I. (2012). Cancer and non-cancer health effects from food contaminant exposures for children and adults in California: A risk assessment. [Article]. *Environmental Health: A Global Access Science Source, 11.*	REV
280	Wang, P., Tian, Y., Wang, X. J., Gao, Y., Shi, R., Wang, G. Q., et al. (2011). Organophosphate pesticide exposure and perinatal outcomes in Shanghai, China. *Environ Int*.	DUPLICATE
281	Wang, P., Tian, Y., Wang, X.-J., Gao, Y., Shi, R., Wang, G.-Q., et al. (2012). Organophosphate pesticide exposure and perinatal outcomes in Shanghai, China. [Article]. *Environment International, 42*, 100-104.	EPI

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
282	Wason, S. C., Julien, R., Perry, M. J., Smith, T. J., & Levy, J. I. (2013). Modeling exposures to organophosphates and pyrethroids for children living in an urban low-income environment. [Article]. *Environmental Research, 124*, 13-22.	EXPO
283	Wason, S. C., Smith, T. J., Perry, M. J., & Levy, J. I. (2012). Using physiologically-based pharmacokinetic models to incorporate chemical and non-chemical stressors into cumulative risk assessment: A case study of pesticide exposures. [Article]. *International Journal of Environmental Research and Public Health, 9*, 1971-1983.	EXPO
284	Whyatt, R. M., Rauh, V., Barr, D. B., Camann, D. E., Andrews, H. F., Garfinkel, R., et al. (2004). Prenatal insecticide exposures and birth weight and length among an urban minority cohort. *Environ Health Perspect, 112*(10), 1125-1132.	EPI
285	Wickerham, E. L., Lozoff, B., Jie, S., Kaciroti, N., Yankai, X. I. A., & Meeker, J. D. (2012). Reduced birth weight in relation to pesticide mixtures detected in cord blood of full-term infants. [article]. *Environment international, 47*, 80-85.	EPI
286	Wickerham, E. L., Lozoff, B., Shao, J., Kaciroti, N., Xia, Y. K., & Meeker, J. D. (2012). Reduced birth weight in relation to pesticide mixtures detected in cord blood of full-term infants. [Article]. *Environment International, 47*, 80-85.	DUPLICATE
287	Williams, A. L., & DeSesso, J. M. (2014). Gestational/ Perinatal chlorpyrifos exposure is not associated with autistic-like behaviors in rodents. [Literature Review]. *Critical Reviews in Toxicology, 44*, 523-534.	REV
288	Wolff, M. S., Engel, S., Berkowitz, G., Teitelbaum, S., Siskind, J., Barr, D. B., et al. (2007). Prenatal pesticide and PCB exposures and birth outcomes. *Pediatr Res, 61*(2), 243-250.	EPI
289	Xiang, H., Nuckols, J. R., & Stallones, L. (2000). A geographic information assessment of birth weight and crop production patterns around mother's residence. *Environ Res, 82*(2), 160-167.	EPI

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
290	Ye, X., Pierik, F. H., Hauser, R., Duty, S., Angerer, J., Park, M. M., et al. (2008). Urinary metabolite concentrations of organophosphorous pesticides, bisphenol A, and phthalates among pregnant women in Rotterdam, the Netherlands: The Generation R study. [Research Support, N.I.H., Extramural]. *Environ Res, 108*(2), 260-267.	EXPO
291	Yolton, K., Cornelius, M., Ornoy, A., McGough, J., Makris, S., & Schantz, S. (2014). Exposure to neurotoxicants and the development of attention deficit hyperactivity disorder and its related behaviors in childhood. [Journal article]. *Neurotoxicology and Teratology, 44*, 30-45.	REV
292	Young, J. G., Eskenazi, B., Gladstone, E. A., Bradman, A., Pedersen, L., Johnson, C., et al. (2005). Association between in utero organophosphate pesticide exposure and abnormal reflexes in neonates. *Neurotoxicology, 26*(2), 199-209.	EPI
293	Yuan, Y., Chen, C., Zheng, C., Wang, X., Yang, G., Wang, Q., et al. (2014). Residue of chlorpyrifos and cypermethrin in vegetables and probabilistic exposure assessment for consumers in Zhejiang Province, China. [Article]. *Food Control, 36*, 63-68.	EXPO
294	Yusà, V., Coscollà, C., & Millet, M. (2014). New screening approach for risk assessment of pesticides in ambient air. [Article]. *Atmospheric Environment, 96*, 322-330.	REV
295	Zhang, X., Wallace, A. D., Du, P., Lin, S., Baccarelli, A. A., Jiang, H., et al. (2012). Genome-wide study of DNA methylation alterations in response to diazinon exposure in vitro. [Article]. *Environmental Toxicology and Pharmacology, 34*, 959-968.	TOX
296	Zhang, Y., Han, S., Liang, D., Shi, X., Wang, F., Liu, W., et al. (2014). Prenatal exposure to organophosphate pesticides and neurobehavioral development of neonates: A birth cohort study in Shenyang, China. [Article]. *PLoS ONE, 9*.	EPI
297	Zhang, Y., Song, H., Liang, D., Shi, X., Wang, F., Liu, W., et al. (2014). Prenatal exposure to organophosphate pesticides and neurobehavioral development of neonates: a birth cohort study in Shenyang, China. [Journal article]. *PLoS ONE, 9*, e88491.	DUPLICATE

Number	Citations	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English
298	Zhao, Q., Gadagbui, B., & Dourson, M. (2005). Lower birth weight as a critical effect of chlorpyrifos: A comparison of human and animal data. *Regul Toxicol Pharmacol, 42*(1), 55-63.	REV
299	Zhou, S., Rosenthal, D. G., Sherman, S., Zelikoff, J., Gordon, T., & Weitzman, M. (2014). Physical, Behavioral, and Cognitive Effects of Prenatal Tobacco and Postnatal Secondhand Smoke Exposure. *Current Problems in Pediatric and Adolescent Health Care, 44*, 219-241.	REV

Appendix 5. Table of Systematic Review Analysis: Second Tier Exclusion Criteria.

Among the 79 potentially relevant epidemiologic studies, 41 were excluded; 17 articles were previously reviewed in 2012; 16 were epidemiological methods papers including exposure validation studies without an original epidemiological risk estimate; and 8 were otherwise not relevant for various reasons. Among the 40 remaining studies, 2 were additionally excluded (one was a duplicate study published a second time; the other did not make a measure of an OP pesticide. Therefore, 38 articles are included in the 2015 literature review.

Number	Citations (Of 79: 17 - part of 3 cohorts, previously reviewed 24-excluded (NOT OP, NOT ND, NOT EPI) 38-include (9 no direct OP measure)	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English	Include? Outcome==pediatric ND/NB/motor control/morphology/motor control; Exposure==OP (biomarker, questionnaire, env media); epi study (cohort/case control/cross sectional (not ecologic))	Include? Rationale
1	Burdorf, A., Brand, T., Jaddoe, V. W., Hofman, A., Mackenbach, J. P., & Steegers, E. A. (2011). The effects of work-related maternal risk factors on time to pregnancy, preterm birth and birth weight: the Generation R Study. *Occup Environ Med, 68*(3), 197-204.	EPI	NO	NO OP; ALL PESTICIDE ONLY
2	Xiang, H., Nuckols, J. R., & Stallones, L. (2000). A geographic information assessment of birth weight and crop production patterns around mother's residence. *Environ Res, 82*(2), 160-167.	EPI	NO	No pesticide measure
3	Ferreira, J. D., Couto, A. C. z., Pombo-de-Oliveira, M. S., & Koifman, S. (2013). In utero pesticide exposure and leukemia in Brazilian children < 2 years of age. [Note]. *Environmental Health Perspectives, 121*, 269-275.	EPI	NO	NOT ND
4	How, V., Hashim, Z., Ismail, P., Said, S. M., Dzolkhifli, O., & Shamsul Bahri, M. T. (2014). Exploring cancer development in adulthood: cholinesterase depression and genotoxic effect from chronic exposure to organophosphate pesticides among rural farm children. [Journal article]. *Journal of Agromedicine, 19*, 35-43.	EPI	NO	not ND
5	Huen, K., Harley, K., Beckman, K., Eskenazi, B., & Holland, N. (2013). Associations of PON1 and Genetic Ancestry with Obesity in Early Childhood. [Article]. *PLoS ONE, 8*.	EPI	NO	not ND

ED_002061_00044425-00157

Number	Citations (Of 79: 17 - part of 3 cohorts, previously reviewed 24-excluded (NOT OP, NOT ND, NOT EPI) 38-include (9 no direct OP measure)	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English	Include? Outcome==pediatric ND/NB/motor control/morphology/motor control; Exposure==OP (biomarker, questionnaire, env media); epi study (cohort/case control/cross sectional (not ecologic))	Include? Rationale
6	Jones, K., Everard, M., & Harding, A. H. (2014). Investigation of gastrointestinal effects of organophosphate and carbamate pesticide residues on young children. [Journal article]. *International Journal of Hygiene and Environmental Health, 217*, 392-398.	EPI	NO	not ND
7	Khan, K., Ismail, A. A., Abdel Rasoul, G., Bonner, M. R., Lasarev, M. R., Hendy, O., et al. (2014). Longitudinal assessment of chlorpyrifos exposure and self-reported neurological symptoms in adolescent pesticide applicators. [Article]. *BMJ Open, 4*.	EPI	NO	not ND
8	Michalakis, M., Tzatzarakis, M. N., Kovatsi, L., Alegakis, A. K., Tsakalof, A. K., Heretis, I., et al. (2014). Hypospadias in offspring is associated with chronic exposure of parents to organophosphate and organochlorine pesticides. [Article]. *Toxicology Letters, 230*, 139-145.	EPI	NO	not ND
9	Rohlman, D. S., Ismail, A. A., Abdel-Rasoul, G., Lasarev, M., Hendy, O., & Olson, J. R. (2014). Characterizing exposures and neurobehavioral performance in Egyptian adolescent pesticide applicators. [Article]. *Metabolic Brain Disease, 29*, 845-855.	EPI	NO	not ND
10	Rojas, M., Agreda, O., & Infante, S. (2008). A preliminary statistical study of whether pesticide use could be related to birth defects in a rural area of Venezuela. *Rev Salud Publica (Bogota), 10*(1), 85-93.	EPI	NO	not ND
11	Simescu, M., Igna, C. P., Nicolaescu, E., Ion, I., Ion, A. C., Caragheorgheopol, A., et al. (2014). MULTIPLE PESTICIDES EXPOSURE OF GREENHOUSE WORKERS AND THYROID PARAMETERS. [article]. *International journal of sustainable development and planning (Print), 9*, 15-28.	EPI	NO	not ND

Number	Citations (Of 79: 17 - part of 3 cohorts, previously reviewed 24-excluded (NOT OP, NOT ND, NOT EPI) 38-include (9 no direct OP measure)	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English	Include? Outcome==pediatric ND/NB/motor control/morphology/motor control; Exposure==OP (biomarker, questionnaire, env media); epi study (cohort/case control/cross sectional (not ecologic))	Include? Rationale
12	Cecchi, A., Rovedatti, M. G., Sabino, G., & Magnarelli, G. G. (2012a). Environmental exposure to organophosphate pesticides: Assessment of endocrine disruption and hepatotoxicity in pregnant women. [Article]. *Ecotoxicology and Environmental Safety, 80*, 280-287.	EPI	NO	not ND directly; maternal ED/thyroid change->adverse fetal (unmeasured)
13	Rohlman, D. S., Lasarev, M., Anger, W. K., Scherer, J., Stupfel, J., & McCauley, L. (2007a). Neurobehavioral performance of adult and adolescent agricultural workers. *Neurotoxicology, 28*(2), 374-380.	EPI	NO	not ND, not OP
14	Carmichael, S. L., Yang, W., Roberts, E. M., Kegley, S. E., Wolff, C., Guo, L., et al. (2013). Hypospadias and residential proximity to pesticide applications. [Article]. *Pediatrics, 132*, e1216-e1226.	EPI	NO	not ND; measured all pesticides, some OP
15	Carmichael, S. L., Yang, W., Roberts, E., Kegley, S. E., Padula, A. M., English, P. B., et al. (2014). Residential agricultural pesticide exposures and risk of selected congenital heart defects among offspring in the San Joaquin Valley of California. [Article]. *Environmental Research, 135*, 133-138.	EPI	NO	not ND; measured all pesticides, some OP
16	Eskenazi, B., Chevrier, J., Rauch, S. A., Kogut, K., Harley, K. G., Johnson, C., et al. (2013). In Utero and Childhood Polybrominated Diphenyl Ether (PBDE) Exposures and Neurodevelopment in the CHAMACOS Study. [Article]. *Environmental Health Perspectives, 121*(2), 257-262.	EPI	NO	not OP
17	Kezios, K. L., Liu, X. H., Cirillo, P. M., Cohn, B. A., Kalantzi, O. I., Wang, Y. Z., et al. (2013). Dichlorodiphenyltrichloroethane (DDT), DDT metabolites and pregnancy outcomes. [Article]. *Reproductive Toxicology, 35*, 156-164.	EPI	NO	not OP

ED_002061_00044425-00159

Number	Citations (Of 79: 17 - part of 3 cohorts, previously reviewed 24-excluded (NOT OP, NOT ND, NOT EPI) 38-include (9 no direct OP measure)	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English	Include? Outcome==pediatric ND/NB/motor control/morphology/motor control; Exposure==OP (biomarker, questionnaire, env media); epi study (cohort/case control/cross sectional (not ecologic))	Include? Rationale
18	Kicinski, M., Vrijens, J., Vermier, G., Hond, E. D., Schoeters, G., Nelen, V., et al. (2015). Neurobehavioral function and low-level metal exposure in adolescents. *International Journal of Hygiene and Environmental Health, 218*, 139-146.	EPI	NO	not OP
19	Ostrea, E. M., Reyes, A., Villanueva-Uy, E., Pacifico, R., Benitez, B., Ramos, E., et al. (2011). Fetal exposure to propoxur and abnormal child neurodevelopment at 2 years of age. *NeuroToxicology*.	EPI	NO	not OP
20	Snijder, C. A., Heederik, D., Pierik, F. H., Hofman, A., Jaddoe, V. W., Koch, H. M., et al. (2013). Fetal growth and prenatal exposure to bisphenol A: The generation R study. [Article]. *Environmental Health Perspectives, 121*, 393-396.	EPI	NO	not OP
21	Tan, J., Loganath, A., Chong, Y. S., & Obbard, J. P. (2009). Exposure to persistent organic pollutants in utero and related maternal characteristics on birth outcomes: A multivariate data analysis approach. *Chemosphere, 74*(3), 428-433.	EPI	NO	not OP
22	Torres-Sanchez, L., Rothenberg, S. J., Schnaas, L., Cebrian, M. E., Osorio, E., Del Carmen Hernandez, M., et al. (2007). In utero p,p'-DDE exposure and infant neurodevelopment: A perinatal cohort in Mexico. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Environ Health Perspect, 115*(3), 435-439.	EPI	NO	not OP
23	Torres-Sanchez, L., Schnaas, L., Cebrian, M. E., Hernandez Mdel, C., Valencia, E. O., Garcia Hernandez, R. M., et al. (2009). Prenatal dichlorodiphenyldichloroethylene (DDE) exposure and neurodevelopment: a follow-up from 12 to 30 months of age. [Research Support, Non-U.S. Gov't]. *NeuroToxicology, 30*(6), 1162-1165.	EPI	NO	not OP

Number	Citations (Of 79: 17 - part of 3 cohorts, previously reviewed 24-excluded (NOT OP, NOT ND, NOT EPI) 38-include (9 no direct OP measure)	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English	Include? Outcome==pediatric ND/NB/motor control/morphology/motor control; Exposure==OP (biomarker, questionnaire, env media); epi study (cohort/case control/cross sectional (not ecologic))	Include? Rationale
24	Acosta-Maldonado, B., Sanchez-Ramirez, B., Reza-Lopez, S., & Levario-Carrillo, M. (2009). Effects of exposure to pesticides during pregnancy on placental maturity and weight of newborns: a cross-sectional pilot study in women from the Chihuahua State, Mexico. *Hum Exp Toxicol, 28*(8), 451-459.	EPI	YES	
25	Andersen, H. R., Debes, F., Wohlfahrt-Veje, C., Murata, K., & Grandjean, P. (2015). Occupational pesticide exposure in early pregnancy associated with sex-specific neurobehavioral deficits in the children at school age. *Neurotoxicology and Teratology, 47*, 1-9.	EPI	YES	
26	Barr, D. B., Ananth, C. V., Yan, X., Lashley, S., Smulian, J. C., Ledoux, T. A., et al. (2010). Pesticide concentrations in maternal and umbilical cord sera and their relation to birth outcomes in a population of pregnant women and newborns in New Jersey. *Sci Total Environ, 408*(4), 790-795.	EPI	YES	
27	Berkowitz, G. S., Wetmur, J. G., Birman-Deych, E., Obel, J., Lapinski, R. H., Godbold, J. H., et al. (2004). In utero pesticide exposure, maternal paraoxonase activity, and head circumference. *Environ Health Perspect, 112*(3), 388-391.	EPI	YES-3COHORTS	
28	Bouchard, M. F., Bellinger, D. C., Wright, R. O., & Weisskopf, M. G. (2010). Attention-deficit/hyperactivity disorder and urinary metabolites of organophosphate pesticides. [Research Support, N.I.H., Extramural]. *Pediatrics, 125*(6), e1270-1277.	EPI	YES	
29	Bouchard, M. F., Chevrier, J., Harley, K. G., Kogut, K., Vedar, M., Calderon, N., et al. (2011). Prenatal exposure to organophosphate pesticides and IQ in 7-year-old children. *Environ Health Perspect, 119*(8), 1189-1195.	EPI	YES-3COHORTS	

Number	Citations (Of 79: 17 - part of 3 cohorts, previously reviewed 24-excluded (NOT OP, NOT ND, NOT EPI) 38-include (9 no direct OP measure)	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English	Include? Outcome==pediatric ND/NB/motor control/morphology/motor control; Exposure==OP (biomarker, questionnaire, env media); epi study (cohort/case control/cross sectional (not ecologic))	Include? Rationale
30	Dabrowski, S., Hanke, W., Polanska, K., Makowiec-Dabrowska, T., & Sobala, W. (2003). Pesticide exposure and birthweight: an epidemiological study in Central Poland. *Int J Occup Med Environ Health, 16*(1), 31-39.	EPI	YES	
31	Ding, G. D., Wang, P., Tian, Y., Zhang, J., Gao, Y., Wang, X. J., et al. (2012). Organophosphate Pesticide Exposure and Neurodevelopment in Young Shanghai Children. [Article]. *Environmental Science & Technology, 46*(5), 2911-2917.	EPI	YES	
32	Engel, S. M., Berkowitz, G. S., Barr, D. B., Teitelbaum, S. L., Siskind, J., Meisel, S. J., et al. (2007). Prenatal organophosphate metabolite and organochlorine levels and performance on the Brazelton Neonatal Behavioral Assessment Scale in a multiethnic pregnancy cohort. *Am J Epidemiol, 165*(12), 1397-1404.	EPI	YES-3COHORTS	
33	Engel, S. M., Wetmur, J., Chen, J., Zhu, C., Barr, D. B., Canfield, R. L., et al. (2011). Prenatal exposure to organophosphates, paraoxonase 1, and cognitive development in childhood. *Environ Health Perspect, 119*(8), 1182-1188.	EPI	YES-3COHORTS	
34	Eskenazi, B., Harley, K., Bradman, A., Weltzien, E., Jewell, N. P., Barr, D. B., et al. (2004). Association of in utero organophosphate pesticide exposure and fetal growth and length of gestation in an agricultural population. *Environ Health Perspect, 112*(10), 1116-1124.	EPI	YES-3COHORTS	
35	Eskenazi, B., Huen, K., Marks, A., Harley, K. G., Bradman, A., Barr, D. B., et al. (2010). PON1 and neurodevelopment in children from the CHAMACOS study exposed to organophosphate pesticides in utero. *Environ Health Perspect, 118*(12), 1775-1781.	EPI	YES-3COHORTS	

ED_002061_00044425-00162

Number	Citations (Of 79: 17 - part of 3 cohorts, previously reviewed 24-excluded (NOT OP, NOT ND, NOT EPI) 38-include (9 no direct OP measure)	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English	Include? Outcome==pediatric ND/NB/motor control/morphology/motor control; Exposure==OP (biomarker, questionnaire, env media); epi study (cohort/case control/cross sectional (not ecologic))	Include? Rationale
36	Eskenazi, B., Kogut, K., Huen, K., Harley, K. G., Bouchard, M., Bradman, A., et al. (2014a). Organophosphate pesticide exposure, PON1, and neurodevelopment in school-age children from the CHAMACOS study. *Environmental Research, 134*, 149-157.	EPI	YES-3COHORTS	
37	Eskenazi, B., Marks, A. R., Bradman, A., Harley, K., Barr, D. B., Johnson, C., et al. (2007). Organophosphate pesticide exposure and neurodevelopment in young Mexican-American children. *Environ Health Perspect, 115*(5), 792-798.	EPI	YES-3COHORTS	
38	Fortenberry, G. Z., Meeker, J. D., Sanchez, B. N., Barr, D. B., Panuwet, P., Bellinger, D., et al. (2014). Urinary 3,5,6-trichloro-2-pyridinol (TCPY) in pregnant women from Mexico City: distribution, temporal variability, and relationship with child attention and hyperactivity. *Int J Hyg Environ Health, 217*(2-3), 405-412.	EPI	YES	
39	Fortenberry, G. Z., Meeker, J. D., Sánchez, B. N., Bellinger, D., Peterson, K., Schnaas, L., et al. (2014). Paraoxonase I polymorphisms and attention/hyperactivity in school-age children from Mexico City, Mexico. [Article]. *Environmental Research, 132*, 342-349.	EPI	YES	
40	Furlong, M. A., Engel, S. M., Barr, D. B., & Wolff, M. S. (2014). Prenatal exposure to organophosphate pesticides and reciprocal social behavior in childhood. *Environ Int, 70*, 125-131.	EPI	YES	
41	Grandjean, P., Harari, R., Barr, D. B., & Debes, F. (2006). Pesticide exposure and stunting as independent predictors of neurobehavioral deficits in Ecuadorian school children. [Research Support, Non-U.S. Gov't]. *Pediatrics, 117*(3), e546-556.	EPI	YES	

Number	Citations (Of 79: 17 - part of 3 cohorts, previously reviewed 24-excluded (NOT OP, NOT ND, NOT EPI) 38-include (9 no direct OP measure)	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English	Include? Outcome==pediatric ND/NB/motor control/morphology/motor control; Exposure==OP (biomarker, questionnaire, env media); epi study (cohort/case control/cross sectional (not ecologic))	Include? Rationale
42	Guodong, D., Pei, W., Ying, T., Jun, Z., Yu, G., Xiaojin, W., et al. (2012). Organophosphate pesticide exposure and neurodevelopment in young Shanghai children. *Environ Sci Technol*.	EPI	YES	
43	Handal, A. J., Harlow, S. D., Breilh, J., & Lozoff, B. (2008). Occupational exposure to pesticides during pregnancy and neurobehavioral development of infants and toddlers. *Epidemiology, 19*(6), 851-859.	EPI	YES	
44	Handal, A. J., Lozoff, B., Breilh, J., & Harlow, S. D. (2007a). Effect of community of residence on neurobehavioral development in infants and young children in a flower-growing region of Ecuador. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Environ Health Perspect, 115*(1), 128-133.	EPI	YES	
45	Handal, A. J., Lozoff, B., Breilh, J., & Harlow, S. D. (2007b). Neurobehavioral development in children with potential exposure to pesticides. *Epidemiology, 18*(3), 312-320.	EPI	YES	
46	Harari, R., Julvez, J., Murata, K., Barr, D., Bellinger, D. C., Debes, F., et al. (2010). Neurobehavioral deficits and increased blood pressure in school-age children prenatally exposed to pesticides. *Environ Health Perspect, 118*(6), 890-896.	EPI	YES	
47	Harley, K. G., Huen, K., Schall, R. A., Holland, N. T., Bradman, A., Barr, D. B., et al. (2011). Association of organophosphate pesticide exposure and paraoxonase with birth outcome in Mexican-American women. *PLoS One, 6*(8), e23923.	EPI	YES-3COHORTS	

Number	Citations (Of 79: 17 - part of 3 cohorts, previously reviewed 24-excluded (NOT OP, NOT ND, NOT EPI) 38-include (9 no direct OP measure)	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English	Include? Outcome==pediatric ND/NB/motor control/morphology/motor control; Exposure==OP (biomarker, questionnaire, env media); epi study (cohort/case control/cross sectional (not ecologic))	Include? Rationale
48	Horton, M. K., Kahn, L. G., Perera, F., Barr, D. B., & Rauh, V. (2012). Does the home environment and the sex of the child modify the adverse effects of prenatal exposure to chlorpyrifos on child working memory? *Neurotoxicol Teratol, 34*(5), 534-541.	EPI	YES-3COHORTS	
49	Kofman, O., Berger, A., Massarwa, A., Friedman, A., & Jaffar, A. A. (2006). Motor inhibition and learning impairments in school-aged children following exposure to organophosphate pesticides in infancy. *Pediatr Res, 60*(1), 88-92.	EPI	YES	NO: LONG TERM EFFECTS OF ONE TIME POISONING
50	Koutroulakis, D., Sifakis, S., Tzatzarakis, M. N., Alegakis, A. K., Theodoropoulou, E., Kavvalakis, M. P., et al. (2014). Dialkyl phosphates in amniotic fluid as a biomarker of fetal exposure to organophosphates in Crete, Greece; association with fetal growth. [Article]. *Reproductive Toxicology, 46*, 98-105.	EPI	YES	
51	Kristensen, P., Irgens, L. M., Andersen, A., Bye, A. S., & Sundheim, L. (1997). Gestational age, birth weight, and perinatal death among births to Norwegian farmers, 1967-1991. *Am J Epidemiol, 146*(4), 329-338.	EPI	YES	
52	Lizardi, P. S., O'Rourke, M. K., & Morris, R. J. (2008). The effects of organophosphate pesticide exposure on Hispanic children's cognitive and behavioral functioning. *J Pediatr Psychol, 33*(1), 91-101.	EPI	YES	
53	Llop, S., Julvez, J., Fernandez-Somoano, A., Santa Marina, L., Vizcaino, E., Iñiguez, C., et al. (2013). Prenatal and postnatal insecticide use and infant neuropsychological development in a multicenter birth cohort study. *Environment International, 59*, 175-182.	EPI	YES	

Number	Citations (Of 79: 17 - part of 3 cohorts, previously reviewed 24-excluded (NOT OP, NOT ND, NOT EPI) 38-include (9 no direct OP measure)	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English	Include? Outcome==pediatric ND/NB/motor control/morphology/motor control; Exposure==OP (biomarker, questionnaire, env media); epi study (cohort/case control/cross sectional (not ecologic))	Include? Rationale
54	Lovasi, G. S., Quinn, J. W., Rauh, V. A., Perera, F. P., Andrews, H. F., Garfinkel, R., et al. (2011). Chlorpyrifos exposure and urban residential environment characteristics as determinants of early childhood neurodevelopment. *Am J Public Health, 101*(1), 63-70.	EPI	YES-3COHORTS	
55	Lu, C., Essig, C., Root, C., Rohlman, D. S., McDonald, T., & Sulzbacher, S. (2009). Assessing the association between pesticide exposure and cognitive development in rural Costa Rican children living in organic and conventional coffee farms. *Int J Adolesc Med Health, 21*(4), 609-621.	EPI	YES	
56	Marks, A. R., Harley, K., Bradman, A., Kogut, K., Barr, D. B., Johnson, C., et al. (2010). Organophosphate pesticide exposure and attention in young Mexican-American children: the CHAMACOS study. *Environ Health Perspect, 118*(12), 1768-1774.	EPI	YES-3COHORTS	
57	Moreno-Banda, G., Blanco-Munoz, J., Lacasana, M., Rothenberg, S. J., Aguilar-Garduno, C., Gamboa, R., et al. (2009). Maternal exposure to floricultural work during pregnancy, PON1 Q192R polymorphisms and the risk of low birth weight. *Sci Total Environ, 407*(21), 5478-5485.	EPI	YES	
58	Nevison, C. D. (2014). A comparison of temporal trends in United States autism prevalence to trends in suspected environmental factors. [Article]. *Environmental Health: A Global Access Science Source, 13*.	EPI	YES	NO: this is a review; ecol comparison
59	Oulhote, Y., & Bouchard, M. F. (2013). Urinary metabolites of organophosphate and pyrethroid pesticides and behavioral problems in Canadian children. [Article]. *Environmental Health Perspectives, 121*, 1378-1384.	EPI	YES	

ED_002061_00044425-00166

Number	Citations (Of 79: 17 - part of 3 cohorts, previously reviewed 24-excluded (NOT OP, NOT ND, NOT EPI) 38-include (9 no direct OP measure)	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English	Include? Outcome==pediatric ND/NB/motor control/morphology/motor control; Exposure==OP (biomarker, questionnaire, env media); epi study (cohort/case control/cross sectional (not ecologic))	Include? Rationale
60	Petit, C., Chevrier, C., Durand, G., Monfort, C., Rouget, F., Garlantezec, R., et al. (2010). Impact on fetal growth of prenatal exposure to pesticides due to agricultural activities: A prospective cohort study in Brittany, France. [Research Support, Non-U.S. Gov't]. *Environ Health, 9*, 71.	EPI	YES	
61	Quiros-Alcala, L., Alkon, A. D., Boyce, W. T., Lippert, S., Davis, N. V., Bradman, A., et al. (2011). Maternal prenatal and child organophosphate pesticide exposures and children's autonomic function. *Neurotoxicology, 32*(5), 646-655.	EPI	YES	
62	Rauch, S. A., Braun, J. M., Barr, D. B., Calafat, A. M., Khoury, J., Montesano, M. A., et al. (2012). Associations of prenatal exposure to organophosphate pesticide metabolites with gestational age and birth weight. [Article]. *Environmental Health Perspectives, 120*, 1055-1060.	EPI	YES	
63	Rauh, V. A., Garfinkel, R., Perera, F. P., Andrews, H. F., Hoepner, L., Barr, D. B., et al. (2006). Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children. *Pediatrics, 118*(6), e1845-1859.	EPI	YES-3COHORTS	
64	Rauh, V. A., Perera, F. P., Horton, M. K., Whyatt, R. M., Bansal, R., Hao, X., et al. (2012). Brain anomalies in children exposed prenatally to a common organophosphate pesticide. *Proc Natl Acad Sci U S A, 109*(20), 7871-7876.	EPI	YES-3COHORTS	
65	Rauh, V., Arunajadai, S., Horton, M., Perera, F., Hoepner, L., Barr, D. B., et al. (2011). Seven-year neurodevelopmental scores and prenatal exposure to chlorpyrifos, a common agricultural pesticide. *Environ Health Perspect, 119*(8), 1196-1201.	EPI	YES-3COHORTS	

Number	Citations (Of 79: 17 - part of 3 cohorts, previously reviewed 24-excluded (NOT OP, NOT ND, NOT EPI) 38-include (9 no direct OP measure)	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English	Include? Outcome==pediatric ND/NB/motor control/morphology/motor control; Exposure==OP (biomarker, questionnaire, env media); epi study (cohort/case control/cross sectional (not ecologic))	Include? Rationale
66	Rohlman, D. S., Arcury, T. A., Quandt, S. A., Lasarev, M., Rothlein, J., Travers, R., et al. (2005). Neurobehavioral performance in preschool children from agricultural and non-agricultural communities in Oregon and North Carolina. *NeuroToxicology, 26*(4 Spec. Iss.), 589-598.	EPI	YES	
67	Ruckart, P. Z., Kakolewski, K., Bove, F. J., & Kaye, W. E. (2004). Long-term neurobehavioral health effects of methyl parathion exposure in children in Mississippi and Ohio. [Comparative Study]. *Environ Health Perspect, 112*(1), 46-51.	EPI	YES	
68	Samarawickrema, N., Pathmeswaran, A., Wickremasinghe, R., Peiris-John, R., Karunaratna, M., Buckley, N., et al. (2008). Fetal effects of environmental exposure of pregnant women to organophosphorus compounds in a rural farming community in Sri Lanka. *Clin Toxicol (Phila), 46*(6), 489-495.	EPI	YES	
69	Savitz, D. A., Arbuckle, T., Kaczor, D., & Curtis, K. M. (1997). Male pesticide exposure and pregnancy outcome. *Am J Epidemiol, 146*(12), 1025-1036.	EPI	YES	
70	Shelton, J. F., Geraghty, E. M., Tancredi, D. J., Delwiche, L. D., Schmidt, R. J., Ritz, B., et al. (2014). Neurodevelopmental disorders and prenatal residential proximity to agricultural pesticides: The charge study. [Article]. *Environmental Health Perspectives, 122*, 1103-1109.	EPI	YES	
71	Suarez-Lopez, J. R., Himes, J. H., Jacobs Jr, D. R., Alexander, B. H., & Gunnar, M. R. (2013). Acetylcholinesterase activity and neurodevelopment in boys and girls. [Article]. *Pediatrics, 132*, e1649-e1658.	EPI	YES	

Number	Citations (Of 79: 17 - part of 3 cohorts, previously reviewed 24-excluded (NOT OP, NOT ND, NOT EPI) 38-include (9 no direct OP measure)	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English	Include? Outcome==pediatric ND/NB/motor control/morphology/motor control; Exposure==OP (biomarker, questionnaire, env media); epi study (cohort/case control/cross sectional (not ecologic))	Include? Rationale
72	Suarez-Lopez, J. R., Jacobs Jr, D. R., Himes, J. H., & Alexander, B. H. (2013). Acetylcholinesterase activity, cohabitation with floricultural workers, and blood pressure in Ecuadorian children. [Article]. *Environmental Health Perspectives, 121*, 619-624.	EPI	YES	
73	Suarez-Lopez, J. R., Jacobs, D. R., Himes, J. H., Alexander, B. H., Lazovich, D., & Gunnar, M. (2012). Lower acetylcholinesterase activity among children living with flower plantation workers. [Article]. *Environmental Research, 114*, 53-59.	EPI	YES	
74	Wang, P., Tian, Y., Wang, X.-J., Gao, Y., Shi, R., Wang, G.-Q., et al. (2012). Organophosphate pesticide exposure and perinatal outcomes in Shanghai, China. [Article]. *Environment International, 42*, 100-104.	EPI	YES	
75	Whyatt, R. M., Rauh, V., Barr, D. B., Camann, D. E., Andrews, H. F., Garfinkel, R., et al. (2004). Prenatal insecticide exposures and birth weight and length among an urban minority cohort. *Environ Health Perspect, 112*(10), 1125-1132.	EPI	YES-3COHORTS	
76	Wickerham, E. L., Lozoff, B., Jie, S., Kaciroti, N., Yankai, X. I. A., & Meeker, J. D. (2012). Reduced birth weight in relation to pesticide mixtures detected in cord blood of full-term infants. [article]. *Environment international, 47*, 80-85.	EPI	YES	
77	Wolff, M. S., Engel, S., Berkowitz, G., Teitelbaum, S., Siskind, J., Barr, D. B., et al. (2007). Prenatal pesticide and PCB exposures and birth outcomes. *Pediatr Res, 61*(2), 243-250.	EPI	YES	
78	Young, J. G., Eskenazi, B., Gladstone, E. A., Bradman, A., Pedersen, L., Johnson, C., et al. (2005). Association between in utero organophosphate pesticide exposure and abnormal reflexes in neonates. *Neurotoxicology, 26*(2), 199-209.	EPI	YES-3COHORTS	

ED_002061_00044425-00169

Number	Citations (Of 79: 17 - part of 3 cohorts, previously reviewed 24-excluded (NOT OP, NOT ND, NOT EPI) 38-include (9 no direct OP measure)	Category (EPI-epi only; EXPO- exposure only; ACR - acute case report or case series; TOX-animal study; REV - review or commentary, no original data; OTH - other, non-English	Include? Outcome==pediatric ND/NB/motor control/morphology/motor control; Exposure==OP (biomarker, questionnaire, env media); epi study (cohort/case control/cross sectional (not ecologic))	Include? Rationale
79	Zhang, Y., Han, S., Liang, D., Shi, X., Wang, F., Liu, W., et al. (2014). Prenatal exposure to organophosphate pesticides and neurobehavioral development of neonates: A birth cohort study in Shenyang, China. [Article]. *PLoS ONE, 9*.	EPI	YES	

Appendix 6. Plausible hypotheses on MOA/AOP for neurodevelopmental outcomes (Extracted from Section 4.4.3. RHHRA for chlorpyrifos)

Numerous studies on the possible mechanistic aspects of neurodevelopmental effects have been published. The results have led some research groups to propose that changes in brain connectivity and/or neurochemistry may underlie the long-term *in vivo* neurobehavioral changes observed into adulthood. While multiple biologically plausible hypotheses are being pursued by researchers, no one pathway has sufficient data to be considered more credible than the others. The SAP concurred with the Agency in 2008 and 2012 about the lack of definable key events in a MOA/AOP leading to neurobehavioral effects. The Agency has considered the new literature since the 2012 SAP related to mechanistic hypotheses as described below (Appendix 11), and note that such a MOA/AOP still cannot be established.

- *Acetylcholinesterase (AChE) as a morphogen*: The classically understood role of AChE is the rapid hydrolysis of acetylcholine at synapses in the brain and at neuromuscular junctions, thereby regulating cholinergic neurotransmission. Consistent with this role, AChE is predominant at cholinergic synapses at neurons and in muscle, and inhibition of its catalytic activity results in the signs and symptoms of cholinergic overstimulation. Several lines of evidence suggest that AChE can also serve as a morphogen, influencing the growth of cells during neurodevelopment distinct from its role as an esterase. Alterations in the expression or structure of the AChE protein can disrupt various aspects of neuronal differentiation and growth, as has recently been shown in vitro (using NG108-15 cell line) following exposure to another OP, paraoxon (Campanha et al., 2014). While perturbation of the morphogenic activity of AChE is a plausible adverse outcome pathway for chlorpyrifos, a number of questions remain, including effective concentrations compared to those that inhibit catalytic activity of AChE. There is, however, no direct evidence showing that disruption of the morphogenic function of AChE can alter axon or dendritic growth *in vivo*. While limited *in vivo* studies using zebrafish indicate that chlorpyrifos or its metabolite chlorpyrifos oxon can disrupt axonal growth (Yang et al. 2011), it has not been demonstrated that this effect is due to alteration of the morphogenic function of AChE versus other potential mechanisms.

- *Cholinergic system:* There are several lines of evidence showing that signaling through cholinergic receptors is involved in neurodevelopment. Activation of muscarinic and/or nicotinic cholinergic receptors can regulate neural progenitor cell proliferation and differentiation (Resende & Adhikari, 2009), and *in vivo* studies demonstrate that cholinergic signaling is likely involved in brain morphogenesis (Hohmann & Berger-Sweeney, 1998). While ChE inhibitors can affect cholinergic signaling by inhibition of the catalytic activity of AChE and subsequent increase in acetylcholine, some inhibitors, including chlorpyrifos and chlorpyrifos oxon, can also directly interact with cholinergic receptors. Thus, direct interaction with cholinergic receptors by chlorpyrifos represents a potential adverse outcome pathway for disruption of neurodevelopment distinct from AChE/ChE inhibition. Some OPs have been shown to directly interact with cholinergic muscarinic receptors at relatively low concentrations. The muscarinic receptors are

members of the G-protein receptor family and five subtypes (m1-m5) have been identified. Ward et al. (1993) examined the relationship between ChE inhibition and direct binding to muscarinic receptors for a series of OPs and their active oxon metabolites. The results indicated a strong correlation between AChE activity of OPs, including chlorpyrifos and chlorpyrifos oxon, and the ability to compete for CD binding sites (m2 receptors) in rat brain homogenates. Binding affinities of the oxons were in the nanomolar range, at or below concentrations that inhibited AChE (Huff et al, 1994); specifically, chlorpyrifos oxon had a binding affinity of 22 nM in rat striatum and 2 nM in rat cortex (Huff, et al., 1994; Ward & Mundy, 1996). In total, these studies suggest that direct interactions with muscarinic receptors, and especially the m2 subtype, represent an alternative site of action for OPs including chlorpyrifos and chlorpyrifos oxon, with the oxon forms having high affinity. Together, the studies cited above outline a plausible adverse outcome pathway for chlorpyrifos and chlorpyrifos oxon to affect brain development via actions at the m2 subtype of muscarinic receptors. However, while there are studies showing that chlorpyrifos oxon can affect neurite outgrowth *in vitro* and decrease cell proliferation and differentiation both *in vitro* (Jameson et al , 2006; Qiao et al, , 2001; Song, et al., 1998) and *in vivo* (Dam et al, , 1998; Qiao, et al., 2003), there is no experimental evidence that these effects are a result of direct actions on the m2 receptor.

- *Endocannabinoid system:* Several lines of research have suggested that disruption of the endocannabinoid (EC) system due to chlorpyrifos exposure could play a role in its acute and/or long-term toxicity, and could also be extended to potential developmental toxicity. The EC system modulates neurotransmission as well as playing a morphogenic role during development of the nervous system. Chemicals (*e.g.*, drugs of abuse) which act on this system, produce long-term neurodevelopmental disorders in animal models and human studies. Chlorpyrifos also interacts with this system, both *in vitro* and *in vivo.* By this hypothesis, the EC system represents a possible adverse outcome pathway for developmental effects of chlorpyrifos. There is a body of studies on the interaction of OPs with relevant enzymes but only two studies have examined the effects of chlorpyrifos on the EC system in developing animals. Carr et al. (2011, 2013) has dosed preweanling rats for 5 or 7 days (1-5 mg/kg/day, p.o.), and showed that endocannabinoid-related enzymes were inhibited in rat brain tissue taken 4 to 48 hours after the last dose. Interestingly, fatty acid amid hydrolase (FAAH) showed a greater degree and more persistent inhibition compared to AChE inhibition measured in the same rats. A more recent publication (Carr et al., 2014) repeated these findings using a lower dose (0.5 mg/kg/d for 7 days), still showing significant FAAH inhibition but with no measurable AChE inhibition. This suggests a greater sensitivity of the EC system, at least in terms of the hydrolase compared to AChE activity, in the pups. However, there were no other ages tested, no downstream or correlative measure of changes in EC system function, and no subsequent neurodevelopmental effects that could be linked to the action. Additional studies along these lines are needed.

- *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.

References Cited

Avila, J., Dominguez, J., & Diaz-Nido, J. (1994). Regulation of microtubule dynamics by microtubule-associated protein expression and phosphorylation during neuronal development. Int J Dev Biol, 38(1), 13-25.

Campanha HM, Carvalho F, Schlosser P. (2014) Active and peripheral anionic sites of acetylcholinesterase have differential modulation effects on cell proliferation, adhesion and neuritogenesis in the NG108-15 cell line. Toxicol Lett epub ahead of print

Carr, R. L., Borazjani, A., & Ross, M. K. (2011). Effect of developmental chlorpyrifos exposure, on endocannabinoid metabolizing enzymes, in the brain of juvenile rats. Toxicol Sci, 122(1), 112-120.

Carr, R. L., Adams, A.L., Kepler, D.R., Ward, A.B., & Ross, M. K. (2013). Induction of

endocannabinoid levels in juvenile rat brain following developmental chlorpyrifos exposure. Toxicol Sci, 135(1), 193-201.

Carr RL, Graves CA, Mangum LC, Nail CA, Ross MK. (2014) Low level chlorpyrifos exposure increases anandamide accumulation in juvenile rat brain in the absence of brain cholinesterase inhibition. Neurotoxicol. 43:82-89.

Crumpton TL, Seidler FJ, Slotkin TA. (2000). Is oxidative stress involved in the developmental neurotoxicity or chlorpyrifos?. Bran Rev Dev Brain Res. 12:189-195.

Dam, K., Seidler, F. J., & Slotkin, T. A. (1998). Developmental neurotoxicity of chlorpyrifos: delayed targeting of DNA synthesis after repeated administration. *Brain Res Dev Brain Res, 108*(1-2), 39-45.

Fredrick, A. L., & Stanwood, G. D. (2009). Drugs, biogenic amine targets and the developing brain. Dev. Neurosci, 31(1-2), 7-22.

Fukushima, N., Furuta, D., Hidaka, Y., Moriyama, R., & Tsujiuchi, T. (2009). Post-translational modifications of tubulin in the nervous system. J Neurochem, 109(3), 683-693.

Hirokawa, N., & Takemura, R. (2004). Molecular motors in neuronal development, intracellular transport and diseases. Curr Opin Neurobiol, 14(5), 564-573.

Hirokawa, N., & Noda, Y. (2008). Intracellular transport and kinesin superfamily proteins, KIFs: structure, function, and dynamics. Physiol Rev, 88(3), 1089-1118.

Hohmann, C. F., & Berger-Sweeney, J. (1998). Cholinergic regulation of cortical development and plasticity. New twists to an old story. Perspect Dev Neurobiol, 5(4), 401-425.

Huff, R. A., Corcoran, J. J., Anderson, J. K., & Abou-Donia, M. B. (1994). Chlorpyrifos oxon binds directly to muscarinic receptors and inhibits cAMP accumulation in rat striatum. *J* Pharmacol Exp Ther, 269(1), 329-335.

Jameson, R. R., Seidler, F. J., Qiao, D., & Slotkin, T. A. (2006). Chlorpyrifos affects phenotypic outcomes in a model of mammalian neurodevelopment: critical stages targeting differentiation in PC12 cells. Environ Health Perspect, 114(5), 667-672.

Le Belle, J. E., Orozco, N. M., Paucar, A. A., Saxe, J. P., Mottahedeh, J., Pyle, A. D., et al. (2011). Proliferative neural stem cells have high endogenous ROS levels that regulate self-renewal and neurogenesis in a PI3K/Akt-dependant manner. Cell Stem Cell, 8(1), 59-71.

Li, Z., Dong, T., Proschel, C., & Noble, M. (2007). Chemically diverse toxicants converge on Fyn and c-Cbl to disrupt precursor cell function. *PLoS Biol, 5*(2), e35.

ED_002061_00044425-00176

Matus, A. (1988). Microtubule-associated proteins: their potential role in determining neuronal morphology. Annu Rev Neurosci, 11, 29-44.

Matus, A. (1990). Microtubule-associated proteins and the determination of neuronal form. J Physiol (Paris), 84(1), 134-137.

NRC (2007). Toxicity Testing in the 21st Century: A Vision and a Strategy. http://www.nap.edu/catalog.php?record_id=11970

Pellegrini, F., & Budman, D. R. (2005). Review: tubulin function, action of antitubulin drugs, and new drug development. Cancer Invest, 23(3), 264-273

Qiao, D., Seidler, F. J., & Slotkin, T. A. (2001). Developmental neurotoxicity of chlorpyrifos modeled in vitro: comparative effects of metabolites and other cholinesterase inhibitors on DNA synthesis in PC12 and C6 cells. Environ Health Perspect, 109(9), 909-913.

Qiao, D., Seidler, F. J., Tate, C. A., Cousins, M. M., & Slotkin, T. A. (2003). Fetal chlorpyrifos exposure: adverse effects on brain cell development and cholinergic biomarkers emerge postnatally and continue into adolescence and adulthood. *Environ Health Perspect, 111*(4), 536-544.

Resende, R. R., & Adhikari, A. (2009). Cholinergic receptor pathways involved in apoptosis, cell proliferation and neuronal differentiation. Cell Commun Signal, *7*, 20.

Sanchez, C., Perez, M., & Avila, J. (2000). GSK3beta-mediated phosphorylation of the microtubule-associated protein 2C (MAP2C) prevents microtubule bundling. *Eur J Cell Biol, 79*(4), 252-260.

Singh V, Panwar R. (2014). In vivo antioxidative and neuroprotective effect of 4-allyl-2-methoxyphenol against chlorpyrifos-induced neurotoxicity in rat brain. Mol Cell Biochem. 388:61-74

Slotkin, T. A., & Seidler, F. J. (2007). Comparative developmental neurotoxicity of organophosphates in vivo: transcriptional responses of pathways for brain cell development, cell signaling, cytotoxicity and neurotransmitter systems. Brain Res Bull, 72(4-6), 232-274.

Slotkin, T. A., MacKillop, E. A., Ryde, I. T., & Seidler, F. J. (2007). Ameliorating the developmental neurotoxicity of chlorpyrifos: a mechanisms-based approach in PC12 cells. Environ Health Perspect, 115(9), 1306-1313.

Song, X., Violin, J. D., Seidler, F. J., & Slotkin, T. A. (1998). Modeling the developmental neurotoxicity of chlorpyrifos in vitro: macromolecule synthesis in PC12 cells. Toxicol Appl Pharmacol, 151(1), 182-191.

Thompson, B. L., & Stanwood, G. D. (2009). Pleiotropic effects of neurotransmission during development: modulators of modularity. J Autism Dev Disord, 39(2), 260-268.

Vallee, R. B., Williams, J. C., Varma, D., & Barnhart, L. E. (2004). Dynein: An ancient motor protein involved in multiple modes of transport. J Neurobiol, 58(2), 189-200.

Vieira, H. L., Alves, P. M., & Vercelli, A. (2011). Modulation of neuronal stem cell differentiation by hypoxia and reactive oxygen species. Prog Neurobiol, 93(3), 444-455.

Ward, T. R., Ferris, D. J., Tilson, H. A., & Mundy, W. R. (1993). Correlation of the anticholinesterase activity of a series of organophosphates with their ability to compete with agonist binding to muscarinic receptors. Toxicol Appl Pharmacol, 122(2), 300-307.

Ward, T. R., & Mundy, W. R. (1996). Organophosphorus compounds preferentially affect second messenger systems coupled to M2/M4 receptors in rat frontal cortex. Brain Res Bull, 39(1), 49-55.

Yang D, Lauridsen H, Buels K, Chi LH, LaDu J, Bruun DA, Olson JR, Tanguay RL, Lein PJ. (2011) Chlorpyrifos-oxon disrupts zebrafish axonal growth and motor behavior. Toxicol. Sci 121:146-159

ED_002061_00044425-00178

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

OFFICE OF
CHEMICAL SAFETY AND
POLLUTION PREVENTION

MEMORANDUM

Date: September 15, 2015

SUBJECT: Literature Review on Neurodevelopment Effects & FQPA Safety Factor Determination for the Organophosphate Pesticides

PC Code: See Below
Decision No.:
Petition No.: None
Risk Assessment Type: None
TXR No.: None
MRID No.: None

DP Barcode: 331251
Registration No.: None
Regulatory Action: None
Case No.: None
CAS No.: See Below
40 CFR: None

FROM: Anna Lowit, Ph.D., Senior Scientist
Khin Swe Oo, MD, DABT, Toxicologist
Elizabeth Holman, DrPH, Physical Scientist
Health Effects Division (7509P)
Office of Pesticide Programs

Virginia C Moser, PhD, DABT, Fellow ATS, Toxicologist
Toxicity Assessment Division
Office of Research and Development

THROUGH: Dana Vogel, Director
Health Effects Division (7509P)

TO: Kelly Ballard, Chemical Review Manager
Neil Anderson, Branch Chief
Risk Management and Implementation Branch 1
Pesticide Re-Evaluation Division (7508P)

This paper supports the use of the 10X FQPA Safety Factor in the individual organophosphate human health risk assessments.

ED_002061_00044426-00001

Chemical	PC Code	CAS No.
Dicrotophos	035201	141-66-2
Fosthiazate	129022	98886-44-3
Coumaphos	036501	56-72-4
Terbufos	105001	13071-79-9
Profenofos	111401	41198-08-7
Bensulide	009801	741-58-2
Diazinon	057801	333-41-5
Ethoprop	041101	13194-48-4
Dimethoate	035001	60-51-5
Malathion	057701	121-75-5
Phosmet	059201	732-11-6
Chlorethoxyfos	129006	54593-83-8
Acephate/ Methamidiphos	103301/ 101201	30560-19-1/ 10265-92-6
Pirimiphos-methyl	108102	29232-93-7
TCVP	083701	961-11-5
Tribufos	074801	78-48-8
Phorate	057201	298-02-2
Phostebupirim	129086	96182-53-5
DDVP	084001	62-73-7
Naled	034401	300-76-5
Trichlorfon	057901	52-68-6
Fenamiphos	100601	22224-92-6
AZM	058001	86-50-0
Methidathion	100301	950-37-8
Propetamphos	113601	31218-83-4
ODM	058702	301-12-2
Disulfoton	032501	298-04-4
Methyl parathion	053501	298-00-0
Temephos	059001	3383-96-8
Chlorpyrifos-methyl	059102	5598-13-0

ED_002061_00044426-00002

September 15, 2015
D331251

Literature Review on Neurodevelopment Effects & FQPA Safety Factor Determination for the Organophosphate Pesticides

Office of Pesticide Programs
US Environmental Protection Agency

ED_002061_00044426-00003

Table of Contents

1.0 Introduction and Background 6
2.0 Literature Review 8
2.1 Developmental Neurotoxicity (DNT) Research on OPs other than Chlorpyrifos: Laboratory Animal Studies 8
2.1.1 Literature Search Strategy & Results 9
2.1.2 Integration of Literature: Neurobehavioral Domains 11
2.1.3 Integration with AChE Inhibition 22
2.1.4 Summary of Findings from the Developmental Neurotoxicity (DNT) Guideline Studies 23
2.1.5 Conclusions on *In Vivo* Laboratory Animal Studies 24
2.2 Epidemiology Research on OPs other than Chlorpyrifos 25
2.2.1 Overview of 2012/2014 and 2015 Literature Reviews 25
2.2.2 Literature Search Methodology 25
2.2.3 Breadth and Depth of the 2015 Literature Review 27
Table 2.2-aStudy Designs, Exposure Measurement Methods, and Outcome Measurement Methods Used across the 31 2015 Review Studies 29
Table 2.2-bDetailed Neurological Outcome Measurement Methods Used across the 31 2015 Review Studies 31
2.2.4 Considerations for Study Quality Evaluation 33
2.2.4.1 Study Designs 33
Table 2.2.4-1 Study Quality Considerations 33
2.2.4.2 Exposure Measures 37
Table 2.2.4-2 CDC Table of Organophosphate Pesticides and Their Dialkyl Phosphate Metabolites (2008) 39
2.2.4.3 Neurological and Other Outcome Measures 40
2.2.4.4 Statistical Analysis 40
2.2.4.5 Confounding 41
2.2.4.6 Risk of Bias 41
2.2.5 Review of Quality Results 42
2.2.5.1 "High" Quality Group 42
Table 2.2.5-1. High Quality Studies: Summary of Study Design Elements Impacting Study Quality Assignment 43
2.2.5.2 "Moderate" Quality Group 45
Table 2.2.5-2. Moderate Quality Studies: Summary of Study Design Elements Impacting Study Quality Assignment 46
2.2.5.3 "Low" Quality Group 49
2.2.6 Assessment of Epidemiological Studies for Relevance to Analysis 49
2.2.7 Birth Outcome Epidemiologic Studies 50
2.2.8 Summary of Epidemiology Studies from 2015 Literature Review Focusing on Neurodevelopmental Outcomes 51
2.2.9 Summary of Three US Children's Cohort Studies (CCCEH, CHAMACOS, Mt. Sinai):

ED_002061_00044426-00004

Focusing on Neurodevelopmental Outcomes Evaluated in 2012/2014 54
2.2.9.1 Overview 54
2.2.9.2 Review of Study Design and Research Methods 55
Table 2.2.9 Detailed Summary Tables of Children's Environmental Health Epidemiology Studies (extracted from USEPA, 2014) 59
2.2.10 Summary & Discussion 74
3.0 Weight of Evidence Analysis: Integration Across Multiple Lines of Evidence 76
3.1 Dose-Response Relationships & Temporal Concordance 76
3.2 Strength, Consistency & Specificity 77
3.3 Biological Plausability & Coherence 79
4.0 10X FQPA Safety Factor for Infants and Children 80
5.0 References 81
6.0 Appendices 90
Appendix 1. Table of *In Vivo* Developmental Neurotoxicity Studies of OPs 91
Appendix 2. Summary of Guideline DNT Studies Submitted to the Agency for OPs other than Chlorpyrifos. 95
Appendix 3. Low Quality Studies: Summary of Study Design Elements Impacting Study Quality Assignment 98

ED_002061_00044426-00005

Literature Review on Neurodevelopment Effects & FQPA Safety Factor Determination for the Organophosphate Pesticides

1.0 Introduction and Background

Organophosphate pesticides (OPs), widely used in agricultural and household pesticidal applications, act by inhibiting acetylcholinesterase (AChE) in nerve cells. Historically the agency has used inhibition of AChE as the point of departure for OP human health risk assessments (HHRAs). This science policy is based on decades of work which shows that AChE inhibition is the initial event in the pathway to acute cholinergic neurotoxicity. AChE inhibition is most often used as the regulatory endpoint for deriving points of departure (PODs) for the single chemical OP HHRAs. In addition, because OPs share the ability to inhibit AChE via phosphorylation of the active site of the enzyme leading to accumulation of acetylcholine and ultimately neurotoxicity, this class of pesticides is subject to assessment of cumulative risk (USEPA, 1999; 2006).

Newer lines of research on OPs in the areas of potential modes of action/adverse outcome pathways (MOAs/AOPs), [1] *in vivo* animal studies, and notably epidemiological studies in mothers and children, have raised uncertainty about the agency's risk assessment approach with regard to the potential for neurodevelopmental effects in fetuses and children. Many of these studies focus on chlorpyrifos and have been the subject of review by the agency over the last several years.

Specific to chlorpyrifos, the agency has taken a stepwise, objective and transparent approach in evaluating, interpreting, and characterizing the strengths and uncertainties associated with all of the available lines of scientific information related to the potential for adverse neurodevelopmental effects in infants and children. The stepwise evaluation began with the September 2008 FIFRA Scientific Advisory Panel (SAP) meeting involving a preliminary review of the literature for chlorpyrifos, with a particular focus on women and children (USEPA, 2008), followed by the draft "Framework for Incorporating Human Epidemiologic & Incident Data in Health Risk Assessment" for integration of epidemiology with other types of experimental data (USEPA, 2010; FIFRA SAP 2010a,b). After the draft framework (2010) was published, the agency released "Chlorpyrifos: Preliminary Human Health Risk Assessment for Registration Review," focusing 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. 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

[1] Mode of action (MOA) and adverse outcome pathways (AOPs) describe a set of measureable key events that make up the biological processes leading to an adverse outcome and the causal linkages between such events.

ED_002061_00044426-00006

on behavior and cognition effects (FIFRA SAP 2012). Similarly, the agency also performed a more in-depth analysis of the biomonitoring data and of epidemiological studies from three major children's health epidemiology cohort studies in the U.S., as well as plausible hypotheses on MOAs/AOPs leading to neurodevelopmental outcomes (USEPA 2012). Following the 2012 SAP meeting, the agency solicited additional input from federal experts in the areas of Magnetic Resonance Imaging (MRI) and neurobehavioral testing in children to further clarify results obtained by examination of the epidemiological studies.[2] In December, 2014, the agency released "Chlorpyrifos: Revised Human Health Risk Assessment for Registration Review" which included the use of a physiologically-based pharmacokinetic/pharmacodynamic (PBPK-PD) model to derive human PODs, which obviated the need for the animal to human extrapolation factor, and refined intra-species factors for some lifestages (USEPA 2014). The chlorpyrifos 2014 revised HHRA also included retention of the 10X FQPA Safety Factor due to uncertainty regarding the degree of protection the endpoint of AChE inhibition provides for potential neurodevelopmental effects (USEPA, 2014).

A review of the scientific literature on potential MOA/AOP leading to effects on the developing brain was conducted for the 2012 FIFRA SAP meeting (USEPA, 2012) and updated for the 2014 chlorpyrifos revised HHRA (USEPA 2014). In short, multiple biologically plausible hypotheses and pathways are being pursued by researchers including: AChE as a morphogen; cholinergic system; endocannabinoid system; reactive oxygen species; serotonergic system; tubulin, microtubule associated proteins and axonal transport. However, no one pathway has sufficient data to be considered more plausible than the others. Among the available studies, there are effects which are either as sensitive as or more sensitive than AChE inhibition. The fact that there are, however, sparse data to support the *in vitro* to *in vivo* extrapolation, or the extrapolation from biological perturbation to adverse consequence, significantly limits their quantitative use in risk assessment. The SAP concurred with the agency in 2008 and 2012 about the lack of definable key events in a MOA/AOP leading to developmental neurobehavioral effects. Since the 2014 literature review, there are no substantive changes in the ability to define and quantify steps in an MOA/AOP leading from exposure to effects on the developing brain. The lack of an established MOA/AOP makes quantitative use of the epidemiology study in risk assessment challenging, particularly with respect to dose-response, critical duration of exposure, and window(s) of susceptibility. The agency will continue to monitor the scientific literature for studies on the AOP for neurodevelopmental effects but this document does not include an updated literature review on this line of evidence.

This document (Section 2.0) provides the literature review of *in vivo* laboratory animal studies and epidemiology studies for OPs other than chlorpyrifos to support the single chemical HHRAs. It also provides an integrated weight of evidence (WOE) analysis for all the OPs to support retention of the 10X FQPA Safety Factor (Section 3.0).

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

ED_002061_00044426-00007

2.0 Literature Review

In recent years, the National Academy of Sciences has encouraged the agency to move towards systematic review processes to enhance the transparency of scientific literature reviews that support chemical-specific HHRAs to inform regulatory decision making[3]. The NRC defines systematic review as "a scientific investigation that focuses on a specific question and uses explicit, pre-specified scientific methods to identify, select, assess, and summarize the findings of similar but separate studies".[4] Consistent with NRC's recommendations, EPA's Office of Chemical Safety and Pollution Prevention (OCSPP) is currently developing systematic review policies and procedures. In short, OCSPP employs fit-for-purpose systematic reviews that rely on standard methods for collecting, evaluating and integrating the scientific data supporting the agency's decisions. The literature review described here uses concepts consistent with systematic review such as detailed tracking of search terms and which literature have been included or excluded.

2.1 Developmental Neurotoxicity (DNT) Research on OPs other than Chlorpyrifos: Laboratory Animal Studies

The literature on neurobehavioral effects of developmental exposure to chlorpyrifos was summarized and discussed at the 2012 FIFRA SAP. More recent studies were added to this summary for the 2014 revised HHRA. At that time, the conclusions were that the animal studies clearly showed neurobehavioral outcomes following developmental exposure to chlorpyrifos, but there were inconsistencies in the types of effects reported (neurological domain altered, direction of change, gender specificity). Furthermore, the studies were conducted with doses that most likely produced at least some amount of AChE inhibition at some time during the exposure based on results of guideline studies submitted for registration. The impact of these observations has lead the agency to evaluate whether or not these conclusions extend to other OP pesticides. In this review, the studies of a number of OP pesticides are summarized.

The search aimed to focus on rodent studies involving prenatal/perinatal exposure to OPs in which the offspring were evaluated with *in vivo* neurobehavioral tests. The search methods and analytical scope for this analysis are consistent with the chlorpyrifos analysis from the 2012 FIFRA SAP and 2014 HHRA. Preweaning measurements of behavioral development were noted but not compiled, since these could reflect effects of current exposure to the pesticide rather than long-term neuronal changes. Information on AChE inhibition in either fetuses/pups or dams during this exposure period was evaluated where available. Sections 2.1.1-2.1.3 describe the studies from the open scientific literature. Section 2.1.4 summarizes relevant results from the DNT guidelines studies submitted for pesticide registration (US EPA guideline 870.6300 and/or OECD guideline 426).

---

[3] NRC 2011. "Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde" ; NRC 2014. "Review of EPA's Integrated Risk Information System (IRIS) Process"

[4] http://dels.nas.edu/Report/Review-Integrated-Risk/18764

ED_002061_00044426-00008

2.1.1 Literature Search Strategy & Results

To review and evaluate the developmentally neurotoxic effects of other OPs, a search of the open literature was undertaken. Due to the limited number of studies available, the agency did not limit the search to currently registered OPs. The agency is aware that some OPs listed below are no longer registered for use in the US. In addition, the data evaluation records (DERs) for existing guideline DNT studies were collected from OPP files and summarized. The literature search strategy was developed and conducted by a US EPA reference librarian. Databases searched were PubMed, Web of Science (WoS) and ScienceDirect using key words described below. Duplicates were eliminated after the total database was generated.

1. PubMed (751 results)
(((((organophos* OR Cholinesterase Inhibitors OR acetylcholinesterase inhibitor))) AND ((prenatal OR neonatal OR perinatal OR in utero OR fetal OR foetale OR newborn OR infant* OR postnatal OR gestational OR pregnancy[MeSH Terms]))))) AND ((neurodevelop* OR attention OR birth outcome* OR health outcome* OR cognitive OR cognition OR developmental disability* OR fetal growth OR fetal growth OR foetal development OR fetal development OR sex OR social OR intelligence OR memory OR neurological functioning OR psychomotor OR neurotoxicity OR neurobehavior OR behavior OR learning OR Nervous System[MeSH Terms]) OR Neurotoxicity Syndromes[MeSH Terms]) AND ((((((guinea pigs[MeSH Terms]) OR rabbits[MeSH Terms]) OR mice[MeSH Terms]) OR rats[MeSH Terms]) NOT fishes[MeSH Terms])

2. Web of Science (427 results)
#5 #4 AND #3 AND #2 AND #1
DocType=All document types; Language=All languages;
#4 TS=(guinea pig* OR rabbit* OR mice OR mouse OR rat* OR rodent*) NOT TS=(fish*)
DocType=All document types; Language=All languages;
#3 TS=(neurodevelop* OR attention OR birth outcome* OR health outcome* OR cognitive OR cognition OR developmental disabilit* OR fetal growth OR fetal growth OR foetal development OR fetal development OR sex OR social OR intelligence OR memory OR neurological functioning OR psychomotor OR neurotoxicity OR neurobehavior OR behavior OR learning OR Nervous System OR Neurotoxicity Syndromes)
DocType=All document types; Language=All languages;
#2 TS=(prenatal OR neonatal OR perinatal OR in utero OR fetal OR foetale OR newborn OR infant* OR postnatal OR gestational OR pregnan*)
DocType=All document types; Language=All languages;
#1 TS=(organophos* OR Cholinesterase Inhibitors OR acetylcholinesterase inhibitor OR chlorpyrifos)
DocType=All document types; Language=All languages;

3. Science Direct (19 results)
(ALL(organophos* OR Cholinesterase Inhibitors OR acetylcholinesterase inhibitor OR chlorpyrifos) and ALL(prenatal OR neonatal OR perinatal OR in utero OR fetal OR foetale OR

ED_002061_00044426-00009

newborn OR infant* OR postnatal OR gestational OR pregnan*)) AND (neurodevelop* OR attention OR birth outcome* OR health outcome* OR cognitive OR cognition OR developmental disabilit* OR fetal growth OR fetal growth OR foetal development OR fetal development OR sex OR social OR intelligence OR memory OR neurological functioning OR psychomotor OR neurotoxicity OR neurobehavior OR behavior OR learning OR Nervous System OR Neurotoxicity Syndromes) AND (ALL(guinea pig* OR rabbit* OR mice OR mouse OR rat* OR rodent) and not ALL(fish*)).

This broad literature search identified 1012 potential papers, which were reviewed individually. Specific criteria were applied to select suitable studies, as was previously done with chlorpyrifos. Since the literature on chlorpyrifos has been previously reviewed, those papers were excluded here. This resulted in 19 relevant papers with the following specifications:

- Exposure occurred during gestation and/or the postnatal time frame, ending no later than weaning.
- Dosing included maternal and/or pup administration.
- Dosing was via oral or subcutaneous injection. One paper with intracisternal injection was excluded.
- Behavioral testing of the offspring occurred after weaning and/or into adulthood.
- Studies involved only single-chemical exposure, and where two or more chemicals were administered together, only the single-chemical data were included in the summaries.
- Test subjects were rats or mice. Several papers in pigs and rabbits were excluded due to the lack of comparative database for those species.
- The test measures of interest were neurobehavioral endpoints. At least two studies involved only electrophysiological measures, and those were excluded. No neurochemical, genomic, or other molecular endpoints were included.

The OPs examined, and the number of papers for each, are listed below. Of particular interest are studies from one laboratory (Duke University) that included parathion and diazinon, and can be directly compared to studies with chlorpyrifos using similar experimental designs. The majority of studies used rats (13), and exposures periods varied about evenly between gestational and postnatal stages.

- Parathion (5)
- Diazinon (5)
- Methyl parathion (3)
- Methamidophos (2)
- Chlormephos (1)
- Dichlorvos (1)
- Fenitrothion (sumithion) (1)
- Oxydemeton-methyl (demeton-S-methyl, metasystox-R) (1)

These papers dated back to 1968, and there was a wide range in study quality. Shortcomings were noted in almost all papers, including cursory methodological information and presentation of results, inappropriate statistical analyses, contradictory statements, and problematic interpretation of the data. Regardless, the literature is summarized below in terms of the functional domains organized by each neurobehavioral evaluation.

ED_002061_00044426-00010

Below are study descriptions and summaries organized by neurological domain. Appendix 1 is an overall summary for each chemical, presenting each endpoint and outcome.

2.1.2 Integration of Literature: Neurobehavioral Domains

Cognition

Fifteen studies measured some aspect of cognition: tests included mazes (radial arm maze, Lashley maze, T-maze spontaneous alternation, M-maze), conditioned response (passive avoidance, conditioned avoidance, operant responding, T-maze), and recognition (novel object). Most of these showed adverse effects of OP exposure, although not always in a consistent or dose-responsive manner.

Rats treated with diazinon (0.5, 2 mg/kg/d, postnatal day (PND) 1-4) showed no differences alternating in a T-maze (Timofeeva *et al.*, 2008a). The same rats were tested several months later in a radial arm maze, and showed increased working memory errors (both males and females), but only at the low dose (0.5 mg/kg/d) with no effect on reference memory performance. Diazinon (1 mg/kg/d) was given to rats on gestational day (GD) 15-18 or PND1-4, and there was no change in the trials to criterion in a passive avoidance test; however, there was clearly decreased step-down latency when tested 24 hr later (Vatanparast *et al.*, 2013). This finding suggests a change in memory but not learning. After *in utero* exposure this effect was only seen in females. In contrast, both genders (greater effect in males) were affected following postnatal exposure. Using a novel object test, male mice (females not tested) exposed postnatally (PND8-11) to diazinon (0.5, 5 mg/kg/d) showed less exploration and discrimination of the new object (Win-Shwe *et al.*, 2013). This was significant at both doses (but no dose-response) when tested at PND49, and only the high dose group showed effects at PND84. Mice exposed to diazinon (0.18, 9 mg/kg/d) throughout gestation were tested in a Lashley III maze, with no changes in the number of errors, suggesting no effect on learning (Spyker and Avery, 1977). Thus, these data on diazinon suggest an effect on learning and/or memory (radial arm maze, passive avoidance, novel object recognition) but no changes in learning a maze task. There was a lack of dose-response across studies.

Parathion exposure in rats (0.1, 0.2 mg/kg/d, PND1-4) produced no changes in T-maze spontaneous alternation (Timofeeva *et al.*, 2008b). These rats showed decreased working memory errors, indicative of improvement, at the low dose only (both males and females), and no changes in reference memory errors when tested at about 3 months of age. However, Levin *et al.* (2008) tested littermates from the Timofeeva study beginning at 14 months, and reported increased working memory errors in male rats treated with the low dose only. Interestingly, there is a difference in direction of change and gender specificity compared to the Timofeeva data. Reference memory errors were also increased at both doses (but no dose-response) in male rats only. When the rats were tested again at 17 months, working memory errors were increased at both doses (but no dose-response), again only in males. There was no effect on reference errors at 17 months, and no change in either parameter when the rats were again

ED_002061_00044426-00011

tested at 19 months. In a study by Stamper *et al.* (1988), rats (only males were tested) exposed postnatally to parathion (1.3, 1.9 mg/kg/d, PND5-20) showed decreased spontaneous alternations in a T-maze at both doses (dose-response evident), and increased working memory errors in a radial arm maze at both doses (but no dose-response). Reference memory errors were not altered. Al-Hachim and Fink (1968) administered parathion (3 mg/kg/d) during either the first, second or third week of gestation in mice, and reported no effect on conditioned avoidance (sex not mentioned) following any exposure period. Overall, the radial arm maze showed effects of parathion in several studies, but the direction of change, specificity of errors, and gender selectivity differed. The results with the T-maze were contradictory, with one study out of two reporting effects.

Radial arm maze performance was affected by methyl parathion given directly to pups PND1-21 using an incrementally increasing dose schedule (Johnson *et al.*, 2009). The middle (0.2 to 0.6 mg/kg/d) and high (0.3-0.9 mg/kg/d) dose groups increased both working and reference memory errors. The lowest dose group (0.2 mg/kg/d throughout dosing) also increased reference memory errors; however, on this measure all dose groups had similar averages. Only males were affected on all measures. Rats dosed with methyl parathion (1 mg/kg/d, GD7-15) were trained to go to a specific side in T-maze, and the correct side reversed five times (Crowder *et al.*, 1980). Treated rats had more trials to criterion only on second and fifth switch (note, the text claimed there was an effect on the 4th switch, but the figure does not show it as significant). Effects on only certain reversals is difficult to interpret, and may be a reflection of the multiple t-tests used to analyze the data. Males and females were tested but data were not provided for each sex separately. There were no effects on passive or active avoidance in rats (no mention of gender) exposed to methyl parathion (1, 1.5 mg/kg/d, GD6-20) (Gupta *et al.*, 1985). The low-dose group only showed slower latency to bar press during operant shaping, and more days to asymptote; however, details of operant training and schedule were not provided, the sample size was extremely small (n=4/group), and high variability was mentioned. Thus, the most consistent effect was seen in the radial arm maze (even though there was no dose-response), and other tests were either negative or the data were inconclusive.

Several other OPs were tested in different cognitive tasks, but there is no more than one report for any specific pesticide. After single-trial passive avoidance training, male rats (females not tested) treated with dichlorvos (8 mg/kg/d, GD6-15) showed faster latency to cross when tested 7 days later, suggesting delayed retention (Lazarini *et al.*, 2004). With fenitrothion, male rats (females not tested) exposed gestationally (5, 10, 15 mg/kg/d, GD7-15) were conditioned to climb a pole to escape shock (Lehotzky *et al.*, 1989). The mid and high dose groups (dose-response evident) showed more escapes, were faster, and reached criterion faster than controls. This same pattern seen with reacquisition after a period of extinction, during which time there were no group differences. There was no effect of oxydemeton methyl (0.5, 1.5, 4.5 mg/kg/d, GD6-15) on M-maze learning or memory in rats (Clemens *et al.*, 1990). There was also no effect on retention of single trial passive avoidance in mice exposed to methamidophos (1 mg/kg/d, PND3-9); however, the retention trial occurred at 3 hr instead of the more standard 24 hr or greater (Lima *et al.*, 2013).

ED_002061_00044426-00012

Table 2.1.1 Summary of Cognitive Outcomes

	Early gestation ~GD1-7, ~GD8-14, GD6-15 GD7-15	Late gestation ~GD15-21, GD6-20, GD15-18	Gestation GD1-birth	Early postnatal PND1-4, PND3-9	Late postnatal PND8-11, PND5-20	Postnatal PND1-21
Radial arm maze				Diazinon: cognitive deficit – rat, low dose only, M & F[1] Parathion: improved cognition – rat, low dose only, M & F[2] Parathion: cognitive deficit – rat, M not F[3]	Parathion: cognitive deficit – rat, no dose-response, only M tested[4]	Methyl parathion: cognitive deficit – rat, dose-response, M only[5]
T-maze spontaneous alternation				Diazinon: no effect – rat, M & F[1] Parathion: no effect – rat, M & F[2]	Parathion: cognitive deficit – rat, dose-response, only M tested[4]	
T-maze learning	Methyl parathion: cognitive deficit – rat, sex not specified[6]					
Lashley III maze			Diazinon: no effect – mouse, sex not specified[7]			
M-maze	Oxydemeton methyl: no effect – rat, M & F[8]					
Active avoidance	Fenitrothion: improved	Methyl parathion: no				

ED_002061_00044426-00013

	cognition – rat, dose-response, only M tested[9] Parathion: no effect – mouse, sex not specified[11]	effect – rat, sex not specified[10] Parathion: no effect – mouse, sex not specified[11]				
Passive avoidance	Dichlorvos: cognitive deficit – rat, only M tested[12]	Diazinon: cognitive deficit – rat, F not M[13] Methyl parathion: no effect – rat, sex not specified[10]		Diazinon: cognitive deficit – rat, M & F[13] Methamidophos: no effect – mouse, sex not specified[14]		
Novel object recognition					Diazinon: cognitive deficit – mouse, no dose-response, only M tested[15]	
Operant responding		Methyl parathion: cognitive deficit – rat, sex not specified[10]				

[1] Timofeeva *et al.*, 2008a
[2] Timofeeva *et al.*, 2008b
[3] Levin *et al.*, 2008
[4] Stamper *et al.*, 1988
[5] Johnson *et al.*, 2009
[6] Crowder *et al.*, 1980
[7] Spyker and Avery, 1977
[8] Clemens *et al.*, 1990
[9] Lehotzky *et al.*, 1989
[10] Gupta *et al.*, 1985
[11] al-Hachim and Fink, 1968
[12] Lazarini *et al.*, 2004
[13] Vatanparast *et al.*, 2013
[14] Lima *et al.*, 2013
[15] Win-Shwe *et al.*, 2013

ED_002061_00044426-00014

Motor Activity

Despite being a commonly tested measure in these studies, only a few of the tested OPs produced changes in motor activity. Rats exposed to dichlorvos (8 mg/kg/d, GD6-15) showed decreased open field locomotion at weaning (males not females), and decreased locomotion and increased immobility as adults (age not specified, only males tested) while rearing was not affected at either age (Lazarini *et al.*, 2004). Male rats exposed to fenitrothion (5, 10, 15 mg/kg/d, GD7-15) showed decreased horizontal activity in the high-dose group only on PND104, with an apparent but not significant effect at PND26, but no effect at PND36 (Lehotsky *et al.*, 1989). Open field testing of rats exposed to methyl parathion (1 mg/kg/d, GD7-15) showed what appeared to be increases only on PND23 and 54, with no differences on PND30, 44, 65 (also not PND18); however, the data are not compelling since statistics are not provided, and the text refers to the data as "a possible change" (Crowder *et al.*, 1980). Methyl parathion-treated rats (1 mg/kg/d, GD6-20) showed a decrease in locomotor activity "accommodation" (apparently the period that is 15-30 min into the activity session) in the low dose group only (Gupta *et al.*, 1985).

Several OPs consistently produced no changes on motor activity, regardless of exposure or test species. No effects were seen with diazinon in rats exposed gestationally (1 mg/kg/d, GD15-18, Vatanparast *et al.*, 2013) or postnatally (0.5, 2 mg/kg/d, PND1-4, Timofeeva *et al.*, 2008a, or 1 mg/kg/d, PND1-4, Vatanparast *et al.*, 2013), or in mice exposed gestationally (0.18, 9 mg/kg/d, GD1-birth, Spyker and Avery, 1977). As with diazinon, there were no motor activity changes following parathion exposure postnatally in rats (0.1, 0.2 mg/kg/d, PND1-4, Timofeeva *et al.*, 2008b, or 1.3, 1.9 mg/kg/d, PND5-20, Stamper *et al.*, 1988) or in mice treated during either the first, second, or third week of gestation (3 mg/kg/d, Al-Hachim and Fink, 1968). There was no effect on measures of activity reported in rats treated with methamidophos (1 mg/kg/d, GD6-15), although high variability of the measures was discussed (deCastro *et al.*, 2000). Methamidophos (1 mg/kg/d, PND3-9) also produced no effect in mice (Lima *et al.*, 2013). Finally, there was no effect of oxydemeton methyl exposure (0.5, 1.5, 4.5 mg/kg/d, GD6-15) on open field activity (Clemens *et al.*, 1990).

In this review, effects on activity are only considered in tests designed specifically for that purpose. During the course of other behavioral tests, e.g., radial arm or T-maze, speed or latency is often measured. These ancillary activity measures were sometimes altered by treatment, but are not included in this domain, since they are not designed to specifically target motor activity.

ED_002061_00044426-00015

Table 2.1.2. Summary of Motor Activity Outcomes

Task/Test Apparatus	Early gestation ~GD1-7, ~GD8-14, GD6-15 GD7-15	Late gestation ~GD15-21, GD6-20, GD15-18	Gestation GD1-birth	Early postnatal PND1-4, PND3-9	Late postnatal PND8-11, PND5-20
Open field	Dichlorvos: decreased activity – rat, only M tested[1] Fenitrothion: decreased activity – rat, dose-response, only M tested[2] Methamidophos: no effect – rat, sex not specified[3] Methyl parathion: increased activity – rat, sex not specified[4] Oxydemeton methyl: no effect – rat, M & F[5] Parathion: no effect – mouse, sex not specified[6]	Diazinon: no effect – rat, M & F[7] Parathion: no effect – mouse, sex not specified[6]	Diazinon: no effect – mouse, sex not specified[8]	Diazinon: no effect – rat, M & F[7] Methamidophos: no effect – mouse, sex not specified[9]	Parathion: no effect – rat, only M tested[10]
Figure-Eight				Diazinon: no effect – rat, M & F[11] Parathion: no effect – rat, M & F[12]	
Donut		Methyl parathion: decreased activity – rat, no dose-response, sex not specified[13]			

ED_002061_00044426-00016

[1] Lazarini *et al*., 2004
[2] Lehotzky *et al*., 1989
[3] deCastro *et al*., 2000
[4] Crowder *et al*., 1980
[5] Clemens *et al*., 1990
[6] al-Hachim and Fink, 1968
[7] Vatanparast *et al*., 2013
[8] Spyker and Avery, 1977
[9] Lima *et al*., 2013
[10] Stamper *et al*., 1988
[11] Timofeeva *et al*., 2008a
[12] Timofeeva *et al*., 2008b
[13] Gupta *et al*., 1985

ED_002061_00044426-00017

Anxiety/Depression

In a series of tests in rats, Roegge *et al*. (2008) showed that early postnatal exposure to diazinon (0.5, 2 mg/kg/d, PND1-4) produced behaviors suggesting higher anxiety at the high dose (decreased open arm time in an elevated plus maze), lower fearfulness (decreased time to start eating in novel environment) in both dose groups, and anhedonia (decreased chocolate milk preference) at the low dose only, but not depression (forced swim test). These effects occurred only in males and did not show a clear dose-response for the novelty eating and chocolate milk preference tests. Using the same tests (same laboratory; Timofeeva *et al*., 2008b), parathion exposure (0.1, 0.2 mg/kg/d, PND1-4) in rats (high dose, both sexes) increased time in the open arm in an elevated plus maze (suggesting decreased anxiety); however, there was also an increase in center crossings, indicating hyperactivity, that may confound overall interpretation. These same rats showed no changes in novelty-suppressed feeding or in chocolate milk preference. Thus, in these two studies the pesticides appear to have different effects on this functional domain.

There are only a few reports of these behaviors with other pesticides. Rats treated with methyl parathion (1, 1.5 mg/kg/d, GD6-20) showed faster emergence from a cage, interpreted by the authors as lowered anxiety, in the low dose group only (Gupta *et al*., 1985). With chlormephos exposure (~0.06, 0.6 mg/kg/d in drinking water, one week premating to weaning), adult mice (both males and females) in the high-dose group showed decreased time spent in the open arms and increased time in the closed arms (no change in latency) of an elevated plus maze, suggesting increased anxiety (Ceh *et al*., 2012). Mice exposed to methamidophos (1 mg/kg/d, PND3-9) showed no differences in time spent in either arm of an elevated plus maze, but spent less time in center (Lima *et al*., 2013). This was interpreted by the authors as effect on choosing arms, which they say is a cognitive effect; however, this measure is often interpreted to reflect only activity levels. The same mice showed increased immobility in forced swim test, suggesting depressive-like behaviors. Overall, these results are varied and did not consistently show a dose-response.

ED_002061_00044426-00018

Table 2.1.3. Summary of Anxiety/Depression Outcomes

	Late gestation ~GD15-21, GD6-20, GD15-18	Perinatal premating-weaning	Early postnatal PND1-4, PND3-9
Elevated plus maze		Chlormephos: increased anxiety – mouse, M & F[1]	Diazinon: increased anxiety – rat, dose-response, M not F[2] Methamidophos: no effect –mouse, sex not specified[3] Parathion: decreased anxiety – rat, dose-response, M & F[4]
Chocolate milk preference			Diazinon: increased anhedonia – rat, no dose-response, M not F[2] Parathion: no effect – rat, M & F[4]
Novelty suppressed feeding			Diazinon: decreased fearfulness – rat, dose-response, M not F[2] Parathion: no effect – rat, M & F[4]
Forced swim			Diazinon: no effect – rat, M & F[2] Methamidophos: increased despair – mouse, sex not specified[3]
Open field behaviors	Methyl parathion: decreased anxiety – rat, no dose-response, sex not specified[5]		

[1] Ceh *et al*., 2012
[2] Roegge *et al*., 2008
[3] Lima *et al*., 2013
[4] Timofeeva *et al*., 2008b
[5] Gupta *et al*., 1985

ED_002061_00044426-00019

Social Behavior

Only one study has used tests of social behavior using these OPs. With exposure to fenitrothion (5, 10, 15 mg/kg/d, GD7-15), rats in mid and high dose (dose-response) spent more time actively interacting in conspecific pairs (Lehotsky *et al.*, 1989). The scarcity of data on this measure prevents any conclusions across OPs.

Sensory Function

In a study measuring response to a tactile stimulus, with and without an acoustic prepulse, male (but not female) rats treated with diazinon (0.5, 2 mg/kg/d, PND1-4) showed less prepulse inhibition at both doses; however, no dose-response was evident (Timofeeva *et al.*, 2008a). Using the same paradigm, rats treated with parathion (0.1, 0.2 mg/kg/d, PND1-4) showed a different pattern: lower response to the stimulus alone (high dose, both sexes), but no change in the inhibition produced by the prepulse (Timofeeva *et al.*, 2008b). Mice treated with diazinon through gestation showed no change in response to noise (auditory startle) or smell (olfactory orientation), but did show change in visual cliff behavior (more steps off a "cliff") which occurred only in females in the low dose group (Spyker and Avery, 1977). Additional studies are needed for general conclusions regarding the effects of these pesticides on sensory function.

Table 2.1.4. Summary of Sensory Outcomes

	Gestation GD1-birth	Early postnatal PND1-4, PND3-9
Auditory	Diazinon: no effect – mouse, sex not specified[1]	
Tactile with or without prepulse		Diazinon: decreased sensory gating – rat, no dose-response, M not F[2] Parathion: decreased startle response – rat, dose-response, M & F[3]
Visual	Diazinon: decreased function – mouse, no dose-response, F not M[1]	
Olfactory	Diazinon: no effect – mouse, sex not specified[1]	

[1] Spyker and Avery, 1977
[2] Timofeeva *et al.*, 2008a
[3] Timofeeva *et al.*, 2008b

ED_002061_00044426-00020

Neuromotor Function

One study assessed neuromotor function in mice exposed to diazinon (0.18, 9 mg/kg/d, GD1-birth), and reported some changes in motor abilities measured at about 2 months of age; however, these were not consistent in dose or direction of change (Spyker and Avery, 1977). Increased ability was suggested by a longer time to cling to a rod (both doses, no dose-response). In contrast, there was less ability to stay on an increasingly inclined plane (both doses, no dose-response) or perhaps on a rotarod (group means not significant due to large variability, suggestive of effect at both doses).

Rats in the high-dose group exposed to fenitrothion (5, 10, 15 mg/kg/d, GD7-15) fell off a rotarod faster on PND26 and PND104, but not PND36. Only males were tested (Lehotsky *et al.*, 1989).

There were no neuromotor changes in terms of rotarod performance in rats following exposure to parathion (1.3, 1.9 mg/kg/d, PND5-20) (Stamper *et al.*, 1988) or methyl parathion (1, 1.5 mg/kg/d, GD6-20) (Gupta *et al.*, 1985). Overall, there is little support for conclusions of neuromotor outcomes following these pesticides, but more studies are needed.

Table 2.1.5. Summary of Neuromotor Outcomes

	Early gestation ~GD1-7, ~GD8-14, GD6-15 GD7-15	Late gestation ~GD15-21, GD6-20, GD15-18	Gestation GD1-birth	Late postnatal PND8-11, PND5-20
Rotarod	Fenitrothion: decreased performance – rat, dose-response, only M tested[1]	Methyl parathion: no effect – rat, sex not specified[2]	Diazinon: no effect – mouse, sex not specified[3]	Parathion: no effect – rat, only M tested[4]
Inclined plane			Diazinon: decreased performance – mouse, dose-response, sex not specified[3] (Spyker)	
Rod cling			Diazinon: increased performance – mouse, no dose-response, sex not specified[3]	

[1] Lehotzky *et al.*, 1989
[2] Gupta *et al.*, 1985
[3] Spyker and Avery, 1977
[4] Stamper *et al.*, 1988

ED_002061_00044426-00021

2.1.3 Integration with AChE Inhibition

There are some data with which to compare effective doses in these DNT studies with doses producing AChE inhibition. A number of studies in this literature review included AChE inhibition (brain and/or blood) in their measurements. Information on diazinon and parathion can be taken from a separate (non-behavioral) study (Slotkin *et al.*, 2006) conducted in the same laboratory with the same dosing paradigm used in several studies (Levin *et al.*, 2008; Roegge *et al.*, 2008, Timofeeva *et al.*, 2008a, 2008b).

Neurobehavioral effects of diazinon were reported in rats at doses of 0.5-2 mg/kg/d (Roegge *et al.*, 2008; Timofeeva *et al.*, 2008a). Slotkin *et al.* (2006) reported that a dose of 0.5 mg/kg/d produced some (<10%, statistically significant) brain AChE inhibition when measured the day after the last dose. At 2 hr after a higher dose (2 mg/kg/d; lower doses were not tested at 2 hr), there was greater brain inhibition (25-30%) compared to 24 hr (10-20%). Thus it is probable that for diazinon, inhibition during and shortly after the dosing period (i.e., within hours) was greater at lower doses. While there is no direct AChE data following diazinon exposure at 1 mg/kg/d (Vataparast *et al.*, 2013), it can be assumed from the Slotkin data that this dose also inhibited brain AChE at some time during/after dosing. No AChE activity was measured in mice by Spyker and Avery (1977), but the high dose of 9 mg/kg/d resulted in depressed weight gain, a sign of maternal toxicity. There is no mention of toxicity at the lower doses (0.5, 5 mg/kg/d) used in mice by Win-Shwe *et al.* (2013).

The parathion studies showing effects at 0.1 and 0.2 mg/kg/d (Timofeeva *et al.*, 2008b; Levin *et al.*, 2008) are also informed by the AChE inhibition presented in Slotkin *et al.* (2006) in which a dose of 0.1 mg/kg/d inhibited brain AChE 5-15% on the day after the last dose. There are no AChE data available for a higher dose, 0.2 mg/kg/d, but Timofeeva noted 5% mortality in that dose group. Much higher doses (1.3, 1.9 mg/kg/d in rats) were reported to produced 35, 68% brain inhibition on the day after the last dose (PND5-20), with 26, 36% inhibition persisting a week later (Stamper *et al.*, 1988).

The methyl parathion postnatal incrementing dose paradigm in rats used by Johnson *et al.* (2009) produced brain AChE inhibition in all dose groups at the end of dosing, persisting for 10 to 20 days later; recovery was evident 30-40 days later. The low dose (0.2 mg/kg/d throughout) produced 13-15% inhibition. During gestational exposure to higher doses of methyl parathion at 1, 1.5 mg/kg/d, the dams had 20, 60% brain inhibition at the end of dosing (GD6-20) (Gupta *et al.*, 1985). At the high dose, cholinergic signs and increased resorptions were noted. Furthermore, the offspring (fostered to control dams) showed brain inhibition as high as 50% in both dose groups when measured at birth and also PND7, 14, 21, and 28. The low dose showed recovery at PND28, but not the high dose.

In the study of rats treated with fenitrothrion, there was postnatal mortality of 16-17.5% at all doses (5, 10, 15 mg/kg/d, GD7-15), compared to 5% in controls (Lehotsky *et al.*, 1989). In separate study of rats, 5, 25 mg/kg on GD19 produced 40, 80% brain AChE inhibition in dams,

and fetal brains showed about 90% inhibition (no dose response) (Sochaski *et al.*, 2007). Assuming similar responses, the postnatal mortality observed in the Lehotsky study could be at least partly due to this high degree of AChE inhibition in both dams and fetuses.

In rats, nonpregnant females dosed methamidophos 1 mg/kg/d for 10 days showed 16% plasma AChE inhibition, but brain AChE was apparently not measured. This suggests that dams treated at the same dose in a study by deCastro *et al.* (2000) most likely experienced some plasma inhibition. Mouse pups dosed with methamidophos 1 mg/kg/d PND3-9 showed ~36, 46% brain inhibition 1, 4 hr after first dose, 53, 61% inhibition at 1, 4 hr after last dose, and ~19% brain inhibition the day after last dose (Lima *et al.*, 2013). The mice therefore experienced considerable brain AChE inhibition throughout dosing.

In the oxydemeton methyl study, dams showed 22-68% brain AChE inhibition on the day after the last dose (0.5-4.5 mg/kg/d, dose response), and 5 days later (GD20) there was 20-54% brain AChE inhibition. Fetal brains taken the day after dosing showed no inhibition (Clemens *et al.*, 1990); however, there were no fetal AChE tissues collected during or shortly after the dosing period when AChE inhibition would be greatest.

Overall, in the studies for which there are direct or comparable data, it is clear that the dosing paradigms produced AChE inhibition and in some cases maternal toxicity. Indeed, there are no studies reporting or even suggesting a lack of AChE inhibition in the dam and/or fetus/pup at any time during dosing. Thus, it is not known whether exposure paradigms that do not inhibit AChE would produce any neurobehavioral effects.

2.1.4 Summary of Findings from the Developmental Neurotoxicity (DNT) Guideline Studies

DNT studies have been submitted for 20 OPs, summarized in Appendix 2. These studies follow the US EPA guideline 870.6300 and/or OECD guideline 426 which require testing of motor activity, acoustic startle response, learning and memory, and brain morphometrics in the offspring around weaning and also in adulthood. In general, these studies provide exposure during development either via diet or oral gavage dosing, including direct dosing of the pup preweaning. As with the literature studies, these submitted studies have shortcomings such as inappropriate statistical analyses, limited methodological information and presentation of results. Many measures tend to show high variability, which reduces their interpretability and utility.

In order to compare the submitted guideline and published studies under the scope identified under Section 2.1 and to be consistent with the chlorpyrifos 2012/2014 review, only changes that occurred after dosing had ended (i.e., shortly after weaning or as adults) were considered here. Across the seven submitted studies that reported effects, there are mostly changes in acoustic startle reactivity, cognitive function, and to a lesser extent, motor activity. Some OPs

ED_002061_00044426-00023

altered multiple domains, others only one. There are both submitted guideline studies and literature studies for only four OPs: diazinon, methyl parathion, methamidophos, and dichlorvos. Diazinon produced cognitive changes but no effects on motor activity or acoustic startle in the DNT: these results are generally in agreement with published studies. Male offspring in the high dose group (~33 mg/kg/d in the dam diet) showed increased errors and longer latency in Biel maze performance at both PND24 and PND62, and similar effects were seen in females but only in the middle dose group (~3.4 mg/kg/d via diet) at PND24. Following methamidophos exposure, female rats in the middle and high dose groups (~1.7, 5.2 mg/kg/d in the dam diet) showed decreased peak amplitudes of the startle response, which was statistically significant at PND38 and apparent but not significant at PND60. In contrast to the literature reports of cognitive and motor effects of methyl parathion, there were no reported changes in the submitted guideline study. The submitted study of dichlorvos was uninterpretable due to high pup mortality in all groups, including control.

In these studies, AChE activity was assessed as part of the DNT itself, or by means of a separate study comparing the response in pups and adults (comparative cholinesterase, or CCA, studies). Thus, in almost all guideline DNT studies there are adequate data describing AChE inhibition in the pups at some time during development. It is clear from these DNT studies that the doses used did produce AChE inhibition in the offspring, sometimes at all doses tested. It was noted that in these studies, most of the reported effects occurred before weaning, which is the period during which there was likely to be ongoing AChE inhibition.

Thus, as with the literature studies, there are scant data that could inform potential neurodevelopmental changes occurring at doses lower than those needed to inhibit AChE. Furthermore, there is little consistency in patterns of effects across studies or chemicals. Thus, there is uncertainty as to whether lower, non-inhibiting exposures are developmentally neurotoxic; this uncertainty was described in the chlorpyrifos reviews and remains applicable for the available data for other OPs as well.

2.1.5 Conclusions on *In Vivo* Laboratory Animal Studies

For chlorpyrifos, there are >30 papers on developmental neurotoxicity; for the remaining OPs, the literature is sparse with very few studies for each OP (including DNT guideline studies). The studies span over decades, and many of the lower quality studies were the earlier ones; however, some very recent papers also have significant deficits. Methodological detail is lacking, inappropriate statistical analyses are applied, results are cursorily described and/or inaccurately presented, and interpretation of some behavioral changes is faulty. Overall, most studies have significant shortcomings and/or are of low quality.

The most commonly tested behaviors considered aspects of cognition. In the majority of studies, some sort of cognitive deficit was detected, especially with working memory performance (radial arm maze) and conditioned response retention (passive avoidance). However, in many cases there was no dose-response, there was some gender specificity which

did not replicate in multiple studies, and cognitive improvement instead of deficit was noted in a few papers. Changes in motor activity in offspring were generally not reported, and the direction of change differed in the papers reporting such effects. There is generally not enough information to make definitive statements about OP effects on other types of neurological disorders.

Few published papers included AChE measurements of the dams and/or offspring, but where measured, all doses used inhibited AChE to some degree. Some papers even reported overt maternal and fetal toxicity. This was also the case in the guideline studies, most of which included concurrent or supplemental data on AChE inhibition. Since there are no studies with low doses that definitively do not inhibit AChE, there is no information in the animal literature that shows whether or not there would be developmentally neurotoxic outcomes at those lower exposures.

2.2 Epidemiology Research on OPs other than Chlorpyrifos

2.2.1 Overview of 2012/2014 and 2015 Literature Reviews

In April 2012, EPA presented to the FIFRA Scientific Advisory Panel (SAP) its review and assessment of several epidemiological investigations of the potential adverse neurodevelopmental outcomes of *in utero* and early life exposure to chlorpyrifos. In this effort, EPA limited its review to studies conducted within three major US based prospective birth cohort studies: 1) Mother's and Newborn Study of North Manhattan and South Bronx conducted by Columbia University, referred to in this document as "Columbia Study/Cohort;" 2) Mount Sinai Inner-City Toxicants, Child Growth and Development Study, or the "Mount Sinai Study/Cohort;" and 3) Center for Health Assessment of Mothers and Children of Salinas Valley (CHAMACOS) conducted by the University of California Berkeley, or "CHAMACOS Study/ Cohort." The conclusion of EPA's evaluation, supported by the FIFRA SAP (2008, 2012), was that "chlorpyrifos likely played a role in the neurodevelopmental outcomes observed in these studies." The major findings of these cohorts are briefly summarized in Section 2.2.9 to provide context for integrating the findings of the 2015 review and for the WOE analysis.

In the current review, the agency has expanded its consideration of the epidemiological data to include studies of any OP pesticide; several different types of development and neurological, neurodevelopmental, and neurobehavioral health outcomes; studies performed in non-U.S. countries as well as US based studies; and non-cohort studies.

2.2.2 Literature Search Methodology

To identify the epidemiological investigations of the association between OP exposure and adverse neurological, neurodevelopmental or neurobehavioral effects, EPA scientists queried PubMed/Medline and Web of Science directly. In this literature search, emphasis was placed

upon identification of all possible epidemiological studies available, and the ability to use the identical search string in both PubMed/Medline and Web of Science The following search string was utilized:

> ((Chlorpyrifos OR Organophosphates) AND (prenatal OR neonatal OR perinatal OR in utero OR fetal OR foetal OR newborn OR infant* OR infancy OR preschool OR child* OR maternal OR mother* OR pregnan*) AND (neurodevelop* OR attention OR birth outcome* OR health outcome* OR birth height OR birth weight OR birth length OR cephalometry OR head circumference OR child development OR cognitive OR cognition OR developmental disability* OR fetal growth OR foetal growth OR foetal development OR fetal development OR intelligence OR memory OR neurological functioning OR psychomotor) AND (human)) (Filter: English language only)

With the aid of an EPA reference librarian, EPA also searched the following databases: PsycInfo, Agricola, Biosis, Embase, Enviroline, Gale Health and Wellness, Global Health, Pascal and Pollution Abstracts. EPA used similar, but modified search terms as listed above. Upon identification of the final set of relevant articles (n=38), limited hand-searching of the reference lists and citation mapping *(Science citation index)* of articles deemed to be most relevant to the review question was performed.

EPA identified 300 articles across these several biomedical search engines. Removing duplicates, there were 243 articles, and 79 were determined to be epidemiological investigations of potential relevance. The 164 studies excluded from the analysis comprised 57 exposure only studies; 51 review articles; 33 reports of acute OP intoxication; 20 studies in non-human systems; and 3 were otherwise not relevant. Among the 79 potentially relevant epidemiologic studies, 41 were excluded; 17 articles were previously reviewed in 2012; 16 were epidemiological methods papers including exposure validation studies without an original epidemiological risk estimate; and 8 were otherwise not relevant for various reasons. Among the 40 remaining studies, 2 were additionally excluded (one was a duplicate study published a second time; the other did not make a measure of an OP pesticide. Therefore, 38 articles are included in this narrative literature review (referred to herein as "2015 Literature Review/Studies"). The determination of relevance to the study question was made by two EPA epidemiologists who agreed by consensus as to article disposition in the literature search.

The following sections provide the results of this literature review. Section 2.2.3 describes the breadth and depth of the 2015 literature review, with Section 2.2.4 summarizing the approach for assigning a quality ranking and Section 2.2.5 providing the results of this quality ranking. In Section 2.2.6, these studies were further analyzed with focus on identification of the most appropriate exposure assessment and relevant outcomes for this assessment. Studies focusing solely on birth outcomes are discussed in Section 2.2.7. However, the emphasis in this assessment is on those studies focusing on neurodevelopmental outcomes, which are discussed in detail in Section 2.2.8 and summarized in Section 2.2.9.

ED_002061_00044426-00026

2.2.3 Breadth and Depth of the 2015 Literature Review

Key features of each of the 38 articles in the current 2015 literature review are summarized in Table 2.2.5-1 and 2.2.5-2, as well as Appendix 3. These articles represent 31 distinct studies covering a wide range of study designs, study locations and time periods, and exposure and outcome measurement approaches and are listed in Table 2.2-a. There were 10 different study designs identified; the majority of the studies utilized a cross-sectional or a prospective birth cohort study design.

In addition to four articles on the previously-reviewed studies (two on the Mt. Sinai Cohort and two on the CHAMACOS Cohort), there were a number of new birth cohorts or named studies in this literature, including (see Table 2.2.5-1, 2.2.5-2, and Appendix 3 for details):

- Denmark, Birth Cohort (Andersen *et al.* 2015)
- Saint Peter's University Hospital, New Brunswick, New Jersey, Birth Cohort (Barr *et al.* 2010)
- Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT), Mexico City, Mexico, Birth Cohort (Fortenberry *et al.* 2014, 2014a)
- EcoSalud Project, Cayambe-Tabacundo region, Ecuador, Infant and Young Child Cohort (Handal *et al.* 2007, 2007b, 2008)
- University Hospital of Heraklion, Crete, Greece, Birth Cohort (Koutroulakis *et al.* 2014)
- Children's Pesticide Survey (CPS), Yuma County, Arizona (Lizardi *et al.* 2008)
- Infancio y Medio Ambiente (INMA) (Environment and Childhood), Spain, Birth Cohort (Llop *et al.* 2013)
- Perturbateurs endocriniens: Étude Longitudinale sur les Anomalies de la Grossesse, l'Infertilité et l'Enfance (PELAGIE), Brittany, France, Birth Cohort (Petit *et al.* 2010)
- Health Outcomes and Measure of the Environment (HOME), Cincinnati, OH, Birth Cohort (Rauch *et al.* 2012)
- Embilipitiya Base Hospital, Southern Sri Lanka, Birth Cohort (Samarawickrema *et al.* 2008)
- Ontario Farm Family Health Study, Ontario, Canada, Birth Cohort (Savitz *et al.* 1997)

ED_002061_00044426-00027

- Childhood Autism Risks from Genetics and the Environment (CHARGE), California, Child Cohort (Shelton *et al.* 2014)

- Secondary Exposure to Pesticides Among Infants, Children and Adolescents (ESPINA), Pedro Moncayo County, Pichincha, Ecuador, Child Cohort (Suarez-Lopez *et al.* 2012, 2013, 2013a)

- Shenyang, China, Birth Cohort (Zhang *et al.* 2014)

Exposures were assessed via environmental samples, biomarkers, and/or proxy methods.

- Only one study used environmental samples as an exposure measure - methyl parathion in household wipe samples (Ruckart *et al.*, 2004).

- Among the studies, 11 biomarkers were used to assess exposure. One study utilized a direct measure of OP pesticide – chlorpyrifos (CPF) in maternal and cord serum (Barr *et al.*, 2010). Several studies looked at specific OP parent metabolites (IMPY, 3,5,6-trichloro-2-pyridinol [TCPy], PNP). The majority of studies used non-specific OP biomarkers: dialkyl phosphates (DAPs), diethyl phosphates (DEPs), and dimethyl phosphates (DMPs). Two effect biomarkers, AChE and butyl cholinesterase (BuChE), were used as the exposure metric.

- Proxy exposure methods included questionnaire and non-questionnaire approaches. For example, questionnaire-based exposures included maternal and or paternal self-report of living with an exposed worker, occupational exposure/ employment, and home pesticide use and child outdoor play exposure. Non-questionnaire-based exposures included Community/area of residence, distance to treated area/farm, percent of area treated with pesticide, level of urbanization, pounds OP pesticide used/year, and pesticide spray season.

Likewise, there were numerous outcome measures examined across the studies, falling into six broad categories: birth characteristics, autonomic nervous system (ANS) effects, Attention Deficit Hyperactivity Disorder (ADHD)/attention problems, autism, general neurodevelopment (cognitive, behavioral, IQ), and physiological effects. The most common outcome measures were birth characteristics (with birth weight, birth length, head circumference and gestational age being the most frequent) and neurodevelopment tests and test batteries. Table 2.2-b lists the many specific neurodevelopment tests employed in the studies. Most studies utilized more than one test, and few tests were utilized in more than one study.

Other features that varied widely among the studies include:

- Study periods ranged from 1991 to 2012 (reports were published from 1997 to 2015).

ED_002061_00044426-00028

- Study locations included Canada (2 studies), China (3 studies), Costa Rica, Israel, Denmark, Ecuador (3 studies), France, Greece, Mexico (3 studies), Poland, Spain, Sri Lanka, and the United States [AZ, CA (2 studies), MS, NJ, NY, NC, OH (2 studies), OR, National (2 studies)].
- Study sizes varied from 25 to 3,159 participants.
- Children's ages ranged from newborns to age 15 years.

Pre-natal exposures were assessed in 19 reports, post-natal exposures in 13 reports, and both pre- and post-natal exposures in 5 reports.

Table 2.2-a Study Designs, Exposure Measurement Methods, and Outcome Measurement Methods Used across the 31 2015 Review Studies

Study design (# studies used)	Exposure measurement (# studies used)	Outcome measurement (# studies used)
Prospective Cohort (10) Retrospective Cohort (3) Case-control (2) Cross-sectional (15) Ecological (1)	**Biomarkers** **AChE** - acetyl cholinesterase (maternal 1, child 2) **BuChE** - butyl cholinesterase (child 1) **CPF** -chlorpyrifos parent (maternal, cord serum 1) **TCPy** - chlorpyrifos metabolite (maternal 1, child 1) **DZN** – diazinon parent (1) **IMPY** – diazinon metabolite (child 1) **MAL** - malathion parent (0) **MDA** - malathion metabolite (maternal 1) **DAP** – dialkyl phosphate (maternal 5, child 7, amniotic fluid 1) **DEP** – diethyl phosphate (maternal 5, child 7, amniotic fluid 1) **DMP** – dimethyl phosphate (maternal 5, child 7, amniotic fluid 1) **OP** - organophosphate (cord blood 1) **PNP** - para-nitrophenol (child 2) **Environmental** Methyl parathion - Household wipe (1) **Proxy (questionnaire)** • Maternal occupational exposure/ employment (6) • Paternal occupational exposure/ employment (2) • Living with exposed worker (1) • Home and outdoor play exposure (1) **Proxy (non-questionnaire)**	**Birth characteristics** (10) • Birth Weight/LBW/FGR (records 7, NP 1, report 2) • Birth Length (records 3) • Head circumference (records 3, NP 1) • Abdominal circumference (records 1) • Gestational age (GA)/preterm (report 2, records 3) • Ponderal index (records 1) • Placental maturity index (1) • Spontaneous abortion/miscarriage (report 1) • Altered sex ratio (report 1) **Autonomic Nervous System** (4) **ADHD/attention problems** (2) • Diagnosis DISC-IV (1) • Use of medication (1) • Screening (CPRS-R, CPT, BASC-PRS) (1) **Autism** (2) • CA Department of Developmental Services (CDDS) reports • US Individuals with Disabilities Education Act (IDEA) reports • Autism spectrum disorders (ASD) • Autism Diagnostic Observation Schedule (ADOS) combined with ADI-R. **Neurodevelopmental (ND) test/battery** - US (3) – see Table 2.2-2b for details **ND test/battery - non-US** (11) – see Table 2.2-2b for details **IQ** (4) - see Table 2.2-2b for details

ED_002061_00044426-00029

	• Community/area of residence (3) • Distance to treated area/farm (2) • Percent of area treated with pesticide (1) • Level of urbanization (1) • Pounds OP used/year (1) • Pesticide spray season (1)	**Physiological** (2) • AChE activity, child • BuChE activity, maternal • Antioxidant status: superoxide dismutase (SOD) activity • Fetal oxidative stress: malondialdehyde (MDA) concentrations) • Fetal DNA fragmentation: electrophoresis

ED_002061_00044426-00030

Table 2.2-b Detailed Neurological Outcome Measurement Methods Used across the 31 2015 Review Studies

Outcome measurement (# studies used)

Neurodevelopmental (ND) test/battery - US (4)

- Pediatric Environmental Neurobehavioral Test Battery (PENTB) : cognitive, motor, sensory, and affect domains
  - Developmental Test of Visual-Motor Integration (VMI)
  - Kaufman Brief Intelligence test (K-BIT)
  - Purdue Pegboard
  - Story Memory and Story Memory-Delay from Wide Range Assessment of Memory and Learning
  - Trail-Making test, Part A and Part B
  - Verbal Cancellation test
- Children's Memory Scale (CMS)
- Behavioral measures:
  - The Child Behavior Checklist/4-18
  - The Teacher Report Form
- Developmental delay (DD):
  - Mullen Scales of Early Learning (MSEL)
  - Vineland Adaptive Behavioral Scale (VABS)
  - Reciprocal social interaction: Social Responsiveness Scale

ND test/battery - non-US (11)

- Bayley Scales of Infant Development - mental and psychomotor development
- Behavioral Assessment and Research System (BARS): Memory and attention, response speed and coordination, visual memory, attention, divided attention, recall and recognition memory, dexterity, hand-eye coordination (2)
- Figure drawing task: child's perception and dexterity
- Long-term memory test
- Ages and Stages Questionnaire (ASQ) – communication, fine motor, gross motor, problem solving, and personal–social skills
- Strengths and Difficulties Questionnaire, parent version (SDQ): behavioral problems
- NEPSY-II test (trained examiners): general assessment battery:
  - attention and executive functioning
  - language
  - memory and learning
  - sensorimotor (visuomotor precision),
  - visuospatial processing
  - Statue and Knock Tap
- Neonatal Behavioral Neurological Assessment (NBNA)
  - Behavior
  - Passive Tone
  - Active Tone
  - Primary Reflexes
  - General Assessment
- Reach-and-grasp, bi-manual coordination: Prehension abilities
- UC Berkeley Preferential Looking Test Cards: Visual acuity skills
- Visual Motor Integration (VMI), Beery-Buktenica, 4th Ed.
- Finger Tapping test: Manual motor speed
- Catsys equipment: Simple reaction time
- Conners' Continuous Performance Test II (CPT II, v5): Attention
- Woodcock-Johnson III Tests of Cognitive Abilities (WJ-III) Verbal Comprehension test: Long-term memory and language function
- Visuospatial performance and memory functions:
  - Raven's Colored Progressive Matrices
  - Stanford–Binet Copying Test, 4th ed
- Physical examination (social response, spontaneous motility, involuntary movements, Romberg's sign, walking straight line, standing on one leg, number hops, biceps and patellar reflexes, finger opposition, diadochokinesis, finger–nose coordination, hearing, vision)
- Gesell Development Schedule (GDS): motor, adaptive, language, and social
- Santa Ana Form Board: Motor coordination
- Child's developmental delay - Parent interview

ED_002061_00044426-00031

IQ (4)

- Wechsler Intelligence Scale for Children-Revised (WISC-R) Digit Span Test, Card sorting Test
- Stanford-Binet Memory for Sentences and Digit String test
- Recall and recognition test

2.2.4 Considerations for Study Quality Evaluation

This section summarizes how specific study characteristics factored in overall quality category. [Note: these study quality considerations are specific to issue of relevance to this document, namely potential for neurodevelopmental effects of OPs. These considerations are considered 'fit for purpose' under this context and could differ in another regulatory or scientific context.] The literature base evaluated is heterogeneous, as noted in Section 2.2.3, consisting of various study designs implemented in U.S. and foreign study populations. Pesticide exposure assessments variously relied on, for example, exposure biomarkers, maternal self-reports, and other proxy indicators of OP pesticide exposures. Outcome assessments were similarly varied, relying, for example, on biomarkers of biological effects, birth records, maternal self-reports, and clinical instruments designed to evaluate neurocognitive and neurobehavioral development. These design elements have potential impacts on study quality and relevance to this document. Each study was therefore judged to be of high, moderate, or low quality in each of the following six domains effecting study quality: Study design, exposure assessment, outcome assessment, confounder control, statistical analysis, susceptibility to bias (See Table 2.2.4-1 for general considerations under each domain).

2.2.4.1 Study Designs

Four basic study designs were used in the literature reviewed for this document: cohort study, case-control study, cross-sectional study, and ecologic study. The first of these two constitute the two basic types of observational (i.e. non-interventional) studies used to evaluate relative incidence of health and disease outcomes by exposure status. The latter two are generally considered descriptive or hypothesis generating study designs, though they too can be used to test hypotheses regarding relative prevalence of health outcomes and, under certain conditions, incidence as well.

Table 2.2.4-1 Study Quality Considerations

Parameter	High (Score 8-12)	Moderate (Score 4-7)	Low (Score 0-3)
Study Design	Prospective, exposure precedes disease	Case Control	Cross sectional Ecological
Exposure assessment	Exposure assessment includes information on specific OP a.i.'s (e.g., CPF, MAL), or urinary metabolite (TCPy, IMPy,), or high quality questionnaire based chemical specific exposure assessment during relevant exposure	Non-specific biomarker of exposure (DAP), or effect (AChE/BuChE), or questionnaire based individual level information on the OP class, or sub-class	Low quality questionnaire based exposure assessment, or ecologic exposure assessment, with or without validation

ED_002061_00044426-00033

	window (pre-natal, early life)		
Outcome Assessment	Standardized tool, validated in study population; or, medical record review with trained staff (birth characteristics)	Standardized tool, not validated in population, or screening tool; or, medical record review, methods unstated (birth characteristics)	Selected sections of test, or maternal report, other; or, maternal/paternal self-report (birth characteristics)
Confounder control	Good control for important confounders relevant to OP-ND question, and standard confounders	Moderately good control confounders, standard variables, not all variables for OP-ND question	Multi-variable analysis not performed, no adjustments
Statistical Analysis	Appropriate to study question and design, supported by adequate sample size, maximizing use of data, reported well (not selective)	Acceptable methods, questionable study power (especially sub-analyses), analytic choices that lose information, not reported clearly	Minimal attention to statistical analyses, comparisons not performed or described clearly
Risk of (other) bias (selection, differential misclassification, other)	Major sources of other potential biases not likely present, present but analyzed, unlikely to influence magnitude and direction of the risk estimate	Other sources of bias present, acknowledged but not addressed in study, may influence magnitude but not direction of estimate	Major study biases present, unacknowledged or unaddressed in study, cannot exclude other explanations for study finding

(Adapted from Munoz-Quezada *et al.* 2013)

Cohort Study

A commonly used design in this literature was the cohort study (See Tables 2.2.5-1, 2.2.5-2, and Appendix 3 for examples). In a typical cohort study, individuals are classified according to exposure status (i.e., presence, absence, or magnitude of exposure), and then followed over time to quantify and compare the development (i.e., incidence) of the health outcome of interest by exposure group. Conceptually, the non-exposed comparison group in a cohort study provides an estimate of the incidence of the outcome among the exposed, had they, counter-to-fact, not been exposed. Apart from chance variations, a valid cohort study comparing exposed individuals to non-exposed individuals provides an estimate of the relative risk (or rate) of the disease associated with exposure. Ideally, the exposed and non-exposed groups are exchangeable, in the sense that switching the exposed to non-exposed, and non-exposed to exposed would yield the same measure of association (e.g., relative risk). If this were the case then, apart from chance, a cohort study would yield a measure of association equivalent to that

ED_002061_00044426-00034

produced in a corresponding (intervention) study where exposure status was randomly assigned.

The chief advantage of the cohort study design is that it affords the investigator the opportunity to avoid and/or adjust for potential biases (i.e., selection bias, information bias, and confounding). Cohort studies also allow for discernment of the chronological relationship between exposure and outcome, and can be particularly efficient for studying uncommon exposures. The primary disadvantage of the cohort study design is logistical inefficiency with respect to the necessary time, expense, and other resources needed to conduct them. Cohort studies are particularly inefficient for evaluating associations with rare outcomes and diseases with long induction or latency periods. Though prospective studies are often logistically less efficient relative to other study designs (e.g., the case-control study), these logistical concerns can be minimized in cohort studies of short duration, such as those used to evaluate prenatal OP pesticide exposure effects on birth outcomes or other outcomes of neonatal development assessed shortly after birth.

Two sub-categories of cohort studies – prospective and retrospective - are often applied to distinguish between studies in which the health outcome has occurred (retrospective study), or has not occurred (prospective study) at the time the investigators initiate the study. This distinction is important primarily as it pertains to the potential differences in the quality (e.g., completeness, accuracy, and precision) of information that can be ascertained by the investigators, and also as it relates to potential sources of bias. Although not always true, the prospective study design is considered the preferable of the two, as investigators can potentially have more choices in determining how exposure, outcome, and covariate information is collected. In a retrospective study conducted to evaluate the same hypothesis, by contrast, the investigators would have to rely on exposure information such as maternal self-report. Such reporting is subject to (human) errors in recall. Moreover, the outcome status of the child (i.e., whether a child has developmental delays that are known to the mother) may influence the recall of prenatal OP pesticide exposure by the mother.

Case-control Study

In a typical case-control study (see, for example, Dawbrowski *et al.*, 2003 in Appendix 3 and Shelton *et al.* 2014 in Table 2.2.5-2), individuals are classified according to their outcome status (i.e., cases who have developed the outcome of interest, and controls who represent the population from which the cases arise). The relative odds of exposure are then compared between cases and controls. The primary advantage of case-control studies is that they are logistically efficient relative to cohort studies. In fact, properly conducted case-control study can be conceptualized as a cohort study with efficient sampling of exposure among the cohort, yet they can often be conducted at a fraction of the cost, in a fraction of the time as a corresponding cohort study. Case-control studies can be used to examine associations between multiple exposures and a given health outcome. They are particularly efficient for evaluating rare outcomes but are inefficient for studying uncommon exposures. The primary weakness of the case-control study is the potential for selection bias, which arises if the exposure distribution among the control subjects is not representative of the exposure distribution

ED_002061_00044426-00035

among the population that gave rise to the cases. Case-control studies that rely on self-reported exposure measures are also susceptible to information bias.

Cross-sectional study

Cross-sectional studies (see, for example, Suarez-Lopez *et al.* in Table 2.2.5-2 and Grandjean *et al.* 2006 in Appendix 3) are used to evaluate associations between exposure and outcome prevalence in a population at a single point in (or period of) time. The primary advantage of a cross-sectional study is logistical efficiency; they are relatively quick and inexpensive to conduct, as a long period of follow-up is not required, and exposure and outcome assessments occur simultaneously. Cross sectional studies have three primary *potential* disadvantages: 1) potential difficulty in discerning the temporal relationships (i.e., whether the exposure precedes the outcome); 2) estimating outcome prevalence rather than incidence of the outcome; and 3) the possible overrepresentation of cases of the outcome with long duration relative to the average in the population, and often with a better prognosis.

Ecological study

Ecological studies are used to evaluate associations between exposures and outcomes using population-level rather than individual-level data. For example, Nevison (2014, Appendix 3) uses annual estimates of pesticides applied to crops and population level autism prevalence to assess the association between OP pesticide exposure and autism. The primary advantages of ecological studies are related to logistical efficiency, as they often rely on pre-existing data sources and require no individual-level exposure, outcome, or covariate assessments. The primary weakness of the ecologic study is the potential for confounding and resultant inappropriate extrapolation of associations observed on the aggregate-level to associations on an individual level. The mistaken belief that associations observed at the population level exist at the individual level is referred to as the ecological fallacy.

In judging an individual study's contribution to the strength of evidence in the epidemiologic literature base, the following hierarchy of observational study designs was considered (from most to least preferred): prospective cohort study, retrospective cohort study, case-control study, cross-sectional study, ecological study. It is important to note, however, that this hierarchy of study designs reflects the *potential* for the collection of high quality information (related to exposure, outcome, confounders, and effect modifiers) and *potential* for efficient and valid estimation of the true association. Thus, in deliberating on quality, care has been taken to consider the circumstances and particulars of each individual study. For example, a well-conducted case-control study of a rare outcome can provide much higher quality evidence vis-à-vis the association of interest than a poorly conducted prospective cohort study of the same relationship. For this report, the placement of the study design in the aforementioned hierarchy of observational study designs was but one facet of the judgment of study design quality. Additional consideration was given to whether the study was *well conducted*, independent of study design type. The particulars of a study's design, specifically the design elements employed to minimize and adjust for biases, were also considered. Finally, the relevance of each study with respect to the association of primary interest in this initiative,

ED_002061_00044426-00036

namely the relationship between prenatal (and early life) OP pesticide exposures and fetal and child neurodevelopment was considered.

2.2.4.2 Exposure Measures

There were three major categories of exposure assessment employed in this literature: exposure biomarkers, participant-reported proxy exposure via questionnaire, and objectively obtained proxy indicators of exposure. Although one study included environmental wipe sampling results in the exposure assessment, urinary biomarker measures were also included and no differentiation of the two approaches was presented, so this exposure assessment category has not been included. The merits and the disadvantages of the three primary exposure assessment strategies are discussed below.

Most of the studies reviewed herein assessed biomarkers of exposure quantified in samples of biological media (most often urine, but also blood, serum, and breast milk). These biomarkers were of three types: 1) OP pesticide residues, 2) metabolites of specific OP pesticides, and 3) non-specific OP metabolites.

The most commonly measured biomarkers were urinary dialkylphosphate metabolites (DAP). These non-specific markers are easily quantified using gas chromatography/mass spectrometry and related methods. Though objective, use of urinary DAPs as biomarkers for OP pesticide exposures has limitations, including substantial temporal variability, often varying substantially over short time scales (i.e., day-to-day). Quantification of DAPs in a single urine sample may not represent an individual's usual exposure to OP pesticides over the time period of interest (e.g., pregnancy) in their utility as biomarkers of OP pesticide exposure. Urinary DAP metabolite levels may also reflect exposure to ambient metabolites in addition to exposure to OP parent compounds. In this literature, errors in DAP as a biomarker of OP pesticide exposure are likely to be non-differential with respect to outcomes. Epidemiologists often distinguish between two mechanisms or types of misclassification – those that are non-differential (or random) and those that are differential (non-random). See Section 2.2.4.6 for further discussion of these exposure misclassifications.

Many studies used questionnaire-based exposure assessments in which study participants (typically mothers) self-reported their exposures, in addition to, or instead of, quantifying OP pesticide biomarkers in samples of biological media. These exposure assessments typically include querying OP pesticide exposure directly, or asked study participants to report on behaviors and conditions associated with pesticide use (e.g., occupation, tasks). Such reporting likely misclassifies actual OP pesticide exposure. If conducted as part of a prospective exposure assessment, these errors are likely to be non-differential with respect to the outcome(s) of interest. In the context of a retrospective assessment in which the mother has knowledge of the outcome status of the child, these errors may be differential or non-differential.

Several studies used proxy measures (including ecological indicators) of pesticide exposure. These included, for example questions about occupational use and exposure to pesticides, distance from residence to fields where pesticides were applied, the proportion of land in a

ED_002061_00044426-00037

specified area dedicated to agricultural uses, and occurrence of pregnancy during the pesticide application season. Again, substantial non-differential exposure measurement error/misclassification of exposure is likely.

For this evaluation, studies employing exposure assessments that quantified biomarkers of specific OP pesticides (e.g., chlorpyrifos, malathion, diazinon), or urinary metabolites of these pesticides (e.g., TCPy, malathion dicarboxylic acid, 2-isopropyl-4-methyl-6-hydroxypyrimidine), or high-quality chemical-specific exposure quantitation during relevant exposure-time windows (i.e. pre-natal, early life) were given the highest weight. Studies that quantified levels of non-specific biomarker of OP pesticide exposure (e.g., DAPs), or exposure effects (e.g., AChE, BuChE,) were given a moderate weight. Studies relying on high-quality survey-based individual-level information on pesticide exposure were also assigned a moderate weight. Exposure assessments that only crudely or subjectively classified pesticide exposures and ecologic and other proxy measures of exposure were assigned a low weight.

Two studies focusing on neurodevelopmental outcomes measured OP exposure using both DAPs and malathion dicarboxylic acid (a metabolite of malathion) (Eskenazi *et al.*, 2007; Engel *et al.*, 2007), with TCPy exposure also being measured in one of these studies (Eskenazi *et al.*, 2007). Additionally, two studies focusing on birth outcomes measured OP exposure by testing for specific OPs, with Whyatt *et al.* (2004) measuring chlorpyrifos and diazinon; and Eskenazi *et al.* (2004) testing for DAPs and for seven pesticide specific metabolites (MDA - derived from malathion; PNP - derived from methyl parathion, parathion, and other nonpesticide chemicals; TCPy - from chlorpyrifos and chlorpyrifos methyl; DEAMPY - from pirimiphos methyl; IMPY - from diazinon; CMHC - from coumaphos and coumaphos methyl; CIT - from isazophos and isazophos methyl). Finally, several method validation studies tested for specific OP pesticides, but did not evaluate the association between these exposures and specific adverse health outcomes (Whyatt *et al.*, 2007; Whyatt *et al.*, 2009; Bradman *et al.*, 2003).

With the exception of the studies discussed above and those focusing exclusively on TCPy or chlorpyrifos, the majority of the epidemiological studies focus on the association of OPs exposure and various neurodevelopmental outcomes. The OP exposure being assessed in these studies used concentrations of urinary dialkyl phosphate metabolites (DAPs). Total DAPs is a non-specific measure of OP exposure and is the sum of six separate molecules - three dimethyl alkylphosphate (DMAP) molecules of DMP, DMTP, DMDTP, and three diethyl alkylphosphate (DEAP) molecules of DEP, DETP, and DEDTP. Each metabolite is a breakdown product from multiple OPs (Table 2.2.4-2). Specifically, DMP, DMTP, and DMDTP are associated with 18, 13, and 5 OPs, whereas DEP, DETP, and DEDTP are associated with 10, 10, and 4 OPs, respectively. Thus, using DAPs as an exposure measure, it is not possible to separate the exposure and associated effects for single, specific OPs. For studies evaluating TCPy (e.g., Fortenberry *et al.*, 2014; Eskenazi *et al.*, 2007; Whyatt *et al.*, 2009), this molecule is a metabolite of chlorpyrifos, chlorpyrifos-methyl, and the herbicide triclopyr. TCPy is a primary environmental degradate of chlorpyrifos, chlorpyrifos-methyl, and triclopyr, and is found on food treated with these pesticides. Studies focusing solely on chlorpyrifos could assess exposure to only this OP (e.g., Lovasi *et al.*, 2010; Whyatt *et al.*, 2004; Rauh *et al.*, 2011).

Table 2.2.4-2 CDC Table of Organophosphate Pesticides and Their Dialkyl Phosphate Metabolites (2008)

Pesticide	DMP	DMTP	DMDTP	DEP	DETP	DEDTP
Azinphos methyl	|	|	|			
Chlorethoxyphos				|	|	
Chlorpyrifos				|	|	
Chlorpyrifos methyl	|	|				
Coumaphos				|	|	
Dichlorvos (DDVP)	|					
Diazinon				|	|	
Dicrotophos	|					
Dimethoate	|	|	|			
Disulfoton				|	|	|
Ethion				|	|	|
Fenitrothion	|	|				
Fenthion	|	|				
Isazaphos-methyl	|	|				
Malathion	|	|	|			
Methidathion	|	|	|			
Methyl parathion	|	|				
Naled	|					
Oxydemeton-methyl	|	|				
Parathion				|	|	
Phorate				|	|	|
Phosmet	|	|	|			
Pirimiphos-methyl	|	|				
Sulfotepp				|	|	
Temephos	|	|				
Terbufos				|	|	|
Tetrachlorviphos	|					
Trichlorfon	|					

DMP = dimethylphosphate; DEP = diethylphosphate; DMTP = dimethylthiophosphate; DMDTP = dimethyldithiophosphate; DETP = diethylthiophosphate; DEDTP = diethyldithiophosphate.

The CHARGE study (Shelton *et al.*, 2015) used a different method for exposure assessment. This study used geospatial analysis to focus on the residential proximity to OP exposure and the association of this exposure with autism spectrum disorders. OP exposure was assessed by Shelton *et al* (2015) using data from the California Department of Pesticide Regulation, with five OPs accounting for a total of 73% of the exposure and each accounting for 10% or more of the exposure (chlorpyrifos, acephate, diazinon, bensulide, and dimethoate); eight OPs

ED_002061_00044426-00039

accounting for a total of 25% of the exposure and each accounting for 1% or more of the exposure (malathion, methyl parathion, azinphos-methyl, phosmet, oxydemeton-methyl, ethephon, naled, and methidathion); and eight OPs accounting for a total of 2% of the exposure and each accounting for 0.1% or more of the exposure (methamidophos, phorate, disulfoton, fenamiphos, coumaphos, parathion, ethoprop, and sulfotep).

2.2.4.3 Neurological and Other Outcome Measures

With some exceptions, the outcomes assessed in this literature fall into three broad categories: 1) neurobehavioral and/or neurodevelopmental status; 2) birth outcomes; and 3) neurodevelopmental diseases and/or disorders (see Table 2.2-b).

There is a broad body of literature available on the use and interpretation of instruments designed to quantify neurological status that is beyond the scope of this summary. Many instruments were used in this literature to assess infant neurodevelopment and neurobehavior (see Table 2.2-b). Importantly, performance on some of these tests can be influenced by the administrator of the assessment. Also of concern is whether these assessments are sensitive enough to distinguish potentially subtle effects of OP pesticide exposure. Some degree of error in the assessment of neurological outcomes is likely in all of the studies, though the errors were unlikely to be related to exposure status in the well conducted studies.

The assessment of birth outcomes in this literature was primarily conducted by reviewing of medical records or birth certificates; these assessments are likely to have minimal errors, and errors that do arise are almost certainly non-differential with respect to exposure status. In some studies, however, birth outcomes were reported by the mother and in these instances, differential misclassification is possible.

The studies that evaluated specific diagnoses, autism spectrum disorders or developmental delay, for example, relied on existing medical records, with some effort to validate diagnoses in a subset of the investigations.

Studies that relied on standardized, validated instruments to assess neurodevelopment, medical records of birth outcomes, or validated diagnosis of disease states were weighted highly in the judgment of study quality. Those that used standardized instruments that had not been validated in the relevant population or screening assessments were given a moderate weight, while studies relying on selected sections of neurodevelopment assessment tools, maternal report of outcome status, or aggregated (ecological) outcome measures were given the lowest weight.

2.2.4.4 Statistical Analysis

Statistical analyses that were appropriate to the study question and study design, supported by adequate sample size, maximized the use of available data, and were well characterized in the report were weighted most highly. Acceptable statistical methods, moderate study power, and analytic choices that resulted in the loss of information or that were not clearly reported were

ED_002061_00044426-00040

given moderate weight. Reports with only minimal attention paid to the conduct and reporting of the statistical analyses were given the lowest weight.

2.2.4.5 Confounding

Risk factors for early life neurodevelopment perturbations that are associated with OP pesticide exposure, but not caused by pesticide exposure, are potential confounders in this literature. Socioeconomic determinants of child development (including, for example, maternal education and access to prenatal and early life health care), quantity and quality of parent-child interactions, and exposure to other environmental toxicants (e.g., lead, PCBs, other pesticides) are difficult to measure and were either not accounted for, or inadequately accounted for in many studies. That said, some studies were relatively homogeneous with respect to these factors and thus limited confounding by design, while others attempted to quantify these factors and adjust for them. Other important potential confounders, such as the child's sex, are easy to identify and adjust for analytically.

2.2.4.6 Risk of Bias

The internal validity of the studies reviewed was judged by noting the design strategies and analytic methods used in each study to constrain or eliminate selection bias, information bias, and confounding. Selection bias occurs when the sampling of the population by the investigator yields a study population that is not representative of the exposure and outcome distributions in the population sampled. Put simply, selection bias occurs if selection of the study sample yields a different estimate of the measure of association than that which would have been obtained, had the entire target population been evaluated. Although there are numerous sources of selection bias, there are several mechanisms that may have induced selection bias in the studies reviewed: less than 100% participation rates of eligible individuals due to non-responsiveness or refusal (self-selection bias); loss to follow-up (i.e. failure to retain all study participants initially enrolled in the study); and, in a case-control study, control selection bias arising because the exposure distribution in the control sample does not represent the exposure distribution of the study base (i.e., the population that gave rise to the cases or more formally, the person-time experience of that population).

Information bias (also referred to as observation bias) arises when study participants are incorrectly categorized with respect to their exposure or outcome status, or when errors arise in the measurement of exposure or outcome, in the case of continuously distributed measures. Epidemiologists often distinguish between two mechanisms or types of misclassification – those that are non-differential (or random) and those that are differential (non-random). Non-differential misclassification of exposure (or non-differential exposure measurement error) occurs when the probability or magnitude of error in the classification or measurement of exposure is independent of the outcome status of the study participants. Similarly, non-differential misclassification of outcome (or outcome measurement error) occurs when the probability or magnitude of error in the assignment of outcome status or level is independent of exposure status. In contrast, differential exposure misclassification (or measurement error) occurs when the error in the exposure assignment is not independent of the outcome status.

ED_002061_00044426-00041

The mechanisms that cause non-differential misclassification in this literature include errors in the medical records, laboratory errors, sampling of biospecimens for biomarker assays, and errors in recall. The mechanisms that induce differential misclassification include recall bias, and interviewer/observer bias. Note that mismeasurement of confounders can result in residual confounding of the association of interest, even when adjustment for that confounder has been conducted in the analysis.

Studies in which major sources of potential biases were not likely to be present, or in which potential sources of bias were present but effectively addressed and analyzed to maximize the study validity, and those in which sources of bias were unlikely to influence the magnitude and direction of the risk estimate were given a high weight. Studies where sources of bias were present and acknowledged by the authors but not addressed in the study and yet may influence the magnitude, but not direction of the association estimate received a moderate weight. A low weight was given to studies in which major biases were present and yet were not acknowledged or addressed in the study, such that they cannot be excluded as an alternative explanation for the study finding.

2.2.5 Review of Quality Results

Each of the studies in the 2015 review was judged to be of high, moderate, or low quality in each of five domains of study design and methodology effecting study quality as discussed above in Section 2.1. The results of the quality assessment are presented separately below for each group. The quality categories represent to the total evaluation. In Section 2.2.6, further evaluation of the study design and exposure assessment of the medium and high quality studies. This further evaluation led to additional studies being removed from the final analysis, with these excluded studies not being considered further in the remaining sections of this document.

2.2.5.1 "High" Quality Group

Six studies (8 articles) were assigned a high quality rating, as shown in Table 2.2.5-1. In general these were prospective birth cohort studies with moderate to high sample size; exposure assessment was based on an objective biomarker measure, the outcome measurement(s) utilized standardized tests and trained data collectors, appropriate statistical analyses were performed, considering relevant covariates, and risks of bias were minimized to the extent possible. For example, Fortenberry *et al.* (2014, Table 2.2.5-1) reported on findings in the ELEMENT study population of 187 mother-child pairs, assessed third trimester maternal urinary TCPy as a biomarker of prenatal chlorpyrifos exposure, and employed a trained and experienced research team to administer a battery of validated ADHD/psychometric assessments (Conner's' Parental Rating Scales-Revised, Conner's' Continuous Performance Test, and the Behavior Assessment System for Children-Parental Rating Scales) that had been translated into Spanish.

ED_002061_00044426-00042

Table 2.2.5-1. High Quality Studies: Summary of Study Design Elements Impacting Study Quality Assignment

Study	Study Location	Cohort Name / Description of Study Population	Study Design	Exposure Assessment	Outcome Assessment	Confounder / Covariate Control	Statistical Analysis	Risk of (other) Bias
Barr *et al*. (2010)	New Brunswick, New Jersey	Mother who underwent elective Cesarean delivery at term and their newborns, Saint Peter's University Hospital	Cross sectional birth cohort, modest sample numbers N=150 Mother-infant pairs	Objective measure, prenatal - parent CPF in maternal and cord serum at birth - exposures do not necessarily precede outcome	Birth outcomes from medical records	Generally appropriate; missing a few, e.g., maternal age, SES, nutrition, pre-natal care, race	Appropriate multivariate analysis	Convenience sample – deemed low probability of selection bias; low potential for exposure or outcome misclassification
Bouchard *et al*. (2010)	U.S. National Population	National Health and Nutrition Examination Survey (NHANES)	Cross-sectional, large sample size N=1139 Age 8-15 years	Objective measure, postnatal – single non-OP specific child urinary DAPs	ADHD from DISC-IV diagnosis or medication in children 8-15 years old, standard protocol, trained interviewers	Appropriate: also included blood lead	Appropriate, accounted for NHANES multistage probability sampling	Low probability of selection bias; some potential for non-differential misclassification of exposure
Fortenberry *et al*. (2014)	Mexico City, Mexico	Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT) study (3 cohorts 1994-1997 (cohort 1), 1997-2000 (cohort 2), and 2001-2005 (cohort 3))	Prospective Birth Cohort Study – modest sample size N=187 Mother-infant pairs	Objective measure - TCPy concentration was measured in third trimester maternal urine samples. In a subset of women randomly selected urinary TCPy in samples collected during all three trimesters of pregnancy was measured.	ADHD - LP - Conner's' Parent Rating Scales-Revised (CPRS-R), Conner's' Continuous Performance Test (CPT), and Behavior Assessment System for Children-Parental Rating Scales (BASC-PRS) – These are screening tools, not diagnostic tools. Standardization and quality control checks were conducted by reviews of videotaped evaluations.	Appropriate. Included continuous maternal IQ, education, socioeconomic status and blood lead one month after delivery, breast feeding (yes/no), child's sex, continuous age at testing, birth length and head circumference at birth.	Appropriate. Multivariable linear regression	History of maternal and paternal exposure to pesticides not included, use of a single urinary measure to estimate exposure, potential for differential exposure misclassification possible as mothers were likely aware of the neurobehavioral status of their children
Fortenberry *et al*. (2014a)	Mexico City, Mexico	Early Life Exposures in Mexico to Environmental Toxicants (ELEMENT) study	Prospective Birth Cohort Study – large sample size N=591 Mother-infant pairs	Objective measure - blood samples from mother–child pairs were analyzed for PON1. OP exposure was not explicitly assessed.	ADHD - LP - Conner's' Parent Rating Scales-Revised (CPRS-R), Conner's' Continuous Performance Test (CPT), and Behavior Assessment System for Children-Parental Rating Scales (BASC-PRS) - These are screening tools, not diagnostic tools.	Appropriate: Included continuous maternal IQ, education, socioeconomic status and blood lead one month after delivery, breast feeding (yes/no), child's sex, continuous age at testing, birth length and head circumference at birth.	Appropriate. Multivariable linear regression	Potential for differential exposure misclassification possible as mothers were likely aware of the neurobehavioral status of their children.

ED_002061_00044426-00043

Table 2.2.5-1. High Quality Studies: Summary of Study Design Elements Impacting Study Quality Assignment (continued)

Study	Study Location	Cohort Name / Description of Study Population	Study Design	Exposure Assessment	Outcome Assessment	Confounder / Covariate Contro	Statistical Analysis	Risk of (other) Bias
Quirós-Alcalá *et al*. (2011)	Salinas Valley, California, USA	Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS)	Prospective Birth Cohort Study Outcomes assessed at: N=149 (6 months) N=149 (1 year) N=97 (3.5 years) N= 274 (5 years)	Objective biomarker, prenatal OP pesticide exposure (DAP) quantified in two maternal spot urine samples at 14 and 27 weeks gestation (on average), and in single child urine sample provided at time of each outcome assessment	Autonomic nervous system dysregulation at ages 6 months, 1 year, 3.5 years, and 5 years; standard protocol, trained, bilingual research staff, appropriate to age of child. May not be sensitive to resolve subtle effects of OP pesticide exposure.	Appropriate. Included maternal and child demographic and SES indicators (age, education).	Appropriate multivariate analysis	Selection bias possible due to considerable loss to follow-up; Residual confounding likely small in magnitude; some potential for non-differential misclassification of exposure (possible explanation for null findings).
Rauch *et al*. (2012)	Cincinnati, Ohio, USA	Health Outcomes and Measure of the Environment (HOME)	Prospective Birth Cohort Study – large sample size. N=306 Age: newborns	Objective biomarker of prenatal OP pesticide exposure (DAP) quantified in two maternal spot urine samples provided at 16 and 26 weeks gestation	Abstracted birth weight from medical records, calculated gestational age from mother's self-reported date of last menstrual period. When gestational age not available, results of an ultrasound was used or a Ballard examination performed just after delivery.	Appropriate. Included income, education, maternal depressive symptoms, maternal IQ, insurance status, area of residence, prenatal care, PON1 genotype, and gestational exposure to alcohol, lead, and tobacco.	Appropriate. multivariable regression	Unable to rule out the possibility that differences in DAP concentrations partially reflect individual variation in metabolism, Recall error
Wolff *et al*. (2007)	New York City, U.S.	Mount Sinai Children's Environmental Health Study	Prospective Cohort – moderate sample size N=404 Mother-infant pairs	Objective biomarker, prenatal OP pesticide exposure (DAP), malathion (MDA) quantified in single maternal spot urine sample provided during the 3rd trimester	Birth outcomes from computerized perinatal database	Appropriate: different covariates for each outcome, e.g., weight: maternal age, race/ethnicity, maternal BMI*pregnancy weight gain, infant sex, gestational age, creatinine	Appropriate PROC GLM, with varying covariates	Selection bias unlikely; some potential for non-differential misclassification of exposure
Furlong *et al*. (2014)	New York City, U.S.	Mount Sinai Children's Environmental Health Study	Prospective Cohort N=136 Age 7-9 years	Objective biomarker, prenatal OP pesticide exposure (DAP) quantified in single maternal spot urine sample provided during the 3rd trimester, simple imputation < lowest level of detection (LLOD)	Reciprocal social impairment at age 7-9 years assessed using the Social Responsiveness Scale, completed by mothers. Designed to assess reciprocal social behaviors in evaluating ASDs, used here as general indicator of impaired social responsiveness.	Appropriate. Included maternal and child demographic and SES indicators (age, education) and ETS (not other environmental toxicants).	Appropriate multivariate analysis	Selection bias possible due to considerable loss to follow-up; Residual confounding likely small in magnitude; some potential for non-differential misclassification of exposure possible explanation for null findings).

ED_002061_00044426-00044

2.2.5.2 "Moderate" Quality Group

Eleven studies (15 articles) were assigned a moderate quality rating, as shown in Table 2.2.5-2. In general, these were cross-sectional or prospective cohort studies with small to high sample size; exposure assessment was based on a non-specific biomarker measure or current self-report, the outcome measurement(s) utilized standardized tests or screening tools, appropriate statistical analyses were performed, considering some but maybe not all relevant covariates, and risks of bias were minimized to some extent. For example, Guodong *et al*. (2012) cross-sectionally evaluated the relationship between DAPs concentrations in urine sampled from 301 young children as an objective, non-specific marker of prenatal OP pesticide exposure and Developmental Quotients based on the Gesell Developmental Schedules adapted for a Chinese population.

ED_002061_00044426-00045

Table 2.2.5-2. Moderate Quality Studies: Summary of Study Design Elements Impacting Study Quality Assignment

Study	Study Location	Cohort Name / Description of Study Population	Study Design	Exposure Assessment	Outcome Assessment	Confounder / Covariate Control	Statistical Analysis	Risk of (other) Bias
Andersen *et al* (2015)	Denmark	School age children living in Denmark	Prospective Birth Cohort, small sample size N=177 (112 maternal occupational pesticide exposure during pregnancy; 65 without) Age: 6-11 years	Prenatal occupational pesticide exposure ascertained by maternal interview at enrollment	Objective standardized clinical exam; neurophysiological status (heart rate variability); Previously validated, neuropsychological testing with demonstrated sensitivity to environmental pollutants; administered and scored by single neuropsychologist	Appropriate. Self-reported by mother. Included age, maternal demographics and risk factors, SES indicator (broad categories), maternal smoking and alcohol use. Possibility of recall errors (residual confounding). Self-reported (by mother)	Appropriate multivariate analysis. Evaluated numerous hypotheses without adjustment for multiple comparisons. Also constructed structural equation model of interaction between child sex and prenatal pesticide exposure on general intellectual ability (parameterized as a latent variable)	Selection bias possible due to loss to follow-up; Residual confounding likely, small in magnitude; potential for non-differential misclassification of exposure
Guodong *et al*. (2012)	Shanghai, China	2-year old children visiting community hospitals	Cross-sectional, large sample size N=301 Age 23-25 months	Objective biomarker, OP exposure (DAP) quantified in single child spot urine sample, simple imputation <LLOD; OP exposure also assessed via questionnaire administered to mothers after delivery	Developmental Quotients based on Gesell Developmental Schedules, adapted for Chinese population	Appropriate. Assessed via mother report via questionnaire, included child sex, maternal demographics and risk factors, SES indicators (maternal education, household income), maternal smoking and alcohol use; Possibility of recall errors	Appropriate multivariate analysis. Assumed linear relationship between log-transformed DAP level and DQ Scores	Selection bias unlikely; Residual confounding likely, small in magnitude; potential for non-differential misclassification of exposure
Handal *et al*. (2007)	Cayambe-Tabacundo region, Ecuador	EcoSalud Project: infants and young children 3 to 61 months of age in lower-altitude communities A&B dominated largely by cut-flower production and in more traditional rural, higher altitude community C.	Cross-sectional, modest sample size from census N=283 Age 3-61 months - 154 high exposure - 129 low exposure	Objective proxy measure: Community of residence	Ages and Stages Questionnaire (ASQ), adapted into local vernacular, 2 trained testers – considered a screening tool	Appropriate: child health status (anemia, stunting) and other characteristics of the home environment (stimulation by 2 methods)	Appropriate: Multiple linear regressions to evaluate associations between community of residence and delayed development, Pairwise t-tests and chi-square to assess mean difference in ASQ score; Effect size Cohen's d	Selection bias unlikely; Residual confounding likely, small in magnitude; potential for non-differential misclassification of exposure
Handal *et al*. (2008)	Cayambe-Tabacundo region, Ecuador	EcoSalud Project: Children 3 to 23 months of age in lower-altitude communities A&B dominated largely by cut-flower production and in more traditional rural, higher altitude community C.	Cross-sectional, modest sample size N:121 Age 3-23 months	Proxy: Distance home to farm, parental employment, pesticide use on domestic crops & within home, child play activities	Ages and Stages Questionnaire (ASQ) (ages 24-61 months), a screening tool; Visual Motor Integration (VMI) Test (ages 48-61 months); two trained testers.	Appropriate: As above	Appropriate: As above	Selection bias unlikely; Residual confounding likely, small in magnitude; potential for non-differential misclassification of exposure

Table 2.2.5-2. Moderate Quality Studies: Summary of Study Design Elements Impacting Study Quality Assignment (continued)

Study	Study Location	Cohort Name / Description of Study Population	Study Design	Exposure Assessment	Outcome Assessment	Confounder / Covariate Control	Statistical Analysis	Risk of (other) Bias
Handal *et al*.(2007B)	Cayambe-Tabacundo region, Ecuador	EcoSalud Project: Children 24 to 61 months of age in lower-altitude communities designated A & B were dominated largely by cut-flower production than in more traditional rural, higher altitude community C.	Cross-sectional, modest sample size N: ASQ - 142 Age 24-61 months VMI - 57 Age 48-61 months	Proxy: Maternal employment during pregnancy, child plays outdoors, Pesticide use on domestic crops , inside home	Ages and Stages Questionnaire (ASQ) (3-23 months) – screening tool; Reach-and-grasp, UC Berkeley Preferential Looking Test Cards; trained tester	Appropriate: As above	Appropriate: As above; Prehension- Logistic regression models, generalized estimation equations (GEE) to account for between-test dependence	Selection bias unlikely; Residual confounding likely, small in magnitude; potential for non-differential misclassification of exposure
Llop *et al*. (2013)	Spain	INMA (Environment and Childhood) Project	Prospective Birth Cohort, large sample size N=1980 Age 14 months	Maternal self-report of prenatal and postnatal indoor pesticide use (pesticide spray or use of a plug-in device assessed via questionnaire)	Mental and psychomotor development at 14 months assessed using validated instrument (Bayley Scales of Infant Development)	Appropriate. Self-reported by mother. Included maternal demographics and risk factors (Age, BMI) SES indicators (maternal education, occupation), maternal smoking and alcohol use. Also childcare behaviors (breast feeding, number of siblings, day care) Possibility of recall errors (residual confounding)	Appropriate multivariate analysis	Selection bias unlikely; Residual confounding likely, small in magnitude; potential for non-differential misclassification of exposure
Oulhote and Bouchard (2013)	Canadian National Population	Canadian Health Measures Survey (CHMS; cycle 1, 2007–2009);	Cross-sectional Study, large national sample size n = 779 children Age 6-11 years	Child urinary DAP, DMP, DEP collected within 2 weeks of survey questionnaire completion by the parents.	Behavioral problems in children based on the parent version of the Strengths and Difficulties Questionnaire (SDQ) (Goodman 1997) - SDQ is a validated screening questionnaire and accepted by parents.	Appropriate: Included sex, age, race/ethnicity, family income, parental education, blood lead levels, maternal smoking during pregnancy, birth weight, maternal age at child's birth, child's BMI, and fasting status (fasting duration at urine collection > 10 hour/≤ 10 hour)	Appropriate: Logistic Regression	Selection bias unlikely; Study population not mixed, single urine sample
Petit *et al*. (2010)	Brittany, France	Newborn children in the PELAGIE Study.	Prospective Birth Cohort, large sample size N= 3,159 Age: newborns	Ecological, proxy indicator of exposure (proportion of municipality devoted to agricultural activity	Objectively measured birth outcomes assessed using hospital records	Appropriate. Maternal report via questionnaire. Included maternal demographics (age, BMI) and pregnancy risk factors (gestational age, hypertension, diabetes, season of pregnancy) SES indicators (district of residence, maternal education, occupation), maternal smoking and alcohol use. Also childcare behaviors (breast feeding, number of siblings, day care); Possibility of recall errors	Appropriate multivariate analysis. Multiple comparisons were conducted for this evaluation, and the authors did not adjust for multiple testing	Selection bias unlikely; Residual confounding likely, small in magnitude; potential for substantial non-differential misclassification of exposure; Misclassification of outcomes unlikely

Table 2.2.5-2. Moderate Quality Studies: Summary of Study Design Elements Impacting Study Quality Assignment (continued)

Study	Study Location	Cohort Name / Description of Study Population	Study Design	Exposure Assessment	Outcome Assessment	Confounder / Covariate Control	Statistical Analysis	Risk of (other) Bias
Ruckart *et al*. (2004)	Mississippi, USA (29 counties), Ohio (one multi-family facility)	Children who were 6 years or younger in MS and OH when homes sprayed with MP	Retrospective cohort, moderate sample size in 2 states, participating/ nonparticipating similar in sex and age MS: N=365 (147 exposed, 218 unexposed) OH: N=287 (104 exposed, 183 unexposed) Age: 1.9-12.5 years old at testing	Specific OP measures: Household methyl parathion (wipe) by approved labs, OR highest urinary PNP level in household, analyzed by CDC	Variety of neuro measures: PENTB, Parenting Stress Index (PSI), Personality Inventory for Children (PIC), Vineland Adaptive Behavior Scales (VABS)	Appropriate: Income, race, site term, ethnicity, mother's use of chemicals at work, mother health/pregnancy conditions, report that child had lead or mercury poisoning (MS only); Raw scores were child age-adjusted	Appropriate: Linear regression (continuous scores), logistic regression (dichotomous scores)	Selection bias possible due to loss of follow-up; Residual confounding likely; substantial potential for differential exposure misclassification (time between spraying and testing, frequency and duration unknown)
Shelton *et al*. (2014)	California, USA	CHARGE Study (3 year-old children)	Case-control, large sample size N = 970 Age 3 years N=486 (ASD) N=168 (DD) N=316 (Controls)	Proxy indicator of prenatal OP pesticide exposure (residential proximity to agricultural pesticide applications defined using ecological pesticide use data)	Clinical outcomes – validated within the study	Appropriate adjustment for demographics (place of birth, race), SES indicators (paternal education) and vitamin intake during pregnancy	Appropriate multivariate analysis.	Selection bias probable; Residual confounding likely; substantial potential for differential misclassification of exposure. Outcome misclassification unlikely.
Suarez-Lopez *et al*. (2012)	Pedro Moncayo County, Pichincha, Ecuador	Secondary Exposure to Pesticides Among Infants, Children and Adolescents (ESPINA) Study, plus new volunteers	Cross-sectional, moderate sample size, from prior census N=277 Exposed: (n=158) Unexposed: (n=119) Age 4-9 years	Proxy: Cohabitation with flower worker >1yr, Adult questionnaire	Child AChE level using commercial kit	Appropriate: Sex, age, height-for-age, hemoglobin concentration, income, pesticide use within household lot, pesticide use by neighbors, examination date, residence, distance to nearest flower plantation	Appropriate: Multiple linear regression (continuous) and logistic regression (polychotomous variables), adjusted models	Selection bias possible due to loss but deemed low; Residual confounding likely small in magnitude; potential for differential exposure misclassification
Suarez-Lopez *et al*. (2013a)	Pedro Moncayo County, Pichincha, Ecuador	Secondary Exposure to Pesticides Among Infants, Children and Adolescents (ESPINA) Study, plus new volunteers	Cross-sectional, moderate sample size, from prior census N=307 Age 4-9 years	Non-specific measures: Child AChE level using commercial kit; Proxy: Cohabitation with flower worker >1yr, Adult questionnaire	Heart rate (HR), Systolic blood pressure (SBP), Diastolic blood pressure (DBP) by standard methods	Appropriate: Sex, age, height-for-age, hemoglobin concentration, income, pesticide use within household lot, pesticide use by neighbors, examination date, residence, distance to nearest flower plantation	Appropriate: Multiple linear regression (continuous), adjusted.	Selection bias possible due to loss but deemed low; Residual confounding likely small in magnitude; potential for differential exposure misclassification
Suarez-Lopez *et al*. (2013)	Pedro Moncayo County, Pichincha, Ecuador	Secondary Exposure to Pesticides Among Infants, Children and Adolescents (ESPINA) Study, plus new volunteers	Cross-sectional, moderate sample size, from prior census N=271 Age 4-9 years	Non-specific measures: Child AChE level using commercial kit	NEPSY-II test (trained examiners): general assessment battery	Model defined a priori: hemoglobin, age, sex, race, height-for-age z score, household income, maternal education, and flower worker cohabitation status	Logistic models (dichotomous and polychotomous) and linear regression models, adjusted; Effect modification according to sex among significant associations; Some imputed values	Selection bias possible due to loss but deemed low; Residual confounding likely small in magnitude; potential for differential exposure misclassification
Wang *et al*. (2012)	Shanghai, China	Pregnant women and newborn children	Cross-sectional Birth Cohort Study N=187 Age: newborns	Objective biomarker of prenatal OP pesticide exposure (DAP) in single maternal spot urine sample provided at onset of labor. Simple imputation of observations < LLOD. OP pesticide exposure also assessed via questionnaire administered after delivery	Gestational age and pre-term delivery appropriately defined and assessed using medical records	Appropriate. Included maternal anthropomorphic, demographic, and SES indicators (income, occupation), predictors of high-risk pregnancy (pregnancy weight gain, gestational age)	Appropriate multivariate analysis	Selection bias unlikely; Residual confounding likely small in magnitude; potential for differential misclassification of exposure
Zhang *et al*. (2014)	Shenyang, China	Newborn children in a birth cohort study	Prospective Cohort, n=249 mother-infant pairs Age: newborns	Biomarker of prenatal OP pesticide exposure (DAP) quantified in single maternal spot urine sample provided at delivery. Many observations < LLOD	Neonatal neurodevelopment assessed using validated instrument (Neonatal Behavioral Neurological Assessment) by trained examiners	Appropriate. Included maternal demographic and SES indicators (age, education), predictors of high-risk pregnancy (BMI, gestational age) and environmental toxicant exposure (cord blood lead); Results stratified by sex	Appropriate multivariate analysis	Selection bias unlikely; Residual confounding likely small in magnitude; considerable potential for non-differential misclassification of exposure (unlikely to account for non-null findings)

2.2.5.3 "Low" Quality Group

Fourteen studies (15 articles) were assigned a low quality rating, as shown in Appendix 3. In general these were small or pilot studies; exposure assessment was based on a proxy measure(s), outcome measurement(s) utilized screening tools or self-report, limited statistical analyses were performed, relevant covariates were not included or discussed, or risks of bias were possible. For example, Acosta-Maldonado *et al.* (2009) conducted a cross-sectional pilot study among 54 women, only nine of whom were considered to have had prenatal exposure to pesticides (defined based on either an exposure history profile or AChE level in blood sampled at the time of admission into the hospital for delivery. The outcome assessed in this study was a standardized but partially subjective assessment of placental maturity (the Placental Maturity Index, PMI). Covariates adjustment of estimated associations between prenatal exposure and PMI in this study was minimal and limited to placental characteristics.

The remaining sections of this document do not discuss further studies identified in the 'low' category. Due to limitations in these studies, they do not provide reliable information evaluating associations between OP exposure and neurodevelopmental outcomes.

2.2.6 Assessment of Epidemiological Studies for Relevance to Analysis

Using the criteria summarized in Section 2.2.4, a total of 38 literature articles were identified in the 2015 literature review and were judged as high, moderate, or low quality. Overall, 8 articles, 15 articles, and 15 articles were judged to be of high, moderate, or low quality, respectively. For the 23 high and moderate quality studies, additional evaluation was conducted as described in this section.

While all of the moderate quality studies had strengths including sample design and outcome assessment, six of these moderate quality studies did not have sufficient exposure assessment methods to determine whether exposure to OPs actually occurred. These studies were conducted on study populations in Spain (Llop *et al.*, 2013), Ecuador (Handal *et al.*, 2007; 2007b; 2008), Denmark (Andersen *et al.*, 2015), and France (Petit *et al.*, 2010). In these studies, participants were considered exposed or unexposed to pesticides based on non-specific exposure measures, such as self-reported occupational exposure, home pesticide spraying, and proportion of municipality devoted to agricultural activity. For all of these proxy exposure assessments, the pesticides used may have included not only OPs, but also pyrethroids, fungicides, and growth regulators. Given the uncertainty about whether OP exposure actually occurred in these studies and whether observed outcomes are associated with OP exposure or with other pesticides, these studies are excluded from further analysis.

The focus of this epidemiological literature review is on the neurodevelopmental outcomes from exposures to low levels of OPs (i.e. below exposures which would result in 10% or more AChE inhibition). Three studies conducted in Ecuador focused on child AChE inhibition and the potential association of AChE inhibition with other measures including parental occupation (Suarez-Lopez *et al.*, 2012), as well as clinical autonomic nervous system (ANS) outcomes such as blood pressure and heart rate (Suarez-Lopez *et al.*, 2013a), and neurodevelopmental outcomes (Suarez-Lopez *et al.*, 2013). The range of AChE activity levels are lower in the first

tertile (range of 1.44 to 2.93 U/mL) compared to the third tertile (range from 3.33 to 4.69 U/mL). Therefore, due to the outcomes assessed and the potentially toxic cholinergic effects that were associated with these outcomes, these studies are not considered to be relevant to this review and are not discussed further here.

In a retrospective cohort study of children exposed to methyl parathion (MP) before age 6 years in Mississippi and Ohio (Ruckart *et al.* 2004), as assessed by household urinary PNP or wipe samples, exposed children performed worse than unexposed children on a few of numerous neurobehavioral development tests conducted. Specifically, participants classified as having had MP exposure had more difficulty with short term memory and attention tasks, and parents reported more behavioral and motor skill problems, relative to unexposed children. However, upon closer inspection of the results across the MS and OH study sites, these neurobehavioral outcomes are not seen consistently. These inconsistencies may be due to differences in how the exposure occurred across the sites, including the fact that MS participants and OH participants were tested 2.5 and 4.5 years after MP spraying in the home. When comparing exposed and unexposed children using general intelligence testing, integrated visual and motor skills testing, and multistep processing, they did not see any differences. The exposure scenario associated with these observations is a critical element in assessing the utility and reliability of this study. Samples were collected in locations from OH and MS where illegal spraying of methyl parathion is known to have occurred during the 1994-1996 time period. Based on the "Revised Organophosphorous Pesticide Cumulative Risk Assessment" (USEPA, 2006), methyl parathion is known to be among the more potent OPs. It is unknown whether study participants were exposed to MP levels that would have induced cholinergic effects. Therefore, given the uncertainty around this illegal use combined with the high potency for cholinergic toxicity, the agency is not emphasizing this study further in the analysis.

In addition, one high quality study on the CHAMACOS birth cohort (Quirós-Alcalá *et al.* 2011) assessed the association between DAPs and autonomic nervous system (ANS) outcomes at ages 6 months, 1 year, 3.5 years, and 5 years. The ANS outcomes assessed included heart rate and respiratory sinus arrhythmia. Overall, while there was some evidence of ANS dysregulation for infants at 6 months, these results were not consistently observed for the other assessed child (1 year, 3.5 years, and 5 years) and maternal OP exposures. There is not a body of literature to compare these results against, making it difficult to put them into context. Furthermore, this study did not focus on neurodevelopmental outcomes, which is the focus of this analysis. Consequently, this Quirós-Alcalá *et al.* 2011 study is not being evaluated further.

2.2.7 Birth Outcome Epidemiologic Studies

Four identified studies, three high quality and one moderate quality, focused solely on OP exposure and adverse birth outcomes related to fetal growth (Barr *et al.*, 2010; Rauch *et al.*, 2012; Wang *et al.*, 2012; Wolff *et al.*, 2007). The birth outcomes assessed included birth weight, birth length, head circumference at birth, and gestational age. The exposure assessment for these studies was conducted using objective measures or biomarkers such as maternal urinary DAPs (Rauch *et al.*, 2012; Wang *et al.*, 2012; Wolff *et al.*, 2007), maternal

urinary malathion (MDA) (Wolff *et al.*, 2007), or maternal and cord blood levels for specific OPs (Barr *et al.*, 2010). The results from these studies are generally inconsistent, with some studies documenting statistically significant associations between OP exposure and birth outcomes whereas others do not. For example, maternal and cord blood serum levels of TCPy were inversely associated in univariate analyses conducted among participants in a prospective cohort study conducted in New Jersey (Barr *et al.* 2010), though the associations did not persist after adjusting for gravidity, maternal pre-pregnancy BMI, infant sex, and gestational age. Chlorpyrifos levels were near the lower limit of detection in this study, though detectable in 98.6% of maternal serum and 62.8% of cord serum samples. Overall, birth length was not associated with third-trimester DAP in the Mount Sinai Cohort (Wolff *et al.* 2007). However, among those with slow-activity paraoxonase-1 (PON1) or PON192, urinary total DMP (but not total DAP or DEP) was statistically significantly associated with shorter birth length (p= 0.032). Birth length was also not associated with maternal urinary DAP sampled at delivery in the cross-sectional investigation conducted in Shanghai, China (Wang *et al.* 2012). Similarly inconsistent results were observed across these studies for birth weight, gestational age, and head circumference at birth.

Overall, in this 2015 literature review, inconsistent evidence of OP exposure and association with adverse birth outcomes/fetal growth was observed (Barr *et al.*, 2010; Rauch *et al.*, 2012; Wang *et al.*, 2012; Wolff *et al.*, 2007). This lack of consistency in the literature was also observed for birth outcomes in the recent chlorpyrifos HHRA (USEPA, 2014) which notes that researchers from the three US birth cohort studies also investigated the possible role of prenatal OP exposure and fetal growth. These results were not consistent across these cohorts. Authors with Columbia Center for Children's Environmental Health (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; Whyatt *et al.*, 2004). Inconsistent results may be due to differences across study groups in exposure profiles as well as dissimilar methods of prenatal OP exposure assessment (Needham, 2005). Given the lack of consistency among cohorts for the fetal growth metrics, the proposed link between fetal growth and OP exposure is tenuous. Therefore, consistent with previous evaluations for chlorpyrifos, EPA is focusing the remainder of this document on neurodevelopmental outcomes. Although the agency is not evaluating these birth outcome studies further at this time, the agency will continue to monitor the scientific literature for advances in this line of research.

2.2.8 Summary of Epidemiology Studies from 2015 Literature Review Focusing on Neurodevelopmental Outcomes

From an initial total of 23 high or moderate quality studies, with the exclusion of six studies for insufficient exposure assessment, four studies with measurable AChE inhibition and potential cholinergic toxicity which are outside the scope of this analysis, one study from an illegal use of a highly potent OP (MP) where cholinergic toxicity cannot be ruled out, four studies with birth

outcome as the only assessment, and one study assessing only PON1 genotype expression and neurobehavioral outcomes (Fortenberry et al, 2014a), a total of seven studies focusing on neurodevelopmental outcomes remain to be evaluated.

Seven total studies focused on OP exposure and either neurodevelopmental or neurobehavioral outcomes, with three high quality (Bouchard *et al.*, 2010; Fortenberry *et al.*, 2014; Furlong *et al.*, 2014) and four moderate quality studies (Guodong *et al.*, 2012; Oulhote and Bouchard, 2013; Zhang *et al.*, 2014; Shelton *et al.*, 2014). The results of these studies are summarized below. With the exception of Shelton *et al.* (2014), all of these studies used biomarker measures for their exposure assessment, including child or maternal urinary DAPs (Bouchard *et al.*, 2010; Furlong *et al.*, 2014; Guodong *et al.*, 2012; Oulhote and Bouchard, 2013; Zhang *et al.*, 2014), child urinary DMP and DEPs (Oulhote and Bouchard, 2013; Zhang *et al.*, 2014; Guodong *et al.*, 2012), or maternal TCPy (Fortenberry *et al.*, 2014). Shelton *et al.* (2014) used pesticide use data from the California Department of Pesticide Regulation and geospatial methods to map the specific pesticide use pattern to the participant residence. The study populations ranged from national in scope (Bouchard *et al.*, 2010; Oulhote and Bouchard, 2013) to mainly urban (Fortenberry *et al.*, 2014; Fortenberry *et al.*, 2014a; Furlong *et al.*, 2014; Guodong *et al.*, 2012; Zhang *et al.*, 2014; Shelton *et al.*, 2014).

In order to summarize the results from these studies, we have separated them into three groups based on the outcomes assessed — two Chinese studies focusing on more generic neurodevelopmental outcomes (Zhang *et al.*, 2014; Guodong *et al.*, 2012); two studies focusing on social responsiveness outcomes or autism spectrum disorders (Furlong *et al.*, 2014; Shelton *et al.*, 2014); and three studies focused on ADHD, behavioral problems, and attention problems (Oulhote and Bouchard, 2013; Bouchard *et al.*, 2010; Fortenberry *et al.*, 2014).

In the first of two Chinese studies focusing on generic neurodevelopmental outcomes, Zhang *et al.* (2014) investigated prenatal exposure to OPs and neurobehavioral development of neonates in a birth cohort study in Shenyang, China. The authors reported that consistent statistically significant associations were observed between all of the quantified urinary biomarkers of prenatal OP pesticide exposure and neonatal neurodevelopment deficits assessed 3 days after birth. A 10-fold increase in total DAPs concentration was associated with an average decrease in Neonatal Behavioral Neurological Assessment (NBNA) summary scores of 1.78 points (95% CI: -2.12 to -1.45). No evidence of departure from linearity of the exposure-response relationship between maternal DAP concentrations and NBNA scores was observed. In the second Chinese study focusing on neurodevelopmental outcomes, Guodong *et al.* (2012) conducted a cross-sectional study, and did not identify any statistically significant associations between the children's urinary DAP metabolite levels and any of the DQ (Developmental quotients) scores. The authors mentioned that their results should be interpreted with caution since OP exposure was quantified in single spot urine sample from children, and should be followed up with a longitudinal study with repeated measurement of exposure levels in urine samples.

ED_002061_00044426-00052

Two studies focused on impaired social responsiveness, autism spectrum disorders, or developmental delays (Furlong *et al.*, 2014; Shelton *et al.*, 2014). Among 7-9 years old children in the Mount Sinai Cohort (Furlong *et al.* 2014), there was no overall statistically significant association between maternal third trimester urinary DAP metabolite levels and reciprocal social responsiveness (a measure linked to many neuropsychiatric conditions that involve impaired social functioning (Constantino and Gruber 2005)). However, some evidence of modification of the association between prenatal OP pesticide exposure and impaired social responsiveness in early childhood was observed by both race/ethnicity and child sex, with an association between DEP and poorer social responsiveness observed among black participants and boys. No association was observed among whites or Hispanics, among girls, or for DAP or DMP biomarker levels.

Shelton *et al.* (2014), investigated autism spectrum disorders (ASD) and developmental delay (DD) in relation to gestational residential proximity to agricultural pesticide applications utilizing the California population-based Childhood Autism Risks from Genetics and Environment (CHARGE) study. The investigators reported that children with ASD were 60% more likely to have OPs applied near the home [1.25 km distance; adjusted OR (aOR) = 1.60; 95% CI: 1.02–2.51] than mothers of normally developing children. They added that as the buffer distance grew larger, these associations became lesser, indicating an exposure-response effect. The authors also mentioned that each 100-lb (45.4 kg) increase in the amount applied over the course of pregnancy (within 1.5 km of the home) was associated with a 14% higher prevalence of ASD (aOR = 1.14; 95% CI: 1.0, 1.32), but no association was identified with DD.

A total of three studies focused on OP exposure and behavioral, memory, or attention/ADHD outcomes (Oulhote and Bouchard, 2013; Bouchard *et al.*, 2010; Fortenberry *et al.*, 2014). In a national cross-sectional study of Canadian children 2007-2009 data for age 6-11 years (Oulhote and Bouchard, 2013), there were no overall statistically significant associations observed between child urinary DAP, DMP, or DEP metabolite levels and parentally reported behavioral problems. In contrast, Bouchard *et al.* (2010), looking at U.S. children age 8-15 years in the 2000-2004 National Health and Nutrition Examination Survey (NHANES),[5] observed a positive association between attention and behavior problems and DAPs and DMPs, but not DEPs. For example, even after controlling for potential confounders such as sex, age, ethnicity, and creatinine concentration, they found that a 10-fold increase in DMAP concentration was associated with a 55 to 72% increased odds of ADHD.

Fortenberry *et al.* (2014) evaluated the relationship between pesticide exposure and ADHD in school age Mexican children, recruiting 187 mother-child pairs from a prospective birth cohort, ELEMENT (Early Life Exposures in Mexico to Environmental Toxicants). The authors reported that, there were no statistically significant associations between tertiles of maternal third trimester urinary TCPy and measures of attention and hyperactivity in children. However, there was suggestive evidence for increases in the ADHD index in relation to TCPy tertiles among boys

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

(the highest TCPy tertile was associated with an ADHD index score that was 5.55 points higher than children in the lowest tertile; p-value = 0.06).

2.2.9 Summary of Three US Children's Cohort Studies (CCCEH, CHAMACOS, Mt. Sinai): Focusing on Neurodevelopmental Outcomes Evaluated in 2012/2014

Detailed summaries and evaluation, associated strengths and limitations, and accompanying detailed evidence table for the CCCEH, CHAMACOS, and Mt. Sinai cohorts can be found in the white paper for the 2012 SAP review and the 2014 chlorpyrifos revised HHRA. Limited summary information is provided here for comparison with the studies identified in the 2015 literature review discussed above.

2.2.9.1 Overview

In the chlorpyrifos revised HHRA, EPA included 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 OPs through the diet and through residential use of the pesticide for indoor pest control. In the residential setting, study populations were most likely exposed through indoor residential use of the pesticide during the study time period and additionally exposed to OPs via the oral route through ingesting residues in the diet and from hand-to-mouth contact with in-home surfaces, as well as possible dermal or inhalation exposure through contact with treated areas in the home environment (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). In each of the three US children's health cohorts, the biological measurements in these cohorts were comparable to the general population NHANES. EPA has considered the strengths and limitations of these

studies, and believes that random or systematic errors in the design, conduct or analysis of these studies were unlikely to fully explain observed positive associations between *in utero* OP exposure and adverse neurodevelopmental effects observed at birth and through childhood (age 7 years). EPA believes these are strong studies which support a conclusion that OPs likely played a role in these outcomes.

2.2.9.2 Review of Study Design and Research Methods

These cohort studies each enrolled pregnant women during roughly the same time period, measured both environmental exposure to the pesticide during pregnancy and also measured biomarkers representing internal dose during pregnancy and at delivery, and prospectively assessed associations in their newborns and young children through age 7 years. Each study includes several hundred (approximately 100-400) mother-infant pairs; these sample sizes are sufficient to perform statistically valid analyses. Investigators from each study cohort utilized a similarly strong study design (prospective birth cohort); measured pesticide exposure using several different methods including environmental indicators as well as specific and non-specific biomarkers of OPs; ascertained developmental outcomes using validated assessment tools well-established in both clinical and research settings; and, measured, analyzed, selected and statistically adjusted for potentially confounding variables including socio-economic status and other environmental exposures using reasonable and appropriate methods. Limitations exist as well. These studies utilized a one-time measure (or the average of two measures) of chlorpyrifos or OP exposure to assess prenatal pesticide exposure throughout the gestational period, were unable to assess the influence of mixtures (co-occurring exposures in the relevant biological time window), and reflect a small sample size to fully evaluate the effect of more than one simultaneous exposure on neurodevelopment, *i.e.*, evidence of effect modification.

As noted, two major uncertainties in environmental epidemiology studies are the accurate and reliable measurement of exposure and potential confounding variables such as the influence of mixtures. The researchers with each of the three cohorts have provided supplemental methodological research to address these areas to the extent possible. Across the three children's health cohorts, study authors measured biomarkers of OP exposure. There is uncertainty as to the extent measurement of non-specific metabolites of OP or chlorpyrifos accurately reflects OP exposure; CCCEH and Mt. Sinai studies do not estimate post-natal exposure to chlorpyrifos among child participants, therefore the influence of early life and childhood OP exposure is unaccounted for in these analyses. The CHAMACOS cohort measured urinary levels of DAPs in young children and did not observe negative significant associations in relation to neurodevelopment from post-natal exposure (Eskenazi *et al.*, 2007). The CHAMACOS cohort investigators also measured AChE and butyl ChE as supplemental indicators of OP exposure.

Potential confounding bias is another major uncertainty within environmental epidemiology studies. Confounding variables, exposures that could be related to OP exposure and neurodevelopmental outcomes such as blood lead, may result in an incorrect epidemiological risk estimate. Across these cohort studies, investigators collected relevant information

concerning demographic characteristics and other environmental exposures, and were, to the extent possible with the existing information, able to effectively hold constant the influence of these other variables when estimating the association between prenatal chlorpyrifos and adverse neurodevelopmental outcomes. Control of these variables is important to reduce the chances of a false positive study result. Overall, statistical analyses were judged to be appropriate and reasonable (not overly large number of statistical model variables) to the research question by EPA and expert Panel reviews (FIFRA SAP 2008 and 2012).

Researchers with both the Mt. Sinai and CHAMACOS cohorts evaluated neonatal neurological functioning in association with prenatal OP exposure; CCCEH did not conduct these measurements. To measure indices of abnormal neonatal behavior and/or neurological integrity authors used outcome measures derived from the Brazelton Neonatal Behavioral Assessment Scale (BNBAS), a neurological assessment of 28 behavioral items and 18 primitive reflexes. This tool was administered to infants 2-5 days post-partum by trained neonatologists in the hospital setting using similar environmental conditions. The authors with both study groups observed an increased number of abnormal reflexes in relation to increasing measures of OP exposure (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.

Researchers across the three children's health cohorts utilized the Bayley Scales of Infant Development II (BSID-II) to generate a Mental Development Index (MDI) and a Psychomotor Development Index (PDI) to assess neurodevelopment in early childhood. In the CCCEH Mothers and Newborn study, Rauh *et al.* (2006) investigated MDI and PDI at 12, 24, and 36 months of age. Children were categorized as having either high (>6.17pg/g) or low ($\leq$6.17pg/g) prenatal chlorpyrifos exposure, using categories informed by results of the previous study on birth characteristics (Whyatt *et al.*, 2004). Authors reported that the difference in MDI scores was "marginally significant" ($p = 0.06$) between the "high" and "low" exposed groups; the high exposed group scoring an average of 3.3 points lower than the low exposed (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, $p = 0.02$) and PDI: 4.9 times greater (95% CI: 1.78-13.72; $p = 0.002$)) than those with lower prenatal exposure (Rauh *et al.*, 2006). Within the Mt. Sinai study, authors administered the BSID-II to participating children at 12 and 24 months and observed that prenatal total DAP metabolite level was associated with a decrement in mental development at 12 months among blacks and Hispanic children; however, these associations either attenuated or were non-existent at the 24-month visit (Engel *et al.*, 2011). In the CHAMACOS 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).

With respect to the findings related to the autism spectrum, from CCCEH, Rauh *et al.* (2006) reported a large odds ratio for pervasive developmental disorder (PDD) (OR=5.39; 95% CI: 1.21-24.11) when comparing high to low chlorpyrifos exposure groups. As described above, among 7-9 years old children in the Mount Sinai Cohort (Furlong *et al.* 2014), there was no overall statistically significant association between maternal third trimester urinary DAP metabolite levels and reciprocal social responsiveness. However, some evidence of modification of the association between prenatal OP pesticide exposure and impaired social responsiveness in early childhood was observed by both race/ethnicity and child sex, with an association between DEAP and poorer social responsiveness observed among black participants and boys. No association was observed among whites or Hispanics, among girls, or for DAP or DMAP biomarker levels. In the CHAMACOS cohort, Eskenazi *et al.* (2010) reported non-significant, but suggestive, increased odds of PDD of 2.0 (0.8 to 5.1; p=0.14), whereas Eskenazi et al (2007) reported a statistically significant association between total DAP exposure and increased odds of PDD.

With respect to attention problems, Rauh *et al.* (2006) also investigated 36-month child behavior checklist (CBCL) (behavioral) scores. Significant differences were observed between the high and low chlorpyrifos exposure groups in the general category of attention-problems (p=0.010), and in the more specific DSM-IV scale for ADHD problems (p = 0.018). The CHAMACOS cohort also investigated attention problems in early childhood using three different assessment tools: maternal report of child behavior at 3.5 and 5 years of age; direct assessment of the child at 3.5 and 5 years; and by a psychometrician's report of the behavior of the child during testing at 5 years. In this study population, higher concentrations of OP metabolites in the urine of pregnant women were associated with increased odds of attention problems and poorer attention scores in their children at age 5 years (Eskenazi *et al.*, 2007).

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. In the Mt. Sinai study, prenatal maternal DEP urinary metabolite concentrations were associated with slight decrements in Full Scale Intelligence Quotient (FSIQ), Perceptual Reasoning, and Working Memory between the ages of 6 and 9 years, and difference in intelligence measures by putative PON1 status were also noted (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.

To ascertain whether observed differences in neurodevelopment after prenatal chlorpyrifos exposure may be explained by differences in brain morphology between exposed groups, investigators compared MRI brain images between high and low chlorpyrifos exposed child study participants (Rauh *et al.*, 2012). Authors determined there were distinct morphological differences in brain areas associated with these neurodevelopmental outcomes. The pilot study included 40 child participants due to strict inclusion and exclusion criteria, and the high cost of performing the imaging studies on each child. EPA convened a Federal Panel of experts to perform a written peer-review of this study.[6] The Federal Panel concurred with the authors' conclusions in general; however the Federal Panel also noted that significantly greater and more sophisticated MRI imaging studies would be needed to link the morphological changes indicated in this study with specific functional outcomes noted in the CCCEH IQ study. Therefore, while generally supportive of the epidemiologic findings, additional study is needed to make specific links with areas of brain development change.

In sum, across these three children's environmental health studies, authors consistently identified associations with neurodevelopmental outcomes in relation to OP exposure. There is evidence of delays in mental development in infants (24-36 months), attention problems and autism spectrum disorder in early childhood, and intelligence decrements in school age children who were exposed to chlorpyrifos or OPs during gestation. Investigators reported strong measures of statistical association across several of these evaluations (odds ratios 2-4 fold increased in some instances), and observed evidence of exposures-response trends in some instances, *e.g.*, intelligence measures.

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

Table 2.2.9 Detailed Summary Tables of Children's Environmental Health Epidemiology Studies (extracted from USEPA, 2014)

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	4 cord plasma chlorpyrifos exposure groups and 4 chlorpyrifos and diazinon exposure groups. Chlorpyrifos only categories, Group 1: levels below LOD (32% of participants); Group 2: lowest 1/3 of detectable levels (20 %); Group 3: middle 1/3 (24%), Group 4: highest 1/3 (25%.) Chlorpyrifos and diazinon together: Group 1: 26%, Group 2: 22 %, Group 3: 26%, Group 4: 26%.	Gestational age, maternal pre-pregnancy weight, maternal net pregnancy weight gain, gender of newborn, parity, race/ethnicity, ETS in home, season, cesarean section	For each log unit increase in cord plasma chlorpyrifos levels, birth weight decreased by 42.6 g (95% CI: –81.8 to –3.8) and birth length decreased by 0.24 cm (95% CI: –0.47 to –0.01). Birth weight averaged 186.3 g less (95% CI: –375.2 to –45.5) among newborns with the highest compared with lowest 26% of exposure levels ($p = 0.01$).	Associations between birth weight and length and cord plasma chlorpyrifos were statistically significant ($p \leq 0.007$) 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 growth and	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. Recall-based exposure assessments were fortified

ED_002061_00044426-00059

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
				gestational age.		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	Birth length, birth weight, head circumference,	Total DAPs: median 136 nmol/L (range:	Gestational age, gestational age squared, maternal age, pregnancy weight gain, week of	Decreases in gestational duration associated with two measures of in utero	Maternal urine collection averaged weeks 14, 26, not creatinine-corrected	Strengths in the study design include the longitudinal design, the use

ED_002061_00044426-00060

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
(N=488)	Gestation Length, Ponderal index	10–6,854); DEP: median 22 nmol/L (range: 2–680 nmol/L); TCPy: median 3.3 nmol/L (range: 0.2–56.1nmol/L) (76% >LOD)	initiating prenatal care, parity, infant sex, mother's country of birth, body mass index, family income, poverty level, smoking, alcohol, illicit drug use, environmental tobacco smoke, caffeine, history of low birth weight, and history of pre-term delivery.	pesticide exposure: levels of metabolites of dimethyl phosphate pesticide compounds and whole blood ChE.		of multiple exposure biomarkers, including quantification of non-specific (DAPs), chlorpyrifos-specific (TCPy) metabolites, and other environmental co-exposures. A reasonable set of exclusion criteria were applied.The selection of the CHAMACOS population, which consists mostly of children from low-income families, served to increase the relative statistical efficiency of the study, as this population is at high risk of neurodevelopmental deficits, compared to the general population.The statistical analysis used to assess the associations between the markers of exposure and neurodevelopment were appropriate.Errors in the assignment of exposure in this prospective study will likely have resulted in attenuation of observed associations.
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	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	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

ED_002061_00044426-00061

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
		was DMP metabolites (GM = 109 nmol/L; 95% CI =98, 120) than DEPs (GM= 23 nmol/L; 95% CI = 21, 25). Allele frequencies: PON1 192 Q allele= 50%; PON1 -108 T allele= 46%.Mean arylesterase activity: For infants: 33.6 U/mL (SD = 16) For mothers: 136.6 U/mL (SD = 44). Mean paraoxonase activity: For infants: 256.6 U/L (SD = 165); For mothers: 989.0 U/L (SD = 616).	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.			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 5: Engel *et al*. (2007) Mt. Sinai (N=311)	Brazelton Neonatal Behavioral Assessment Scale (BNBAS), primitive reflexes (neurological integrity) measured before hospital discharge.	DEP: 24.7 nmol/L; Total DAP: 82 nmol/L	Maternal age, race, marital status, education, cesarean delivery, delivery anesthesia, infant age at examination, infant gender, infant jaundice, smoking (yes/no), alcohol consumption, caffeine consumption, illicit drug use during pregnancy, and the examiner.	No adverse associations were found for DAPs and any measured behavior. Relative to the first quartile, quartiles 2–4 of total DEPs, DMPs, and DAPs were associated with an increased proportion of abnormal reflexes, although the associations did not increase monotonically and varied in their strength and precision.	Used non-specific biomarker DEP/DAP	This was a well conducted prospective study conducted in a young, predominantly minority population. The study design, analytic approach, and statistical analyses were appropriate. Pesticide metabolites evaluated are not specific for chlorpyrifos. The BNBAS was administered before hospital discharge only on a subset of children in the cohort (n =311/404). Factors related to weekend delivery (e.g., fewer inductions) would be

ED_002061_00044426-00062

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
						underrepresented among the tested subjects, and may induce bias, reduce the degree of precision with which associations were estimated, and limit the generalizability of the study findings.The statistical analysis was largely appropriate. Imputing of missing data may affect precision of association estimates and result in attenuated effect estimates as a result of exposure measurement error. Assessment and control for confounding were appropriate. However, confounding by unmeasured (and mis-measured) risk factors for abnormal growth that are related to pesticide exposure would bias the results in this study. Such factors may be related to socioeconomic status of the study participants, a cofactor which is difficult to define, no less measure in an epidemiologic study.
Article 6: Young *et al*. (2005) CHAMACOS (N=381)	Neurodevelopment, Brazelton Neonatal Behavioral AssessmentScale (BNBAS), abnormal reflexes	DAP (average during pregnancy): median 222nmol/L (range: 7–21,867 nmol/L); DEP (average during pregnancy): median 21 nmol/L (range: 2–680	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,	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 =	Associations seen pre-natal OP, not post-natal OP exposure, Maternal urine collection averaged weeks 14, 26	Strengths: Longitudinal design, measurement and consideration of many confounders, the prenatal exposure measures were, with some exceptions, the average of two measurements, and thus may better reflect chronic exposure during the pregnancy

ED_002061_00044426-00063

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
		nmol/L)	infant sex, age in days at BNBAS, minutes since last feed at BNBAS, and BNBAS examiner.	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).		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 7: Rauh *et al*. (2006) Columbia U. (N=254)	Neurodevelopment: The Bayley Scales of Infant Development II (BSID-II), Mental Development Index (MDI) and Psychomotor Development Index (PDI) at 12, 24, and 36 months of age. • Behavior: Child Behavior Checklist (CBCL) at 12, 24, and 36 months. • Quality of the child-care environment: The Home Observation for Measurement of the Environment (HOME)	Exposure levels were categorized as low (≤ 6.17 pg/g) or high (>6.17 pg/g)	Data were collected regarding lead exposure, demographics, education and occupational history, income, active and passive smoking, alcohol and drug use during pregnancy, and residential pesticide use. Final models included prenatal environmental tobacco smoke (ETS) exposure, gender, ethnicity, gestational age at birth, quality of home care-taking environment, maternal education, and maternal IQ.	At the 36 month milestone, the likelihood of highly exposed children developing mental delays were 2.4 times greater (95% CI: 1.12-5.08, p = 0.02) and motor delays were 4.9 greater (95% CI: 1.78-13.72; p = 0.002) than those with lower prenatal exposure. The GLM analysis for PDI scores showed a significant effect of chlorpyrifos exposure over time with an estimated deficit of approximately 7 points by age 36 months (p = 0.01).	The authors summarize three main findings: 1) by age 3, the more highly exposed children demonstrated mental and motor delays; 2) the observed developmental trajectories for PDI and MDI scores confirmed that the adverse impact on cognitive and motor development increased over time; and 3) by age 3, highly exposed children were more likely to demonstrate clinically significant attention problems.	• Only 53% of the children reached the three year milestone with study data collected. It is unclear what percentage of these children did not survive, were lost to follow-up, or too sick to participate. • Reliance on a single exposure level (prenatal/cord blood.) • No control for exposure over the subsequent 3 years • Creation of a dichotomous exposure variable brings limitations due to the amount of within-group variation. • Limitations of the sensitivity and predictive validity of the developmental tests, especially among children less than 3 years of age. • No discussion of whether this 7-point deficit is clinically relevant. • Due to the pervasive, non-specific nature of neurological effects, it is difficult to attribute causality.

ED_002061_00044426-00064

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
Article 8: Lovasi *et al*. 2010 Columbia U. (N=266)	Bayley scores (MDI/PDI) 12 months, 24 months, 36 months	N/A	Neighborhood characteristics: The percentage of housing units without complete plumbing, the percentage of vacant housing units, the percentage of residents below the federal poverty line, the percentage of residents older than 25 years of age who completed high school, the percentage of households receiving public assistance, the percentage of housing units with one or more residents per room, racial composition, the percentage of residents born outside the United States, the percentage of Spanish-speaking residents, and the percentage of residents who were linguistically isolated	Neighborhood characteristics did not confound the observed association between chlorpyrifos levels and cognitive development.	Hierarchal regression analysis of potential confounding by SES	Direct measurement of chlorpyrifos.The statistical analyses were generally appropriate. Missing data on covariates were estimated using multiple imputation, and the variance estimates presented appropriately reflect the degree of uncertainty caused by missing covariate data. Robust standard errors were used.The setting of the investigation in a sample drawn from low-income African American and Dominican communities is both a strength (increases the power, restriction of confounders) and a limitation of the study (reduced generalizability).
Article 9: Engel *et al*. (2011) Mt. Sinai (N=276)	Bayley scores (MDI/PDI) at 12 months, 24 months.	DEP: 24.7 nmol/L; Total DAP: 82 nmol/L (same as Engel 2007)	Maternal age, race/ethnicity, marital status, education, breast-feeding, child sex, alcohol, smoking, or drug use during pregnancy, maternal IQ, a score based on assessment of the home environment (HOME), season of urine collection, language spoken in the home, age at testing, examiner, and urinary creatinine level.	An observed association between prenatal total dialkylphosphate metabolite level and a decrement in mental development at 12 months among blacks and Hispanics.	Used non-specific biomarker DEP/DAP; some evidence of effect modification by PON1 genotype	Limitations include use of non-specific markers of chlorpyrifos pesticide exposure (DAPs), use of only a single (third-trimester) urine sample, and the large proportion of loss to follow-up. Statistical analysis was appropriate. Imputing of missing data may affect precision of association estimates and result in attenuated effect estimates as a result of exposure measurement error, although these are offset by the further categorization of the exposure levels (at the

ED_002061_00044426-00065

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
						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) CHAMACOS (N=372)	Neurodevelopment, Bayley Index (MDI, PDI), Maternal behavior checklist at 6, 12, and 24 months	DEP: geom. Mean in mother 18.1 nmol/L (95% CI = 16.7–19.7); DEP geometric mean in child at 24 months 10.5 nmol/L (95% CI =8.8–12.6); TCPy median 3.54 ug/l	Psychometrician, location of assessment, exact age at assessment, sex, breast-feeding duration (months), HOME score, and household income, parity, maternal PPVT, maternal age, education, depressive symptoms, active/passive smoking exposure during pregnancy, regular alcohol use during pregnancy, marital status, father's presence in home, housing density, maternal work status, ≥ 15 hours out-of-home childcare/week, birth weight, gestational age, abnormal reflexes, PCBs, lead, DDT, β-hexachlorocyclohexane, and hexa-chlorobenzene	DAP metabolite levels during pregnancy, particularly from dimethyl phosphate pesticides, may be negatively associated at 24 months with mental development (MDI) on the Bayley Scales and an increase in risk of maternally reported PDD.	No strong associations identified with DE or TCPy, Maternal urine collection averaged weeks 14, 26	Strengths: Longitudinal design, measurement and consideration of many confounders (including other environmental chemicals); the prenatal exposure measures were, with some exceptions, the average of two measurements, and thus may better reflect chronic exposure during the pregnancy Weaknesses: Potential for residual confounding, potential for exposure misclassification, potential selection bias (not all children had the outcome measures), study population not

ED_002061_00044426-00066

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
						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 U/mL (SD = 44). Mean paraoxonase activity: For infants: 256.6 U/L (SD = 165); For mothers: 989.0 U/L (SD = 616).	Psychometrician, location of assessment, exact age at assessment, sex, breast-feeding duration (months), HOME score, and household income, parity, maternal PPVT, maternal age, education, depressive symptoms, active/passive smoking exposure during pregnancy, regular alcohol use during pregnancy, marital status, father's presence in home, housing density, maternal work status, ≥ 15 hours out-of-home childcare/week, birth weight, gestational age, abnormal reflexes, PCBs, lead, DDT, β-hexachlorocyclohexane, and hexa-chlorobenzene	Decrease MDI (24 months) $PON1_{108TT}$ –5.7 (–9.0 to –2.5) 9=0.01; Decrease PDI (24 months) $PON1_{108TT}$ –2.8 (–5.7 to 0.2) p=0.07; increased odds PDD 2.0 (0.8 to 5.1) p=0.14; no association $PON1_{192}$; no association PON1 activity measured newborn, 2 years, maternal and MDI, PDI, PDD. Evidence of decreasing MDI score by number of $PON1_{108}$ variant alleles: $PON1_{108CC}$ –3.2 (–9.8 to 3.5), CT –3.7 (–8.0 to 0.6), TT –5.5 (–11.1 to 0.1), p-interaction 0.98.	In this study population, evidence PON1 may influence MDI score, but not PDI or PDD risk at two-years. Non-significant evidence of decreasing MDI score by increasing DAP levels across strata of the number of $PON1_{108}$ variant alleles, interaction non-significant. Similar trend with prenatal DEP levels and MDI, PDI by PON108 alleles, but less pronounced. Overall, limited, non-definitive evidence of effect modification by PON1 status in the relation between mental and psychomotor effects and prenatal DAPs.	Strengths: Longitudinal design, measurement and consideration of many confounders (including other environmental chemicals); the prenatal exposure measures were, with some exceptions, the average of two measurements, and thus may better reflect chronic exposure during the pregnancy Weaknesses: Potential for residual confounding, potential for exposure misclassification, potential selection bias (not all children had the outcome measures), study population not generalizable to the whole US. Study may be under-powered to evaluate effect modification by *PON1* status.
Article 12: Marks *et al*. (2010) CHAMACOS (N=348)	CBCL; K-CPT; ADHD confidence index; Hillside behavioral rating scale;	DAP (geometric mean) pregnancy 109.0 nmol/L; DEP 17.7 nmol/L	Psychometrician, exact age at assessment, sex, maternal education, depressive symptoms, PPVT (continuous), ≥	Prenatal DAPs were non-significantly associated with maternal report of attention problems and	Marked effect modification by gender: 11-fold increase ADHD composite indicator in boys, less than 2-fold in	Strengths: Longitudinal design, measurement and consideration of many confounders (including

ED_002061_00044426-00067

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
	composite ADHD indicator		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	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].	girls, however unstable estimates; weak evidence of association DAPs at 3.5, 5 years and attention	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 using assay-specific limit of detection (LOD) values to impute an approximate level.	Data were collected regarding lead exposure, demographics, education and occupational history, income, active and passive smoking, alcohol and drug use during pregnancy, and residential pesticide use. Final models included prenatal environmental tobacco smoke (ETS) exposure, gender, ethnicity, gestational age at birth, quality of home care-taking environment, maternal education, and maternal IQ.	Full-Scale IQ: (B) of -0.003, CI = 0.006, 0.001, p= 0.064 Working Memory Index: (B) of -0.006, CI = 0.009, 0.002, p<0.001. The investigators articulated these results as showing that a 1 pg/g increase in chlorpyrifos exposure was associated with a 0.006 point decrease in the log-transformed Working Memory score and a 0.003 point decrease in the log-transformed Full-Scale IQ score. The investigators concluded that for each standard deviation increase in exposure (4.61pg/g) there is a 1.4% reduction in Full-Scale IQ and a 2.8% reduction in Working Memory.	For each standard deviation increase in exposure (4.61pg/g) there is a 1.4% reduction in Full-Scale IQ and a 2.8% reduction in Working Memory.	Strengths • Direct assessment of chlorpyrifos levels using maternal serum and cord blood. • Analysis using a continuous CPF level, which, in contrast to dichotomous CPF levels, provides a more meaningful look at potential threshold effects and dose-response trends. • The investigators rigorously evaluated their methods for imputing values for undetectable CPF levels which in the end, were validated. • The authors describe an elegant and methodologically sound statistical analysis,

ED_002061_00044426-00068

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
						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

ED_002061_00044426-00069

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
						psychological or educational plans.
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:	Wechsler-IV	Total DAPs	Maternal intelligence, measures	The authors observed	Prenatal measures taken	Strengths: the longitudinal

ED_002061_00044426-00070

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
Bouchard *et al.* (2011) CHAMACOS (N=329)	Intelligence Scale (verbal comprehension; perceptual reasoning, working memory, processing speed, full scale intelligence) measured at age 7 years	(quintiles): Q1 (39 nmol/L); Q2 75 nmol/L; Q3 126 nmol/L; Q4 221 nmol/L; Q5 508 nmol/L. Geometric mean DAP 131 nmol/L	of how stimulating the environment is, and known or suspected neurotoxins were measured prenatally. Maternal intelligence was assessed via the Peabody Picture Vocabulary Test (PPVT). To measure the quality and extent of stimulation available to a child in the home environment, the Infant-Toddler HOME (Home Observation for Measurement of the Environment) inventory was completed at the 6-month, 1, 2, 3.5, 5, and 7 year visits; known or suspected neurotoxicants, polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), p,p'-dichlorodiphenyltrichlorethane (DDT), p,p'-dichlorodiphenyltrichlorethylene (DDE), and lead.	evidence of an association between prenatal exposures to OP pesticides as measured by urinary DAP metabolites in women during pregnancy, and decreased cognitive functioning in children at age 7.	later half of pregnancy more significantly associated intelligence than early; little evidence post-natal OP exposure associated with intelligence; 7 point reduction in full scale intelligence DAP Q5/Q1 (SS)	design, the measurement of urinary DAP at multiple time points and following children to age seven when tests of cognitive function are reportedly more reliable. The authors were able to adjust for or consider many factors related to cognitive function, such as prenatal exposure to other environmental agents, socioeconomic indicators, maternal intelligence and education, and child stimulation. The cohort had a relatively homogenous socioeconomic profile, reducing the potential for uncontrolled confounding.
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)	None	The limit of detection (LOD)	N/A	Meconium TCPy concentrations were	TCPy in maternal urine samples was not reliable,	Comprehensive exposure assessment including actual

ED_002061_00044426-00071

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
Columbia U. (N=102)		of chlorpyrifos in blood samples was 0.5–1 pg/g plasma. The LOD of TCPy in urine samples was 0.26 ng/mL urine. The LOD for TCPy in meconium was 0.2 ng based on a sample weighing 0.5 g. Exposure marker levels below the LOD were given a value of half the level of detection, and were then log10 transformed.		positively correlated with chlorpyrifos in maternal and cord blood ($r = 0.25$–0.33, $p < 0.05$) and with TCPy in maternal urine ($r = 0.31$, $p < 0.01$).	but the maternal and cord blood chlorpyrifos as well as the TCPy levels in meconium were reliable measures of exposure	blood chlorpyrifos levels, the repeated sampling, and the environmental sampling. Weaknesses: only included participants recruited in the post-cancellation period, use of nonparametric, rank-based statistics is appropriate but the large number of observations below the level of detection receiving equal rank, may be problematic; no dietary assessment
Rauh et al.(2012), (n=40)	Morphological change in the pediatric brain in regions of the brain known to be associated with learning, cognition and social behavior	Tertile 3 (≥4.39 pg/g), compared to Tertiles 0, 1, 2 (<4.39 pg/g, including those not exposed to CPF)	Age, sex	Authors report differences in brain structure (regional cerebral size and thickness) by CPF exposure groups, and the differences (high>low CPF) in regional brain size is likely due to enlargement of underlying white matter. Statistical interaction by gender reported.	Authors concluded that the evidence from the study illustrated changes in brain morphology in association with higher CPF exposure, and that changes observed were in areas of the brain that subserve those learning, cognition and social behavioral, supported by previous observational and experimental literature.	Study supports general hypothesis of CPF influence on brain morphology, but lacks specific hypotheses regarding particular areas of the cerebrum affected; limited and somewhat unbalanced depiction of the available rodent experimental data; statistical methods appropriate, correction for multiple statistical comparisons a strength; MRI image readers blinded to exposure status enhances study validity; lack of information on other validation practices; small sample size, pilot study, low statistical power; external validity limited; one time measure

ED_002061_00044426-00072

Author, Year, Sample Size	Outcome Assessed	Exposure Measurement	Potential confounders considered	Primary Result	Conclusions/Uncertainties	Strengths and Weaknesses
						of pre-natal exposure

ED_002061_00044426-00073

2.2.10 Summary & Discussion

Across the eight studies identified in this 2015 literature review within the scope of this analysis and with adequate quality in design and exposure assessment, it is important to note that these studies used study methods that were highly variable, including different exposure measurement, outcome assessment, study design, and geographical location. These differences make it challenging to compare the results across studies. In comparison to the studies from the three US birth cohorts, results from the new studies (e.g., ELEMENT, CHARGE) provide some supportive evidence for the findings from CCCEH, Mt. Sinai and CHAMACOS but in general the new studies are not as robust as those from CCCEH, Mt. Sinai and CHAMACOS.

In the two Chinese studies, EPA does not know how the OP exposures from these studies relate to the currently registered use pattern for OPs used in the U.S./North America. In the case of these two Chinese studies (Guodong *et al.,* 2012; Zhang *et al.*, 2014), there may be differences in the study population and outcome measurements that may account for the observed differences in study results, with Zhang *et al.* (2014) documenting statistically significant associations for total DEAPs, total DMAPs, and total DAPs and Guodong *et al.* (2012) observing no association with these exposures. The Zhang *et al.* (2014) study was conducted in Shenyang, with a study population reported as 87% urban and 13% rural, whereas the Guodong *et al.* (2012) study was conducted in Shanghai with a 99% urban and 1% suburban study population. Given the higher percentage of study participants from rural areas, the study participants from Zhang *et al.* (2014) may have had different pattern and magnitude of OP exposures compared to those from Guodong *et al.* (2012). In addition, it is noted that different outcome measurements were made in these studies, with Guodong *et al.* (2012) assessing 23-25 month old children using a developmental quotient score and Zhang *et al.* (2014) assessing 3 day old infants using a Neonatal Behavioral Neurological Assessment. Given the different outcome assessments, exposure potential, study designs (cohort vs. cross-sectional), and ages of the participants in these two studies, it is difficult to draw conclusions on how these study results compare. It is notable that the results from the Zhang *et al.* (2014) study focusing on neonates are consistent with those from other studies which reported statistically significant associations between delayed neurological development measured in newborns and total DEAP, total DMAP, and total DAP exposure (Engel *et al.*, 2007; Young *et al.*, 2005). However, it is noted that neurological development was measured within a few days of birth for Zhang *et al.* (2014) and Engel *et al.* (2007), whereas Young *et al.* (2005) conducted their measurements within two months of birth.

In this 2015 literature review, two studies were identified that documented either suggestive or statistically significant associations between OP exposure and autism spectrum disorders (Furlong *et al.*, 2014; Shelton *et al.*, 2014). Specifically, Furlong *et al.* (2014) reported suggestive, but not statistically significant, evidence of an association between total DEAP exposure and reciprocal social responsiveness among black participants and boys. These results are consistent with previous studies conducted on the CCCEH and CHAMACOS cohorts, with these studies also showing statistically significant associations between OP exposure and

ASD (Rauh *et al.*, 2006; Eskenazi *et al.*, 2007).[7] Specifically, Eskenazi *et al.* (2007) reported a statistically significant association between PDD and total DAP exposure, whereas Rauh *et al.* (2006) showed a significant association between PDD and specifically chlorpyrifos exposure. Both PDD and reciprocal social responsiveness are related to the autism spectrum disorder. Using a different exposure assessment method (geospatial analysis and residential proximity to total OP exposure), Shelton *et al.* (2014) also documented statistically significant associations between total OP exposure and ASD. While these studies vary in the magnitude of the overall strength of association, they have consistently observed a positive association between OP exposure and ASD.

A total of three studies focusing on ADHD/behavioral/attention problems were identified in this 2015 literature review (Oulhote and Bouchard, 2013; Bouchard *et al.*, 2010; Fortenberry *et al.*, 2014). It is noted that the Fortenberry *et al.* (2014) study is a prospective cohort study on Mexican children, with this study adding an additional North American cohort to the previously identified U.S. cohort studies (CHAMACOS, Mt. Sinai, and CCEH). Of the four studies focusing on ADHD and OP exposure, three found statistically significant associations, with only Oulhote and Bouchard (2013) finding no association with total DMAP, DEAP, or total DAP exposure. In contrast, Bouchard *et al.* (2010) observed an association with total DMAP and total DAP exposure and ADHD. Fortenberry *et al.* (2014) found suggestive, but not statistically significant, evidence of an association with TCPy and ADHD in boys. Overall, the Fortenberry and Bouchard study results are consistent with that of earlier studies from the CCCEH (Rauh *et al.*, 2006) and CHAMACOS (Eskenazi *et al.*, 2007; Marks *et al.*, 2010). Specifically, statistically significant associations were observed by Rauh *et al.* (2006) with chlorpyrifos exposure and ADHD, Eskenazi *et al.* (2007) with total DMAPs and total DAPs and ADHD, and Marks *et al.* (2010) with total DEAP, DMAP, and total DAP exposure.

It is important to put into context the specific outcome measures used in the assessment of attention and neurobehavioral problems. For example, Bouchard *et al.* (2010) identified statistically significant associations between OP exposure and ADHD/behavioral problems, whereas Oulhote and Bouchard (2013) did not. It is valuable to compare these studies given that they are both cross-sectional studies using large population level datasets with biomarker information, with Oulhote and Bouchard (2013) using a Canadian dataset and Bouchard et al (2010) using a U.S. dataset. Bouchard *et al.* (2010) used criteria for ADHD from DSM-IV, whereas Oulhote and Bouchard (2013) used a "Strengths and Differences Questionnaire (SDQ)," with the SDQ being a more generic assessment of mental health status than the DSM-IV criteria. When Oulhote and Bouchard (2013) compared their results to Bouchard *et al.* (2010), they noted that their outcome measurements may not have been as sensitive and that this may account for the difference in study results.

[7] The DSM-V defines ASD (autism spectrum disorder) which now encompasses several disorders that were different diagnoses in DSM-IV, including PDD (pervasive developmental disorder, a catch-all where the other categories didn't fit). Depending on when the study was conducted, the authors may use the PDD or ASD criteria and terminology.

ED_002061_00044426-00075

Across epidemiology studies looking at ADHD/behavioral problems, a suggestive or statistically significant positive association was observed in multiple studies between OP exposure and these neurobehavioral outcomes (Bouchard *et al.*, 2010; Fortenberry *et al.*, 2014; Rauh *et al.*, 2006, Eskenazi *et al.*, 2007). While these studies have differences in the years that the exposure occurred, study design, exposure assessment, and outcome assessment, the commonality in their results is striking.

When all the evidence is considered together, there are uncertainties with respect to a number of factors such as exposure assessment, lack of ability to make strong causal linkages, and unknown window(s) of susceptibility. The epidemiology studies reviewed in the 2012/2014 and 2015 literature reviews represent different investigators, locations, points in time, exposure assessment procedures, and outcome measurements. Despite all these uncertainties and differences in study design, authors consistently identified associations with neurodevelopmental outcomes such as ADHD/behavioral problems and autism spectrum, in relation to OP exposure.

3.0 Weight of Evidence Analysis: Integration Across Multiple Lines of Evidence

In 2010, OPP developed a draft "Framework for Incorporating Human Epidemiologic and 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) and has recently been applied to chlorpyrifos. This document extends the chlorpyrifos WOE to other OPs.

One of the key components of the agency's draft framework is the use the MOA/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 MOA/AOP, and explicitly considers such concepts as strength, consistency, dose response, temporal concordance and biological plausibility in a weight of evidence analysis; sections 3.1-3.3 below summarize the available evidence based on these principles.

3.1 Dose-Response Relationships & Temporal Concordance

Since the MOA(s)/AOP(s) is/are not established for neurodevelopmental outcomes (USEPA, 2012, 2014), it is not possible to describe the concordance in key events or biological steps leading to neurodevelopmental outcomes. As such, the quantitative linkages between molecular initiating events (MIE)s, intermediate steps, and ultimately the adverse outcome (i.e., neurodevelopmental effects) cannot be determined.

ED_002061_00044426-00076

With respect to the timing of exposure, across the epidemiology database of studies the maternal urine, cord blood and other (meconium) measures provide evidence that exposure did occur to the fetus during gestation, but the actual level of such exposure during the critical window(s) of susceptibility is not known. While significant uncertainties remain about the actual exposure levels experienced by mothers and infant participants in the children's health cohorts, it is unlikely that these exposures resulted in AChE inhibition. As part of the CHAMACOS study, Eskenazi *et al.* (2004) measured AChE activity and showed that no differences in AChE activity were observed. The biomarker data from the Columbia University studies are supported by the agency's dose reconstruction analysis using the PBPK-PD model. Following the recommendation of the FIFRA SAP (2012), the agency conducted a dose reconstruction analysis of residential uses available prior to 2000 for pregnant women and young children inside the home (USEPA, 2014). Based on the output from the PBPK-PD model, for the highest exposure considered (i.e., indoor broadcast use of a 1% chlorpyrifos formulation), <1% RBC AChE inhibition in pregnant women would be expected. While uncertainty exists as to actual OP exposure at (unknown) critical windows of exposure, EPA believes it is unlikely individuals in the epidemiology studies experienced RBC AChE inhibition.

Within the Columbia University epidemiology studies, the relationship in time between prenatal chlorpyrifos exposure and adverse neurodevelopmental outcomes is concordant. The time period under study within the Columbia University (CCCEH) study, spanned the point in time in which pesticide manufacturers voluntarily cancelled the use of chlorpyrifos in the home environment, and researchers were able to show the change in exposure before (high use period) and after (low/no use period) the period of removal of chlorpyrifos products from the residential marketplace. Moreover, prior to the voluntary cancellation there were >80% detectable levels of chlorpyrifos in cord blood but in the time period after the cancellation only 16% of the measured values were greater than the level of detection (LOD); there was only one child born in the time period subsequent to the voluntary cancellation of chlorpyrifos in the residential marketplace for whom the cord blood chlorpyrifos level was in the upper-tertile of pre-cancellation exposure levels. The significantly reduced proportion of measured values greater than the LOD as well as the observation of an absence of an association between prenatal chlorpyrifos exposure among infants born after the voluntary cancellation of chlorpyrifos and neurodevelopmental effects support the hypothesis that chlorpyrifos is related to these outcomes. However, as noted by study authors, EPA and the FIFRA SAP (2012), this could also be due to inadequate sample size to detect a small to modest effect among the group of infants born after the voluntary cancellation. It is notable that epidemiology studies from other research groups have not included analyses across different years of exposure.

3.2 Strength, Consistency & Specificity

Published and submitted laboratory animal studies have been reviewed for OPs. The >30 papers on chlorpyrifos provide evidence of long-lasting neurodevelopmental disorders in rats and mice; however, there was no clear consistency in terms of pattern, timing, or dose

response for these effects. The additional toxicological literature and guideline DNT studies with the other OPs provide more evidence for the same conclusions, with again the same caveats and uncertainties. While overall cognitive function and motor activity appeared to be altered the most often, it is apparent that these behaviors were also the most often evaluated.

Among the epidemiology studies, two of the cohorts (CCCEH and ELEMENT) have focused on chlorpyrifos whereas the other studies (Mt. Sinai cohort, CHAMACOS cohort, CHARGE study, Bouchard *et al.*, 2007) have focused on less specific biomarkers (i.e., DAPs) and are not specific to any particular OP. When considered in concert, the epidemiology studies provide consistent findings for some outcomes. Specifically, with regard to the three US children's environmental health epidemiology studies and the ELEMENT cohort in Mexico, each of the four 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. As noted above, the CHAMACOS and Mt. Sinai cohorts that measured neurological effects at birth (the Brazelton index), observed a putative association with OPs (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), the three US cohorts each reported evidence of impaired mental and psychomotor development. Attentional problems and ADHD were reported by CCCEH, Mt. Sinai, CHAMACOS, and ELEMENT investigators with additional support from Bouchard *et al.* (2010). In addition, several studies have now documented suggestive or positive associations between OP exposure and autism spectrum disorders (Rauh *et al.*, 2006; Shelton *et al.*, 2014; Furlong *et al.*, 2014; Eskenazi *et al.*, 2007; Eskenazi et al, 2010). Finally, each of the three US childrens cohort study authors observed an inverse relation between the respective prenatal measures of chlorpyrifos and intelligence measures at age 7 years.

As stated in the EPA neurotoxicity guidelines[8], direct extrapolation of developmental neurotoxicity results from laboratory animals to humans is limited by the lack of knowledge about underlying toxicological mechanisms and the relevance of these results to humans. EPA notes consistencies across the databases of *in vivo* laboratory animal studies and epidemiology studies, although challenges of making a direct comparison between neurodevelopmental domain inter-species remain. It can be assumed that developmental neurotoxicity effects in animal studies indicate the potential for altered neurobehavioral development in humans, although the specific types of developmental effects seen in experimental animal studies may not be the same as those that may be produced in humans. However, considering the toxicological and epidemiological data in the context of three major neurodevelopmental domains (specifically, cognition, motor control, and social behavior), insights can be gained. Previously reviewed studies of chlorpyrifos in rats and/or mice reported impaired cognition, changes in locomotor activity levels, altered social interaction, and to a lesser extent, changes in neuromotor function (FIFRA SAP 2012; USEPA, 2014). While there are fewer studies for all the other OPs, behavioral effects in the same functional domains were again reported. The most commonly reported outcome was cognitive dysfunction, and although it was overall consistent there were again differences in cognitive specificity, gender differences, or dose

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

ED_002061_00044426-00078

response. Quite a few studies also report changes in motor activity and sensory function in offspring, but there generally fewer studies that assess social interactions for OPs other than chlorpyrifos. It is notable that the laboratory animal studies vary in experimental designs such as species, strain, gender, dosing regimens (age, routes, vehicle), and test parameters (age, protocol). Likewise, observational epidemiology studies vary by population characteristics (race/ethnicity, SES, and pesticide use/exposure profile), co-exposures (mix of chemicals, windows of exposure), and method of exposure and outcome assessment. Given the differences across laboratory animal and epidemiology studies, the qualitative similarity in research findings is striking.

In contrast, quantitatively, there are notable differences between animals and humans. Specifically, in animals, the doses most often used in these studies are sufficient to elicit ≥10% brain and RBC inhibition depending on the study design, age of the animal, and sampling time. In the epidemiology studies, based on the comparisons with biomonitoring data, reported AChE data from CHAMACOS and the results of the chlorpyrifos dose-reconstruction analysis, it is unlikely that RBC AChE would have been inhibited by any meaningful or measurable amount, if any at all, and most likely none in the brain. This key difference in dose response between the experimental toxicology and epidemiology studies poses challenges in interpreting such data. There are a number of possible hypotheses such as: 1) limitations of experimental laboratory studies which have limited statistical power due to relatively small sample sizes; 2) humans display a broader array of behaviors and cognitive abilities than rats, thus limiting the sensitivity of the rat studies; and 3) in the epidemiology studies, the timing of OP application and blood collections are not coupled—thus higher levels of blood OPs were likely missed.

3.3 Biological Plausability & Coherence

EPA's cancer guidelines (2005) includes guidance which are also applicable to this current evaluation of OPs. In fact, the Guidelines indicate:

"*evaluation of the biological plausibility of the associations observed in epidemiologic studies reflects consideration of both exposure-related factors and toxicological evidence relevant to identification of potential modes of action (MOAs). Similarly, consideration of the coherence of health effects associations reported in the epidemiologic literature reflects broad consideration of information pertaining to the nature of the biological markers evaluated in toxicologic and epidemiologic studies*. [p. 39]."

The Cancer Guidelines further state that "*lack of mechanistic data, however, is not a reason to reject causality* [p. 41]."

At this time, a MOA(s)/AOP(s) has/have not been established for neurodevelopmental outcomes. This growing body of literature does demonstrate, however, that OPs are biologically active on a number of processes that affect the developing brain. Moreover, there

ED_002061_00044426-00079

is a large body of *in vivo* laboratory studies which show long-term behavioral effects from early life exposure, albeit at doses which cause AChE inhibition. EPA considers the results of the toxicological studies relevant to the human population, as qualitatively supported by the results of epidemiology studies. The lack of established MOA/AOP pathway does not undermine or reduce the confidence in the findings of the epidemiology studies. When all the evidence is considered together, there are uncertainties with respect to a number of factors such as exposure assessment, lack of ability to make strong causal linkages, and unknown window(s) of susceptibility. The epidemiology studies reviewed in the 2012/2014 and 2015 literature reviews represent different investigators, locations, points in time, exposure assessment procedures, and outcome measurements. Despite all these uncertainties and differences in study design, multiple investigators have identified associations with neurodevelopmental outcomes such as ADHD/behavioral problems and autism spectrum, in relation to OP exposure. There is evidence of delays in mental development in infants (24-36 months), attention problems and autism spectrum disorder in early childhood, and intelligence decrements in school age children who were exposed to OPs during gestation. Investigators reported strong measures of statistical association across several of these evaluations (odds ratios 2-4 fold increased in some instances), and observed evidence of exposures-response trends in some instances, *e.g.*, intelligence measures. Thus, with respect to biological plausibility and coherence, although uncertainties remain, these uncertainties are diminished in the context of the qualitative similarity between the epidemiology studies.

4.0 10X FQPA Safety Factor for Infants and Children

As section 408(b)(2)(C) of the FFDCA instructs EPA, in making its "reasonable certainty of no harm" finding, that in "the case of threshold effects, an additional tenfold margin of safety for the pesticide chemical residue and other sources of exposure shall be applied for infants and children to take into account potential pre- and postnatal toxicity and completeness of data with respect to exposure and toxicity to infants and children." Section 408 (b)(2)(C) further states that "the Administrator may use a different margin of safety for the pesticide chemical residue only if, on the basis of reliable data, such margin will be safe for infants and children." Given the totality of the evidence, there is sufficient uncertainty in the human dose-response relationship for neurodevelopmental effects which prevents the agency from reducing or removing the statutory 10X FQPA Safety Factor. For the preliminary human health risk assessments for the OPs, a value of 10X will be applied. Similarly, a database uncertainty factor of 10X will be retained for occupational risk assessments. The agency will continue to evaluate the epidemiology studies and pursue approaches for quantitative or semi-quantitative comparisons between doses which elicit AChE inhibition and those which are associated with neurodevelopmental outcomes prior to a revised human health risk assessment. **The FQPA 10X Safety Factor will be retained for OPs for the population subgroups that include infants, children, youths, and women of childbearing age for all exposure scenarios.**

5.0 References

Acosta-Maldonado, Brenda, Sánchez-Ramírez, Blanca, Reza-López, Sandra, and Levario-Carrillo, Margarita. Effects of exposure to pesticides during pregnancy on placental maturity and weight of newborns: A cross-sectional pilot study in women from the Chihuahua State, Mexico. Human and Experimental Toxicology 28(8) 451–459, 2009.

al-Hachim, G.M., Fink, G.B., 1968a. Effect of DDT or parathion on open-field behavior of offspring from DDT- or parathion-treated mothers. Psychol Rep **22**, 1193-1196.

al-Hachim, G.M., Fink, G.B., 1968b. Effect of DDT or parathion on condition avoidance response of offspring from DDT or parathion-treated mothers. Psychopharmacologia **12**, 424-427.

Andersen, Helle R., Debes, Fróði, Wohlfahrt-Veje, Christine, Murata, Katsuyuki, Grandjean, Philippe. Occupational pesticide exposure in early pregnancy associated with sex-specific neurobehavioral deficits in the children at school age. Neurotoxicology and Teratology 47:1-9, 2015.

Ankley, G. T., Bennett, R. S., Erickson, R. J., Hoff, D. J., Hornung, M. W., Johnson, R. D., *et al.* (2010). Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. *Environ Toxicol Chem, 29*(3), 730-741.

Barr DB, Ananth CV, Yan X, Lashley S, Smulian JC, Ledoux TA, Hore P, Robson MG. Pesticide concentrations in maternal and umbilical cord sera and their relation to birth outcomes in a population of pregnant women and newborns in New Jersey. Sci Total Environ. 2010;408:790–795.

Berkowitz GS, Wetmur JG, Birman-Deych E, Obel J, Lapinski RH, Godbold JH, Holzman IR, Wolff MS. In Utero Pesticide Exposure, Maternal Paraoxonase Activity, and Head Circumference. Environmental Health Perspectives. 2004;112:388–391.

Boobis, A. R., Cohen, S. M., Dellarco, V., McGregor, D., Meek, M. E., Vickers, C., *et al.* (2006). IPCS framework for analyzing the relevance of a cancer mode of action for humans. *Crit Rev Toxicol, 36*(10), 781-792.

Boobis, A. R., Doe, J. E., Heinrich-Hirsch, B., Meek, M. E., Munn, S., Ruchirawat, M., *et al.* (2008). IPCS framework for analyzing the relevance of a noncancer mode of action for humans. *Crit Rev Toxicol, 38*(2), 87-96.

Bouchard M.F., Bellinger D.C., Wright R.O., Weisskopf M.G., (2010). Attention-Deficit/Hyperactivity Disorder and Urinary Metabolites of Organophosphate Pesticides. PEDIATRICSDOI:10.1542/peds.2009-3058.

Bouchard, M. F., Chevrier, J., Harley, K. G., Kogut, K., Vedar, M., Calderon, N., *et al.* (2011). Prenatal exposure to organophosphate pesticides and IQ in 7-year-old children. Environ Health Perspect, 119(8), 1189-1195.

Ceh, K., Jezek, D., Zuzek, M.C., Cebulj-Kadunc, N., Majdic, G., 2012. Effects of organophosphate chlormephos on development and function of testes and brain. Slovenian Veterinary Research **49**, 41-49.

Chen L, Zhao T, Pan C, Ross JH, Krieger RI. (2012) Preformed biomarkers including dialkylphosphates (DAPs) in produce may confound biomonitoring in pesticide exposure and risk assessment. J Agric Food Chem. 2012 Sep 12;60(36):9342-51. doi: 10.1021/jf303116p. Epub 2012 Sep 4.

Clemens, G.R., Hartnagel, R.E., Bare, J.J., Thyssen, J.H., 1990. Teratological, neurochemical, and postnatal neurobehavioral assessment of METASYSTOX-R, an organophosphate pesticide in the rat. Fundam Appl Toxicol **14**, 131-143.

Crowder, L.A., Lanzaro, G.C., Whitson, R.S., 1980. Behavioral effects of methyl parathion and toxaphene exposure in rats. J Environ Sci Health B **15**, 365-378.

Dąbrowski, Sławomir, Hanke, Wojciech, Polańska, Kinga, Makowiec-Dąbrowska, Teresa, and Sobala, Wojciech. Pesticide exposure and birthweight: An epidemiological study in Central Poland. International Journal of Occupational Medicine and Environmental Health 16(1): 31-39, 2003.

de Castro, V.L., Chiorato, S.H., Pinto, N.F., 2001. Relevance of developmental testing of exposure to methamidophos during gestation to its toxicology evaluation. Toxicol Lett **118**, 93-102.

Engel SM, Berkowitz GS, Barr DB, Teitelbaum SL, Siskind J, Meisel SJ, Wetmur JG, Wolff MS. Prenatal Organophosphate Metabolite and Organochlorine Levels and Performance on the Brazelton Neonatal Behavioral Assessment Scale in a Multiethnic Pregnancy Cohort. American Journal of Epidemiology. 2007;165:1397-1404.

Engel SM, Wetmur J, Chen J, Zhu C, Barr DB, Canfield RL, Wolff MS. Prenatal Exposure to Organophosphates, Paraoxonase 1, and Cognitive Development in Childhood. Environmental Health Perspectives. 2011;119:1182-1188.

Eskenazi B, Harley K, Bradman A, Weltzien E, Jewell NP, Barr DB, Furlong CE, Holland NT. Association of in Utero Organophosphate Pesticide Exposure and Fetal Growth and Length of Gestation in an Agricultural Population. Environmental Health Perspectives. 2004;115:792–798.

Eskenazi B, Marks AR, Bradman A, Harley K, Barr DB, Johnson C, Morga N, Jewell NP. Organophosphate Pesticide Exposure and Neurodevelopment in Young Mexican-American Children. Environmental Health Perspectives. 2007;115:792–798.

Eskenazi, B., Huen, K., Marks, A., Harley, K. G., Bradman, A., Barr, D. B., & Holland, N. (2010). PON1 and neurodevelopment in children from the CHAMACOS study exposed to organophosphate pesticides in utero. Environ Health Perspect, 118(12), 1775-1781.

ED_002061_00044426-00082

FIFRA Scientific Advisory Panel. (2008b). "The Agency's Evaluation of the Toxicity Profile of Chlorpyrifos." Report from the FIFRA Scientific Advisory Panel Meeting of September, 2008. FIFRA Scientific Advisory Panel, Office of Science Coordination and Policy, Office of Prevention, Pesticides and Toxic Substances, U.S. Environmental Protection Agency. Washington, DC. Available: http://www.epa.gov/scipoly/sap/meetings/2008/index.html

FIFRA Scientific Advisory Panel. (2010). February 2 - 4, 2010: Incorporation of Epidemiology and Human Incident Data into Human Risk Assessment.

FIFRA Scientific Advisory Panel. (2012). Transmittal of the Meeting Minutes from the FIFRA Scientific Advisory Panel Meeting of April 10-12, 2012. FIFRA Scientific Advisory Panel, Office of Science Coordination and Policy, Office of Prevention, Pesticides and Toxic Substances, U.S. Environmental Protection Agency. Washington, DC. Available: http://www.epa.gov/scipoly/sap/meetings/2012/041012meeting.html

Fortenberry G.Z., Meeker J.D., Sanchez B.N., *et al.*, (2014). Urinary 3,5,6-tichloro-2-pyridinol (TCPY) in pregnant women from Mexico City: Distribution, temporal variability, and relationship with child attention and hyperactivity. International Journal of Hygiene and Environmental Health 217 (2014) 405-412.

Fortenberry, GZ, Meeker, JD, Sánchez, BN, Barr, DB, Peterson, K, Bellinger, D, Schnaas, L, Solano-González, M, Ettinger, AS, Hernandez-Avila, M, Hu, H, Tellez-Rojoj, MM. Paraoxonase I polymorphisms and attention/hyperactivity in school-age children from Mexico City, Mexico. (2014a.) Environmental Research 132:342–349.

Furlong, Melissa A., Engel, Stephanie M., Boyd Barr, Dana, Wolff, Mary S. Prenatal exposure to organophosphate pesticides and reciprocal social behavior in childhood. 2014. Environment International 70:125-131.

Grandjean, Philippe, Harari, Raul, Barr, Dana B., Debes, Frodi. Pesticide exposure and stunting as independent predictors of neurobehavioral deficits in Ecuadorian school children. 2006. Pediatrics 117(3):e546-e556.

Guodong D., Pei W., Ying T., Jun Z., *et al.*, (2012). Organophosphate Pesticide Exposure and Neurodevelopment in Young Shanghai Children. Environ Sci. Technol. 2012, 46, 2911-2917.

Gupta, R.C., Rech, R.H., Lovell, K.L., Welsch, F., Thornburg, J.E., 1985. Brain cholinergic, behavioral, and morphological development in rats exposed in utero to methylparathion. Toxicol Appl Pharmacol **77**, 405-413.

Handal A.J., Lozoff B., Breilh J., Harlow S.D., (2007). Effect of community of residence on neurobehavioral development in infants and young children in a flower-growing region of Ecuador. Environ Health Perspect. 2007 Jan;115(1): 128-33.

Handal A.J., Lozoff B., Breilh J., Harlow S.D., (2007B). Neurobehavioral Development in Children with Potential Exposure to Pesticides. Epidemiology May 2007; 18: 312-320.

Handal A.J., Lozoff B., Breilh J., Harlow S.D., (2008). Occupational Exposure to Pesticides During Pregnancy and Neurobehavioral Development of Infants and Toddlers. Epidemiology, Volume 19, number 6, November 2008.

Harari, Raul, Julvez, Jordi, Murata, Katsuyuki, Barr, Dana, Bellinger, David C., Debes, Frodi, and Grandjean, Philippe. Neurobehavioral Deficits and Increased Blood Pressure in School-Age Children Prenatally Exposed to Pesticides. 2010. Environmental Health Perspectives 118(6): 890-896.

Harley KG, Huen K, Schall RA, Holland NT, Bradman A, *et al*. Association of Organophosphate Pesticide Exposure and Paraoxonase with Birth Outcome in Mexican-American Women. PLoS ONE 2011; 6(8)1-10.

Hill AB (1965). The Environment and Disease: Association or Causation? Proc R Soc Med. May 1965; 58(5): 295–300.

Johnson, F.O., Chambers, J.E., Nail, C.A., Givaruangsawat, S., Carr, R.L., 2009. Developmental chlorpyrifos and methyl parathion exposure alters radial-arm maze performance in juvenile and adult rats. Toxicol Sci **109**, 132-142.

Kofman, Ora, Berger, Andrea, Massarwa, Ali, Friedman, Alon, and Abu Jaffar, Abed. Motor Inhibition and Learning Impairments in School-Aged Children Following Exposure to Organophosphate Pesticides in Infancy. Pediatric Research. 60:88-92, 2006.

Koutroulakis, D., Sifakis, S., Tzatzarakis, M.N., Alegakis, A.K., Theodoropoulou, E., Kavvalakis, M.P., Kappou, D., Tsatsakis, A.M. Dialkyl phosphates in amniotic fluid as a biomarker of fetal exposure to organophosphates in Crete, Greece; association with fetal growth. Reproductive Toxicology 46 (2014) 98–105.

Lazarini, C.A., Lima, R.Y., Guedes, A.P., Bernardi, M.M., 2004. Prenatal exposure to dichlorvos: physical and behavioral effects on rat offspring. Neurotoxicol Teratol **26**, 607-614.

Lehotzky, K., Szeberenyi, M.J., Kiss, A., 1989. Behavioral consequences of prenatal exposure to the organophosphate insecticide sumithion. Neurotoxicol Teratol **11**, 321-324.

Levin, E.D., Timofeeva, O.A., Yang, L., Petro, A., Ryde, I.T., Wrench, N., Seidler, F.J., Slotkin, T.A., 2009. Early postnatal parathion exposure in rats causes sex-selective cognitive impairment and neurotransmitter defects which emerge in aging. Behav Brain Res **208**, 319-327.

Lima, C.S., Dutra-Tavares, A.C., Nunes, F., Nunes-Freitas, A.L., Ribeiro-Carvalho, A., Filgueiras, C.C., Manhaes, A.C., Meyer, A., Abreu-Villaca, Y., 2013. Methamidophos exposure during the early postnatal period of mice: immediate and late-emergent effects on the cholinergic and serotonergic systems and behavior. Toxicol Sci **134**, 125-139.

ED_002061_00044426-00084

Lizardi, Patricia Sanchez, O'Rourke, Mary Kay, and Morris, Richard J. The effects of organophosphate pesticide exposure on Hispanic children's cognitive and behavioral functioning. Journal of Pediatric Psychology 33(1): 91–101, 2008.

Llop S., Julvez J., Marina L.S., Vizcaino E., *et al.*, (2013). Prenatal and postnatal insecticide use and infant neuropsychological development in a multicenter birth cohort study. Environment International 59(2013) 175-182.

Lovasi GS, Quinn JW, Rauh VA, Perera FP, Andrews HF, Garfinkel R, Hoepner L, Whyatt R, Rundle A. Chlorpyrifos Exposure and Urban Residential Environment Characteristics as Determinants of Early Childhood Neurodevelopment. American Journal of Public Health. 2010;101(1):63-70. doi: 10.2105/AJPH.2009.168419. Epub 2010 Mar 18.

Lu, *et al*. Assessing the association between pesticide exposure and cognitive development in rural Costa Rican children living in organic and conventional coffee farms. Int J Adolesc Med Health. 2009; 21(4): 609-621

Marks AR, Harley K, Bradman A, Kogut K, Barr DB, Johnson C, *et al*. Organophosphate Pesticide Exposure and Attention in Young Mexican-American Children: The CHAMACOS Study. Environmental Health Perspectives. 2010;118:1768-1774.

Meek ME, Boobis A, Cote I, Dellarco V, Fotakis G, Munn S, Seed J, Vickers C. 2014. New developments in the evolution and application of the WHO/IPCS framework on mode of action/species concordance analysis. *J Appl Toxicol.* 2014 Jan;34(1):1-18.

Moreno-Banda, G., Blanco-Muñoz, J., Lacasaña, M., Rothenberg, S.J., Aguilar-Garduño, C., Gamboa, R., Pérez-Méndez, O. Maternal exposure to floricultural work during pregnancy, PON1 Q192R polymorphisms and the risk of low birth weight. Science of the Total Environment 407: 5478–5485, 2009.

Muñoz-Quezada MT, Lucero BA, Barr DB, Steenland K, Levy K, Ryan PB, Veronica Iglesiase V, Alvaradoe S, Conchaf C, Rojas E, Catalina Vega C. Neurodevelopmental effects in children associated with exposure to organophosphate pesticides: A systematic review. Neurotoxicology 39: 158-168.

Naksen W, Prapamontol T, Mangklabruks A, Chantara S, Thavornyutikarn P, Srinual N, Panuwet P, Ryan PB, Riederer AM, Barr DB. Associations of maternal organophosphate pesticide exposure and PON1 activity with birth outcomes in SAWASDEE birth cohort, Thailand. Environmental Research142: 288–296, 2015.

Nevison. A comparison of temporal trends in United States autism prevalence to trends in suspected environmental factors. Environmental Health. 2014; 13(73): 16 pp

Oulhote, Youssef and Bouchard, Maryse F. Urinary Metabolites of Organophosphate and Pyrethroid Pesticides and Behavioral Problems in Canadian Children. Environmental Health Perspectives. 121:1378-1384 (2013)

Petit, Claire, Chevrier, Cécile, Durand, Gaël, Monfort, Christine, Rouget, Florence, Garlantezec, Ronan, Cordier, Sylvaine. Impact on fetal growth of prenatal exposure

ED_002061_00044426-00085

to pesticides due to agricultural activities: a prospective cohort study in Brittany, France. Environmental Health 9:71-83, 2010.

Quiros-Alcala L., Alkom A.D., Boyce W.T., Lippert S., *et al.*, (2011). Maternal prenatal and child organophosphate pesticide exposures and children's autonomic function. NeuroToxicology 32 (2011) 646-655.

Racke J.D. Degradation of organophosphorous insecticidesin environmental matrices. In: Organophosphates Chemistry, Fate and Effects. Academic Press, Inc., San Diego, CA, 1992, pp 47–72.

Rauch, Stephen A., Braun, Joe M., Boyd Barr, Dana, Calafat, Antonia M., Khoury, Jane, M., Montesano, Angela, Yolton, Kimberly, and Lanphear, Bruce P. Associations of Prenatal Exposure to Organophosphate Pesticide Metabolites with Gestational Age and Birth Weight. Environmental Health Perspectives. 120:1055–1060 (2012).

Rauh VA, Garfinkel R, Perera FP, Andrews HF, Hoepner L, Barr DB, Whitehead R, Tang D, Whyatt RW. Impact of Prenatal Chlorpyrifos Exposure on Neurodevelopment in the First 3 Years of Life Among Inner-City Children. Pediatrics. 2006;18(6): 1835-1859.

Rauh V, Arunajadai S, Horton M, Perera F, Hoepner L, Barr DB, *et al.* 2011. Seven-Year Neurodevelopmental Scores and Prenatal Exposure to Chlorpyrifos, a Common Agricultural Pesticide. Environmental Health Perspectives. 2011;119:1196-1201.

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 Natl Acad Sci U S A, 109(20), 7871-7876.

Roegge, C.S., Timofeeva, O.A., Seidler, F.J., Slotkin, T.A., Levin, E.D., 2007. Developmental diazinon neurotoxicity in rats: later effects on emotional response. Brain Res Bull **75**, 166-172.

Rohlman, Diane S., Arcury, Thomas A., Quandt, Sara A., Lasarev, Michael, Rothlein, Joan, Travers, Rachelle, Tamulinas, Alys, Scherer, Jennifer, Early, Julie, Marín, Antonio, Phillips, Jacki, McCauley, Linda. Neurobehavioral performance in preschool children from agricultural and non-agricultural communities in Oregon and North Carolina. Neurotoxicology 26:895-598, 2005.

Ruckart, *et al.* Long-Term Neurobehavioral Health Effects of Methyl Parathion Exposure in Children in Mississippi and Ohio. Environmental Health Perspectives. 2004; 112(1):46-51.

Samarawickrema, Nirma, Pathmeswaran, Arunasalam, Wickremasinghe, Rajitha, Peiris-John, Roshini, Karunaratna, Madawa, Buckley, Nick, Dawson, Andrew, and De Silva, Janaka. Fetal effects of environmental exposure of pregnant women to organophosphorus compounds in a rural farming community in Sri Lanka. Clinical Toxicology 46:489–495, 2008.

Savitz, David A., Arbuckle, Tye, Kaczor, Diane, and Curtis, Kathryn M. Male pesticide

ED_002061_00044426-00086

exposure and pregnancy outcome. American Journal of Epidemiology 146(12):1025-1036, 1997.

Shelton, Janie F., Geraghty, Estella M., Tancredi, Daniel J., Delwiche, Lora D., Schmidt, Rebecca J., Ritz, Beate, Hansen, Robin L., and Hertz-Picciotto, Irva. Neurodevelopmental disorders and prenatal residential proximity to agricultural pesticides: The CHARGE Study. Environmental Health Perspectives 122:1103–1109, 2014.

Simon TW, Simons SS Jr, Preston RJ, Boobis AR, Cohen SM, Doerrer NG, Fenner-Crisp PA, McMullin TS, McQueen CA, Rowlands JC; RISK21 Dose-Response Subteam. 2014. The use of mode of action information in risk assessment: Quantitative key events/dose-response framework for modeling the dose-response for key events. *Crit Rev Toxicol.* 2014 Aug;44 Suppl 3:17-43.

Slotkin, T.A., Tate, C.A., Ryde, I.T., Levin, E.D., Seidler, F.J. 2006. Organophosphate insecticides target the serotonergic system in developing rat brain regions: Disparate effects of diazinon and parathion at doses spanning the threshold for cholinesterase inhibition. Environ Health Perspec **114**, 1542-1546.

Sochaski, M.A., McManus, B.M., Struve, M.F., Wallace, D.G., Dorman, D.C. 2007. Inhibition and recovery of maternal and foetal cholinesterase enzymes following fenitrothion administration in CD rats. Xenobiotica **37**, 19-29.

Spyker, J.M., Avery, D.L., 1977. Neurobehavioral effects of prenatal exposure to the organophosphate Diazinon in mice. J Toxicol Environ Health **3**, 989-1002.

Stamper, C.R., Balduini, W., Murphy, S.D., Costa, L.G., 1988. Behavioral and biochemical effects of postnatal parathion exposure in the rat. Neurotoxicol Teratol **10**, 261-266.

Suarez-Lopez J. R., Jacobs D.R., Himes J.H., Alexander B.H., Lazovich D., *et al.*, (2012). Lower acetylcholinesterase activity among children living with flower plantation workers. Environmental Research 114 (2012) 53-59.

Suarez-Lopez J. R., Jacobs D.R., Himes J.H., Alexander B.H., (2013). Aectylcholinesterase activity and neurodevelopment in boys and girls. PEDIATRICS Volume 132, number 6, December 2013.

Suarez-Lopez J. R., Jacobs D.R., Himes J.H., Alexander B.H., (2013a). Acetycholinesterase activity, cohabitation with floricultural workers, and blood pressure in Ecuadorian Children. Environmental Health Perspectives, volume 121, number 5, May 2013.

Timofeeva, O.A., Roegge, C.S., Seidler, F.J., Slotkin, T.A., Levin, E.D., 2008a. Persistent cognitive alterations in rats after early postnatal exposure to low doses of the organophosphate pesticide, diazinon. Neurotoxicol Teratol **30**, 38-45.

Timofeeva, O.A., Sanders, D., Seemann, K., Yang, L., Hermanson, D., Regenbogen, S., Agoos, S., Kallepalli, A., Rastogi, A., Braddy, D., Wells, C., Perraut, C., Seidler, F.J., Slotkin, T.A., Levin, E.D., 2008b. Persistent behavioral alterations in rats neonatally

ED_002061_00044426-00087

exposed to low doses of the organophosphate pesticide, parathion. Brain Res Bull **77**, 404-411.

U.S. Environmental Protection Agency. (2005). Guidelines for Carcinogen Risk Assessment. Risk Assessment Forum, U.S. Environmental Protection Agency. Washington, DC. EPA/630/P-03/001F.

U.S. Environmental Protection Agency. (2006). Revised Organophosphorous Pesticide Cumulative Risk Assessment; July 31, 2006. Office of Pesticide Programs, U.S. Environmental Protection Agency. Washington, D.C. Available http://www.epa.gov/pesticides/cumulative/rra-op.

U.S. Environmental Protection Agency. (2008). Draft Science Issue Paper: Chlorpyrifos Hazard and Dose Response Characterization. http://www.epa.gov/scipoly/sap/meetings/2008/091608_mtg.htm

U.S. Environmental Protection Agency. (2010). Draft Framework for Incorporating Human Epidemiologic and Incident Data in Health Risk Assessment, January 7, 2010.

U.S. Environmental Protection Agency. (2011a). Chlorpyrifos: Preliminary Human Health Risk Assessment for Registration Review. June 30, 2011. D388070.

U.S. Environmental Protection Agency. (2011b). Chlorpyrifos: Occupational and Residential Exposure Assessment, EPA Barcode: D388165,. In Office of Pesticide Programs (Ed.). Washington D.C.

U.S. Environmental Protection Agency. (2012). Draft Issue Paper: Scientific Issues Concerning Health Effects of Chlorpyrifos. http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2012-0040-0002

U.S. Environmental Protection Agency. (2014). Chlorpyrifos: Revised Human Health Risk Assessment for Registration Review. December 29, 2014. D424485.

Vatanparast, J., Naseh, M., Baniasadi, M., Haghdoost-Yazdi, H., 2012. 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.

Wang, Pei, Tian, Ying, Wang, Xiao-Jin, Gao, Yu, Shi, Rong, Wang, Guo-Quan, Hu, Guo-Hua, Shen, Xiao-Ming. Organophosphate pesticide exposure and perinatal outcomes in Shanghai, China. 4 Environment International 2:100–104, 2012.

Whyatt, R. M., Barr, D. B., Camann, D. E., Kinney, P. L., Barr, J. R., Andrews, H. F., . . . Perera, F. P. (2003). Contemporary-use pesticides in personal air samples during pregnancy and blood samples at delivery among urban minority mothers and newborns. Environ Health Perspect, 111(5), 749-756.

Whyatt RM, Rauh V, Barr DB, Camann DE, Andrews HF, Garfinkel R, Hoepner LA, Diaz D, Dietrich J, Reyes A, Tang D, Kinney PL, Perera FP. Prenatal Insecticide Exposures and Birth Weight and Length among an Urban Minority Cohort. Environmental

ED_002061_00044426-00088

Health Perspectives. 2004;112:1125–1132.

Whyatt RM, Garfinkel R, Hoepner LA, Holmes D, Borjas M, Williams MK, *et al*. Within- and Between-Home Variability in Indoor-Air Insecticide Levels during Pregnancy among an Inner-City Cohort from New York City. Environmental Health Perspectives 2007;115:383-389.

Whyatt RM, Garfinkel R, Hoepner LA, Andrews H, Holmes D, Williams MK, *et al*. A Biomarker Validation Study of Prenatal Chlorpyrifos Exposure within an Inner-City Cohort during Pregnancy. Environmental Health Perspectives. 2009;117:559-567.

Wickerham, Erin L., Lozoff, Betsy, Shao, Jie, Kaciroti, Niko, Xia, Yankai, Meeker, John D. Reduced birth weight in relation to pesticide mixtures detected in cord blood of full-term infants. Environment International 47:80–85, 2012.

Win-Shwe, T.T., Nakajima, D., Ahmed, S., Fujimaki, H., 2012. Impairment of novel object recognition in adulthood after neonatal exposure to diazinon. Arch Toxicol **87**, 753-762.

Wolff, MS, Engel, S, Berkowitz, G, Teitelbaum, S, Siskind, J, Barr, DB, Wetmur, J. Prenatal Pesticide and PCB Exposures and Birth Outcomes. 2007. Pediatric Research 61(2):243-250.

Young JG, Eskenazi B, Gladstone EA, Bradman A, Pedersen L, Johnson C, Barr DB, Furlong CE, Holland NT. Association Between In Utero Organophosphate Pesticide Exposure and Abnormal Reflexes in Neonates. NeuroToxicology. 2005;26:199–209.

Zhang Y., Han S., Liang D., Shi X., Wang F., *et al*., (2014). Prenatal exposure to organophosphate pesticides and neurobehavioral development of neonates: A birth cohort study in Shenyang, China. Plos ONE 9(2):e88491. doi:10.1371/journal.pone.0088491.

Zhang X, Driver JH, Li Y, Ross JH, Krieger RI. (2008) Dialkylphosphates (DAPs) in fruits and vegetables may confound biomonitoring in organophosphorus insecticide exposure and risk assessment. J Agric Food Chem. 2008 Nov 26;56(22):10638-45. doi: 10.1021/jf8018084.

ED_002061_00044426-00089

6.0 Appendices

1. Table of *In Vivo* Developmental Neurotoxicity Studies of OPs.
2. Summary of Guideline DNT Studies Submitted to the Agency for OPs other than Chlorpyrifos.
3. Low Quality Studies: Summary of Study Design Elements Impacting Study Quality Assignment

Appendix 1. Table of *In Vivo* Developmental Neurotoxicity Studies of OPs.

(Effects described are only those measured after weaning. Bold indicates functional domains that were reported to show treatment effects.)

OP	Study	Species & strain	Dose, route, vehicle	Dosing period	ChE inhibition	Domain	Age of testing	Outcomes	NOEL, LOEL	Notes & Study Problems
Chlormephos	Ceh *et al.*, 2012	mouse BALB/c	3.5, 0.35 ug/ml in drinking water of dams ~ 0.6, 0.06 mg/kg/d (@ 5ml/d, 30 g)	7 day premating to weaning	No	**Anxiety & Emotion**	PND70-80	Increased time in closed arms & decreased time in open arms in elevated plus maze, ~0.6 mg/kg/d, M&F	NOEL=0.35 ug/ml in water, ~0.06 mg/kg/d	Litter not unit of statistical analysis No pup allocation described but had to have used some littermates M & F responses appear similar but not statistically compared
Diazinon	Roegge *et al.*, 2008	rat Sprague Dawley	0.5, 2 mg/kg/d sc DMSO	PND1-4	Cites Slotkin *et al.* (2006): 0.5 mg/kg/d produced <10% brain inhibition on PND5, 2 mg/kg/d produced 25-30% brain inhibition 2 hr after dose on PND4, and 10-20% inhibition on PND5	**Anxiety & Emotion**	PND52-56	Decreased time in open arms in elevated plus maze, 2 mg/kg/d, M only	No NOEL LOEL=0.5 mg/kg/d	Pups & dams redistributed daily No effect in F Accepts p<0.1 as significant for interactions No dose-response in feeding or milk preference studies Abstract misstates sex differences
							PND64-67, 78-79	Decreased latency to eat in novelty suppressed but not home-cage feeding, 0.5 & 2 mg/kg/d, M only		
							PND73-74	Decreased chocolate milk preference, 0.5 mg/kg/d only, M only		
							PND86-87	No effect on forced swim test		
Diazinon	Spyker and Avery, 1977	mouse F2 hybrid (NCTR cross bred)	0.18, 9 mg/kg/d in peanut butter	GD1-birth	No	**Sensory**	PND38	Increased errors on visual cliff, 0.18 mg/kg/d only, F only No effect acoustic startle or olfactory responses	No NOEL LOEL=0.18 mg/kg/d	No pup allocation described but had to have used littermates Not clear when both sexes tested and/or compared Statistics not described Maternal weight gain lowered at both doses Weight gain of high dose pups decreased Preweaning testing: decreased contact placing, 0.18 mg/kg/d only No dose-response for some measures Twice as many controls as treated Looks like decreased rotarod endurance PND65, not significant due to high variability
						Neuromotor	PND50, 60, 65,75	No effect swimming ability Increased rod cling endurance, 0.18 & 9 mg/kg/d, sex not specified Decreased inclined plane performance, 0.18 & 9 mg/kg/d, sex not specified		
						Activity	PND75-76	No effect open field		
						Cognition	PND87	No effect errors in Lashley maze		
Diazinon	Timofeeva *et al.*, 2008	rat Sprague Dawley	0.5, 2 mg/kg/d sc DMSO	PND1-4	Cites Slotkin *et al.* (2006): 0.5 mg/kg/d produced <10% brain inhibition on PND5, 2 mg/kg/d produced	Activity	PND28-42	No effect in figure-8 chamber, M&F	No NOEL LOEL=0.5 mg/kg/d	Littermates of those used in Roegge *et al.* 2008 Pups & dams redistributed daily Accepts p<0.1 as significant for interactions
						Sensory	PND77-84	Decreased prepulse inhibition, 0.5 & 2 mg/kg/d, M only		

ED_002061_00044426-00091

					25-30% brain inhibition 2 hr after dose on PND4, and 10-20% inhibition on PND5	**Cognition**	PND28-35, 91-126	No effect on T-maze spontaneous alternation, M&F Increased working memory errors, 0.5 mg/kg/d only, M&F		No mention of sex effects in T-maze No dose-response for some measures
Diazinon	Vatanparast *et al.*, 2013	rat Wistar	1 mg/kg/d sc DMSO	GD15-18 or PND1-4	No	Activity	PND60	Gestational: No effect in open field, M&F Postnatal: No effect in open field, M&F	No NOEL LOEL=1 mg/kg/d	1 M & 1 F per litter but sex not nested within litter in statistics F only affected with gestational exposure, both sexes affected with postnatal, looks like M more affected Large effect sizes Number of dams not mentioned Discrepancy in text on pup sample sizes
						Cognition	PND60-63	Gestational: Decreased latency to cross and increased time spent in dark side on retention trial, no effect acquisition in passive avoidance, F only Postnatal: Decreased latency to cross and increased time spent in dark side on retention trial, no effect acquisition in passive avoidance, M&F		
Diazinon	Win-Shwe *et al.*, 2013	mouse C3H/HeN	0.5, 5 mg/kg/d sc DMSO	PND8-11	No	**Cognition**	PND46-49, 81-84	PND46-49: Decreased novel object exploration and discrimination, 0.5 & 5 mg/kg/d PND81-84: Decreased novel object exploration and discrimination, 5 mg/kg/d only	No NOEL LOEL=0.5 mg/kg/d	M only tested Separate mice at two test times Litter allocation to dose group not described Assumes that ChE inhibition reported by Slotkin in rats would be same as in mice Larger sample size at later age
Dichlorvos	Lazarini *et al.*, 2004	rat Wistar	8 mg/kg po (dilution of technical product); vehicle from formulation	GD6-15	No	**Activity**	PND21, "adult"	PND21: Decreased locomotion open field, M only "Adult": Decreased locomotion and increased immobility, only M tested	No NOEL LOEL=8 mg/kg/d	Data analyzed as litter but sex not nested within litter in statistics No effect on physical and reflex preweaning development Only M tested as after PND21 Adult age not given
						Cognition	"Adult"	Decreased latency to cross on retention trial in passive avoidance		
Fenitrothion (sumithionR, 50% ai)	Lehotzky *et al.*, 1989	rat Lati	5, 10, 15 mg/kg/d po sunflower oil	GD7-15	No	**Neuromotor**	PND26, 36, 104	Decreased latency to fall off rotarod PND26, 104, not 36, 15 mg/kg/d	NOEL=5 mg/kg/d LOEL=10 mg/kg/d	Postnatal mortality at all doses (16-17.5%) No pup allocation described but had to have used some littermates Only M tested Statistics not described Measured startle, righting, contact placing on PND22 but no results given Shorter latency in cognitive task hard to interpret Nonsignificant decrease in activity at PND26
						Activity	PND26, 36, 104	Decreased activity in open field PND104, 15 mg/kg/d		
						Cognition	PND42, 104	Shorter escape latency in conditioned response during acquisition, 10 & 15 mg/kg/d		
						Social behavior	PND62	Increased time in social interaction, 10 & 15 mg/kg/d		

ED_002061_00044426-00092

Methamidophos	deCastro *et al*., 2000	rat Wistar	1 mg/kg/d po water	GD6-15	Pilot in nonpregnant F dosed for 10 d gives 17% plasma inhibition at 1 mg/kg/d	Activity	PND40	No effect in open field, sex no mentioned	NOEL=1 mg/kg/d	Used 2 pups/litter but no mention of sex, apparently used as independent observations No effect on preweaning swimming performance Decreased immobility time PND 14 only Open field measures with really high variability, not reliable
Methamidophos	Lima *et al*., 2013	mouse Swiss	1 mg/kg/d sc DMSO	PND3-9	Pilot showed for 1 mg/kg/d: ~19% brain inhibition on PND10; ~36%, 46% brain inhibition 1, 4 hr after dosing on PND3; ~53, 61% brain inhibition 1, 4 hr after dosing on PND9; no brain inhibition in PND60 adults	Activity	PND61	No effect in open field, sex not mentioned	No NOEL LOEL=1 mg/kg/d	1 M & 1 F per litter No data for M & F separately or mention of statistical differences Dosing by litter High variability especially with passive avoidance
						Anxiety & Emotion	PND60-61	Increased immobility time in forced swim, sex not mentioned No effect on elevated plus maze, sex not mentioned		
						Cognition	PND63	No effect on passive avoidance, sex not mentioned		
Methyl parathion	Crowder *et al*., 1980	rat Sprague Dawley	1 mg/kg/d po corn oil	GD7-15	No	**Activity**	PND23, 30, 44, 54, 65	Increased activity in open field, only PND23 and 54, sex not mentioned to criterion	No NOEL LOEL=1 mg/kg/d	Preweaning testing: possibly decreased wire cling time (not analyzed), no effect on righting, startle, placement response Increased postnatal mortality (30%) Littermates used Only 3 litters used Small sample size for maze testing Statistics not mentioned except for maze transfer test, just used t-test Sex not mentioned except for maze transfer test, data not given for M & F Methods & results cursory
						Cognition	>PND68	Slower transfer on 1st, 4th direction change in T-maze learning transfer, sex not mentioned		
Methyl parathion	Gupta *et al*., 1985	rat Wistar-Furth F mated with F344 M	1 mg/kg/d in peanut butter, 1.5 mg/kg/d po peanut oil	GD6-20	Dams on GD19 show 20, 60% brain inhibition at 1, 1.5 mg/kg/d Pups show brain inhibition up to 50% on PND1, 7, 14, 21, 1 & 1.5 mg/kg/d; on PND28 only 1.5 mg/kg/d	**Cognition**	PND60	No effect on passive avoidance No effect on shuttle box avoidance Slower latency to bar press & increased days to asymptote on operant task (no schedule given), 1 mg/kg/d only, sex not mentioned	No NOEL LOEL=1 mg/kg/d but no effects at 1.5 mg/kg/d	High dose dams had cholinergic signs, increased resorptions Pups moved to foster mothers at 24 hr No effect on preweaning reflexive behaviors Pup allocation not described Statistics barely described M & F apparently tested but data for each not shown or mentioned Methods cursory No dose-response for behavior but there is dose-response for ChE inhibition
						Neuromotor	PND60	No effect on rotarod		
						Activity	PND60	Decreased activity, 1 mg/kg/d only, sex not mentioned		
						Anxiety & Emotion	PND60	Faster cage emergence, 1 mg/kg/d only, sex not mentioned		

						Sensory	PND120	No effect on acoustic startle response		Only 4/dose for operant testing
Methyl parathion	Johnson *et al*., 2009	rat Sprague Dawley	Incrementing doses: low 0.2 mg/kg/d throughout; mid 0.2, 0.4, 0.6 mg/kg/d every 5-6 d; high 0.3, 0.6, 0.9 mg/kg/d every 5-6 d	PND1-21	Low dose showed 13-15% brain inhibition and high dose showed 63, 20, 18% brain inhibition on PND20, 30, 40; all doses recovered by PND50	**Cognition**	PND29-60	Increased working memory errors, mid and high dose, M only Increased reference memory errors, all doses, M only	No NOEL LOEL=0.2 mg/kg/d	Split-litter dose design Included litter as random effect in statistics No effect on preweaning measures of reflex development No effect in F
Oxydemeton methyl (metasystoxR, 91% ai)	Clemens *et al*., 1990	rat CD	0.5, 1.5, 4.5 mg/kg/d water	GD6-15	Dams on GD16 show 30, 54, 72% plasma inhibition (RBC similar) & 22, 52, 68% brain inhibition at 0.5, 1.5, 4.5 mg/kg/d Dams on GD20 show 20, 39, 54% brain inhibition at 0.5, 1.5, 4.5 mg/kg/d, 40% RBC inhibition at 4.5 mg/kg/d, and no plasma inhibition Fetuses on GD20 show no brain inhibition	Cognition	PND25, 26, 35	No effect on M-maze	NOEL=4.5 mg/kg/d	High dose dams had tremors 1 M & 1 F per litter No data for M & F separately or mention of statistical differences Statistics barely described No effect on preweaning reflex or sensory tests
						Activity		No effect in open field		
Parathion	Al-Hachim & Fink, 1968	mouse CF1	3 mg/kg/d po Corn oil	3 dosing times: 1st, 2nd, or 3rd trimester	No	Cognition	PND30-37	No effect on conditioned avoidance learning	NOEL=3 mg/kg/d	Very similar experiment as other papers, maybe same study Pup allocation not clear but littermates probably used Inadequate statistics No mention of sex
Parathion	Al-Hachim & Fink, 1968	mouse CF1	3 mg/kg/d po Corn oil	3 dosing times: 1st, 2nd, or 3rd trimester	No	Activity	PND60-66	No effect in open field	NOEL=3 mg/kg/d	Very similar experiment as other papers, maybe same study Pup allocation not clear but littermates probably used Inadequate stats No mention of sex
Parathion	Levin *et al*., 2010	rat Sprague Dawley	0.1, 0.2 mg/kg/d sc DMSO	PND1-4	Cites Slotkin *et al*. 2006: 0.1 mg/kg/d produced 5-15% brain inhibition on PND5, no data for 0.2 mg/kg/d	**Cognition**	PND420, 510, 570	PND420: Increased working memory errors in radial arm maze, 0.1 mg/kg/d, M only; increased reference memory errors, 0.1 & 0.2 mg/kg/d, M only PND510: Increased working memory errors in radial arm maze, 0.1 & 0.2 mg/kg/d, M only PND570: No effect in radial arm maze	No NOEL LOEL=0.1 mg/kg/d	Littermates of those used in Timofeeva 2008 Pups & dams redistributed daily 5% mortality high dose No effect in F Accepts p<0.1 as significant for interactions No dose-response for several measures

ED_002061_00044426-00094

Parathion	Stamper *et al.*, 1988	rat Long Evans	1.3, 1.9 mg/kg/d sc corn oil	PND5-20	35, 68% brn inh PND21; 26, 36% brn in PND28	Activity	PND24	No effect in open field	No NOEL LOEL=1.3 mg/kg/d	Split-litter dose design Pup allocation not clear but littermates probably used High dose produced cholinergic signs, says doses are 33 and 50% of LD50 in PND5 rat Decreased weight gain with both doses Preweaning, increased cliff avoidance latency, no effect righting, negative geotaxis, open field M only tested No post-hoc comparison of groups when significant, but looks like effects in both doses No dose-response in working memory errors
						Neuromotor	PND24	No effect on rotarod		
						Cognition	PND24, PND 35-37	Decreased alternation rate in T-maze, 1.3 & 1.9 mg/kg/d, only M tested Increased working memory errors, 1.3 & 1.9 mg/kg/d, only M tested		
Parathion	Timofeeva *et al.*, 2008	rat Sprague Dawley	0.1. 0.2 mg/kg/d sc DMSO	PND1-4	Cites Slotkin *et al.* 2006: 0.1 mg/kg/d produced 5-15% brain inhibition on PND5, no data for 0.2 mg/kg/d	Activity	PND58-61	No effect in figure-8 chambers	No NOEL LOEL=0.1 mg/kg/d	Pups & dams redistributed daily 5% mortality high dose Accepts p<0.1 as significant for interactions All radial arm maze effects only in low dose
						Cognition	PND35-45 PND112-182	No effect in T-maze spontaneous alternation Decreased working memory errors in radial arm maze, 0.1 mg/kg/d only, M&F		
						Anxiety & Emotion	PND50-53 PND64-72 PND81-94	Increased time in open arms in elevated plus maze, 0.2 mg/kg/d, M&F No effect on novelty suppressed feeding No effect on chocolate milk preference		
						Sensory	PND78-81	Decreased tactile startle, 0.2 mg/kg/d, M&F No effect on prepulse inhibition		

Appendix 2. Summary of Guideline DNT Studies Submitted to the Agency for OPs other than Chlorpyrifos.

(Only changes that were observed after exposure had ended (post weaning, adult) are listed. 'X' indicates no significant changes on tests for each domain.)

Chemicals	Cognition	Motor activity	Acoustic startle	Neuromotor (FOB)	Notes
Acephate	X	X	X	X	
Azinphos-methyl	X	X	X	X	
Coumaphos	X	X	X	X	

ED_002061_00044426-00095

Diazinon	Biel maze: increase errors & latency high dose (~33.1 mg/g,/d, dam diet) M, PND24 & PND62; also mid dose (~3.4 mg/kg/d, dam diet) F, PND24	X	X	X	
Dichlorvos[1]	--	--	--	--	
Dicrotophos	X	X	X	X	
Dimethoate	X	X	X	X	
Disulfoton	X	X	X	X	
Ethoprop	M maze: increase trials to criterion high dose (~29.3 mg/kg/d, dam diet) M, PND60	X	X	X	
Fenamiphos	X	X	X	X	
Malathion	X	increased rearing in FOB open field mid dose (50 mg/kg/d to dams & pups), F, significant at PND45 only (maybe also PND60); no change automated motor activity	increased peak amplitude later blocks (perhaps habituation effect) all doses (5, 50, 150 mg/kg/d to dams & pups), F only, PND23; increased peak amplitude without prepulse low dose only, F only, PND60	altered gait mid & high dose (50, 150 mg/kg/d to dams & pups), M & F, PND60 but not earlier	
Methamidophos	~X	X	decreased peak amplitude early blocks mid & high dose (~1.65, 5.2 mg/kg/d dam diet), F only, significant at PND38, looks same but not significant at PND60	X	PA PND24: report says decreased latency but nothing significant & table is questionable; M maze PND60: report says increased trials to criterion (M) and increased (M) or decreased (F) errors, but nothing significant and table is questionable
Methyl parathion	X	X	X	X	
Naled	X	X	increased peak amplitude & decreased latency middle blocks low dose only (0.4 mg/kg/d to dams & pups), F only, PND60; report says decreased amplitude but not significant all blocks high dose (10 mg/kg/d to dams & pups), M only, PND23 & PND60	X	only swimming time in Y maze reported, varied significances, no mention of errors or other performance measures

ED_002061_00044426-00096

Phorate	M maze: decrease number reaching criterion with relearning, low & mid dose (0.03 & 0.1 mg/kg/d to dams & pups), M only significant, PND30; not seen in second study with higher dose	X	decreased peak amplitude all blocks high dose (0.1 mg/kg/d to dams & pups), M only, PND60; not seen in second study with higher dose	X	combined two studies; one with low & mid dose, other with high dose; some data didn't agree
Profenofos	X	X	X	X	
Terbufos	X	X	X	X	
Tetrachlorvinphos	X	X	X	X	
Tribufos	X	X	X	X	
Trichlorfon	PA: decreased latency to enter on retention high dose (~205.1 mg/kg/d dam diet), M only, PND29; M maze: increased average errors second trial high dose, F only, PND60	decreased activity middle blocks (maybefaster habituation) mid dose (~76.2 mg/kg/d dam diet) only, F only, PND60	decreased peak amplitude all blocks high dose (~205.1 mg/kg/d dam diet), early blocks mid dose (~76.2 mg/kg/d dam diet), M & F, PND22; decreased amplitude middle blocks (not consistent) all doses (~23.3, 76.2, 205.1 mg/kg/d dam diet), F only, PND38; for M decreased amplitude apparent but not significant high dose PND38 & PND60	X	

[1] high pup mortality in all groups, including control, negated any valid neurotoxicity assessments of dichlorvos

Appendix 3. Low Quality Studies: Summary of Study Design Elements Impacting Study Quality Assignment

Study	Study Location	Cohort Name / Description of Study Population	Study Design	Exposure Assessment	Outcome Assessment	Confounder / Covariate Control	Statistical Analysis	Risk of (other) Bias
Acosta-Maldonado *et al*. (2009)	Chihuahua, Mexico	Singleton pregnancies	Cross-sectional, small pilot study – only 9 women exposed, participant selection and exclusion not detailed N=54 mothers (9 exposed, 45 comparison mothers)	Proxy indicator of exposure - Residence in agricultural community were pesticides had been applied; or home located < 5 km from a pesticide application zone; or cohabitating with worker exposed to pesticides or agricultural labor Also, AChE activity – Objective biomarker of exposure/altered function	Standardized but partially subjective assessment of placental maturity	Minimal. Adjustment for placental characteristics.	Appropriate multivariate analysis; Corrected hypothesis test results for multiple comparisons.	Selection bias unlikely; Residual confounding likely; substantial potential for differential misclassification of exposure.
Dawbrowski *et al*. (2003)	Lodz, Poland	Newborn children among Polish farmers	Case-control, large sample size N=389 Age: newborns	Prenatal pesticide exposure assessed via questionnaire (retrospective self-report). Site visit *after* delivery to evaluate pesticide exposure	Pregnancy outcomes assessed using birth records	Appropriate. Included maternal demographics, predictors of high-risk pregnancy (duration, maternal weight) and environmental toxicant exposure (ETS). No adjustment for SES indicators	Appropriate multivariate analysis.	Selection bias unlikely; Residual confounding likely small in magnitude; considerable potential for non-differential misclassification of exposure (unlikely to account for non-null findings). Minimal misclassification of outcome.
Grandjean *et al*.(2006)	Tabacundo, Ecuador	Healthy 2nd and 3rd grade children	Cross-sectional, small sample size N=72 Age <9 years	Prenatal occupational exposure assessed via questionnaire; Also recent child exposure biomarker assessment (DAP).	Objective anthropometric and other clinical outcomes; Numerous neurobehavioral outcomes evaluated using easy-to-administer screening instruments; Age appropriate. May be insensitive to subtle effects of OP pesticide exposure effects.	Appropriate. Homogenous population limited confounding by design; Included child demographics (age, sex, weight); SES indicators (maternal race, housing, running water, sewage), diet (meals/day), environmental toxicants (maternal alcohol and smoking) and medical history.	Appropriate multivariate analysis; Numerous hypotheses evaluated without correction for multiple comparisons.	Selection bias unlikely; Residual confounding likely small in magnitude; considerable potential for non-differential misclassification of exposure (likely to account for null findings). Potential misclassification of outcome.

ED_002061_00044426-00098

Appendix 3. Low Quality Studies: Summary of Study Design Elements Impacting Study Quality Assignment (continued)

Study	Study Location	Cohort Name / Description of Study Population	Study Design	Exposure Assessment	Outcome Assessment	Confounder / Covariate Control	Statistical Analysis	Risk of (other) Bias
Harari *et al.* (2010)	Tabacundo, Ecuador (Andean plateau north of Quito)	6- to 8-year-old children in the two lowest grades (called second and third) of one of two schools	Cross-sectional, small sample size n = 81 Age 6- 8 years	Child Current Exposure - DMP metabolites measured in a spot urine samples. A blood sample was analyzed for AChE. Maternal Exposure – Interview by skilled interviewers	Blood pressure and neurophysiologic measures - the instruments used for neurophsychologic measures were validated to avoid cross-cultural influences.	Appropriate: child's sex, age, BMI, number of daily meals (only in current exposure), stunting, hematocrit, school grade, having repeated one grade, maternal education level, family living in a traditional house, drinking water supply, and paternal education and employment	Appropriate: Standard parametric tests and logistic regression	Errors in exposure classification status since it was based on the maternal self-report, mothers likely being aware of the neurobehavioral status of their children, exposure assessment based on a spot urine sample
Kofman *et al.* (2006)	Israel (Negev region)	Bedouin population (Children aged 6 to 12 at the time of the study, who were victims of poisoning before age three)	Retrospective cohort study, small sample size N = 52 9-Exposed to OP; 17-Exposed to Kerosene/paint thinner 26-Controls Aged 6-12 years	OP poisoning was confirmed by low serum butyrylcholinesterase activity based on hospital records	Neuropsychological evaluation and structured interview of parents. Errors in assessment minimized as psychologists were qualified individuals, language and cultural differences taken into consideration, each child tested on same day and in same place as matched control.	Age, sex, background (cultural and demographic)	Difference in means	Errors is outcome classification likely since psychologists who administered the tests knew which children were exposed, small sample size
Koutroulakis *et al.* (2014)	Crete, Greece	Women with singleton pregnancies, permanent residents for at least two years, referral for amniocentesis to the Fetal-Maternal Unit, Department of Obstetrics and Gynecology, University Hospital of Heraklion	Prospective Cohort Study – large sample size, ethical issues n = 415 Age: newborns	Objective. DAP measurement in a single amniotic fluid samples collected at either 16th or 20th weeks of gestation – Novel biomarker. Questionnaire was also used.	Birth weight and head circumference. Unclear how the outcome information was obtained.	Neonatal sex, maternal age, agricultural activities, and gestational age at amniocentesis – Rationale for confounder selection not provided	Appropriate: multiple linear regression	Comparing exposure measurements in AF against known biomarker (e.g., OP metabolite levels in urine) for validation of AF not conducted. Smoking status of participants before/during pregnancy, high risk pregnancies, gestational diabetes, PON1 enzyme activity in the fetuses not considered.

Appendix 3. Low Quality Studies: Summary of Study Design Elements Impacting Study Quality Assignment (continued)

Study	Study Location	Cohort Name / Description of Study Population	Study Design	Exposure Assessment	Outcome Assessment	Confounder / Covariate Control	Statistical Analysis	Risk of (other) Bias
Lizardi *et al*. (2008)	Yuma County, Arizona, USA	Children's Pesticide Survey (CPS)	Cross-sectional, small sample size N=48 Age 7 years	Objective Biomarker of prenatal OP pesticide exposure (DAP) quantified in single child spot urine sample provided at the time of the cognitive assessment.	Cognitive assessment using battery of test instruments considered valid and reliable in similar populations. Spanish translation as necessary.	None	Correlation coefficients (type unspecified). Statistically significant confounders were not robust due to influential outliers	Selection bias unlikely; Residual confounding likely; considerable potential for non-differential misclassification of exposure Potential misclassification of outcome.
Lu *et al*. (2009)	Cota Brus, Costa Rica	4-10 yr. old children whose parents worked in organic coffee farm (La Amistad) and conventional coffee farms (Las Mellizas)	Cross-Sectional (pilot), low sample numbers N=35: 17 Organic farm 18 Conventional farms Age 4-10 years old	Good measure (urinary PNP, IMPY, TCPy), but no major differences between exposure groups	Good measure (CBARS) but different SES and demographic characteristics for exposure groups	Limited number (group, age, sex, handedness, grade)	Appropriate: Linear mixed effects for significant test outcomes from paired t-test analyses	Somewhat high - Convenience sample with different recruitment methods; exposure misclassification
Moreno-Banda *et al*. (2009)	Mexico (Villa Guerrero, Coatepec de Harinas, Tenancingo (Mexico); Cuernavaca, Cuautla, Jiutepec, Temixco, (Morelos)	Newborn children of floricultural workers and families	Cross-sectional, large sample size N=328 Age: newborns	Proxy indicator of prenatal occupational OP pesticide exposure (self-reported floricultural occupation)	Objectively measured birth outcomes assessed using birth certificate (partial). Self-reported by mother if birth certificate unavailable.	Appropriate, though minimal – history of adverse reproductive outcomes, infant sex, maternal smoking and alcohol use during pregnancy.	Appropriate multivariate analysis	Selection bias likely; Residual confounding likely small in magnitude; considerable potential for non-differential misclassification of exposure (likely accounts for null findings). Potential outcome misclassification (self-report in subset of participants).
Nevison (2014)	U.S. National population	Children with birth years 1970–2005 in 1) California Department of Developmental Services (CDDS) reports 2) Individuals with Disabilities Education Act (IDEA) reports	Ecological (time trend) N=NP (national database)	Non-specific, proxy OP exposure measure (lbs/yr)	Two large reporting DBs: CA Department of Developmental Services (CDDS), US Individuals with Disabilities Education Act (IDEA)	Limited: differences in autism definitions, changes in diagnostic criteria	Appropriate: ratio of age-resolved snapshot; tracking trend slopes; correlation coefficient between temporal trend and composite autism prevalence curve	Selection bias unlikely; do not know individual exposure

ED_002061_00044426-00100

Appendix 3. Low Quality Studies: Summary of Study Design Elements Impacting Study Quality Assignment (continued)

Study	Study Location	Cohort Name / Description of Study Population	Study Design	Exposure Assessment	Outcome Assessment	Confounder / Covariate Control	Statistical Analysis	Risk of (other) Bias
Rohlman *et al*. (2005)	Oregon and North Carolina, USA	Latino children of immigrant parents living in Oregon or North Carolina	Cross-Sectional, small sample size N= 78 Age 48-71 months	Proxy indicator of chronic OP pesticide exposure - Residence in highly agricultural communities	Battery of neurocognitive development; Screening tools; Some instruments likely not appropriate for use in study population - Not all participants able to complete all evaluations. Poor administration. Tests administered twice; only performance on 2nd evaluation considered in analysis.	Self-reported covariate information collected via questionnaire. Adjustment for age, SES indicator (maternal education). No environmental toxicants.	One-sided hypothesis tests. Numerous hypotheses evaluated without correction for multiple comparisons.	Selection bias unlikely; Residual confounding likely; substantial potential for differential misclassification of exposure and outcome. Inadequate presentation of study results. Seemingly post-hoc evaluation of effect modification.
Samarawickrema *et al*. (2008)	Southern Sri Lanka	Pregnant women delivering at Embilipitiya Base Hospital	Cross-sectional Birth Cohort, small sample size N=41 end of two pesticide spraying seasons; N=25 at beginning of spraying season	Proxy indicator of prenatal OP pesticide exposure (delivery during pesticide spray season); Objective pesticide biomarkers assessed (OP pesticide residues), but detected in only two subject's specimens – not evaluated	Objective biomarkers of early biological effect outcomes - Maternal and fetal butyrylcholinesterase (BuChE) activity; antioxidant status; fetal oxidative stress; fetal DNA fragmentation	No adjustment for potential confounders (though comparison groups were considered to be relatively homogeneous).	Largely appropriate. Assumptions of some statistical tests likely violated.	Selection bias unlikely; Residual confounding likely; substantial potential for differential misclassification of exposure; Seemingly post-hoc evaluation of effect modification.
Savitz *et al*. (1997)	Ontario, Canada	Newborn children in the Ontario Farm Family Health Study;	Retrospective Birth Cohort, large sample size N= 1,898 couples; 3,984 Age: newborns	Proxy indicator of pre-conception paternal para-occupational OP pesticide exposure (self-reported male farm activities in 3-month period prior to conception.	Objectively measured birth outcomes assessed by maternal self-report	Appropriate. Included family/child demographics (sex, weight, maternal age, ethnicity); SES indicators (maternal and paternal education and occupation, per capita income) race, housing, running water, sewage), diet (meals/day), pregnancy risks (maternal caffeine, alcohol and smoking) and medical history.	Inappropriate multivariate regression analysis. Likely misspecification of true variance; No adjustment for multiple comparisons.	Selection bias probable; Residual confounding likely; substantial potential for differential misclassification of exposure and outcome.
Wickerham *et al*. (2012)	Zhejiang Province, China	Newborn children delivered at the Fuyang Maternal and Children's hospital	Cross-sectional, small pilot study n=116 Age: Full term infants	Objective biomarker of pesticide exposure (pesticide residues in cord blood) – parameterized as number of pesticide residues detected. Methods unlikely suitable for detecting low levels.	Birth weight assessed using birth records and maternal report	Appropriate. Assessed using questionnaire (maternal self-report) and medical records.	Appropriate multivariate analysis.	Selection bias unlikely; Residual confounding likely; substantial potential for differential misclassification of exposure. Some outcome misclassification unlikely .
Naksen et al (2015)	Fang district, Chiang Mai province, Thailand	Pregnant women delivering at Fang Hospital	Prospective Cohort, small pilot study n=52 Age: newborns	Objective biomarkers of pesticide exposure (DAPs). Also AChE, BChE, and PON1 genotype expression measurement. Maternal blood and urinary samples taken, plus cord blood. Questionnaire to assess other exposures and covariates.	Birth outcomes (Body weight and length, and head circumference) abstracted from medical records.	Appropriate. Assessed using questionnaire (maternal self-report) and medical records.	Some errors in statistical analysis were identified; e.g., for gestation age, log total DEAP at 32 weeks of pregnancy, a 0.7 beta was reported, but the confidence interval is reported as (-0.1, -1.4)]. No adjustment for multiple comparisons.	Inadequate presentation of study results. Selection bias possible due to loss to follow-up; Residual confounding likely, small in magnitude; potential for non-differential misclassification of exposure.

ED_002061_00044426-00101

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

APR 15 2011

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

Wendelyn Jones, Ph.D.
Senior Director, Human Health Policy
CropLife America
1156 15th St. N.W.
Washington, DC 20005

Re: Petition For Rulemaking To Establish Criteria For Acceptance Of Epidemiological Evidence Into the Pesticide Risk Assessment Process For Human Health Effects

Dear Dr. Jones:

In a letter dated December 28, 2010, you transmitted to EPA a petition from CropLife America (CLA) requesting that EPA promulgate a regulation establishing criteria for evaluating whether epidemiological evidence will be accepted for use in pesticide risk assessments. For the reasons detailed below, CLA's request is denied.

EPA is in the process of preparing guidelines regarding use of epidemiological data in pesticide risk assessments. On January 7, 2010, EPA released a draft guideline on this matter entitled "Draft Framework for Incorporating Human Epidemiologic & Incident Data in Health Risk Assessment" ("Draft Framework"). This document proposes "a framework to describe the scientific considerations that EPA will weigh in evaluating how such studies and scientific information can be integrated into risk assessments of pesticide chemicals."[1] In February 2010, EPA sought review of the Draft Framework from the Scientific Advisory Panel (SAP) created under the Federal Insecticide, Fungicide, and Rodenticide Act, 7 U.S.C. 136 et seq. The SAP issued a report on the Draft Framework on April 22, 2010.[2] SAP review is a public process. CLA as well as other representatives of the pesticide industry filed written comments on that review and also appeared before the Panel to present oral remarks. EPA is currently reviewing the SAP's report on the Draft Framework and plans to release a revised version of the Framework for public comment this year.

---

[1] Draft Framework at 6.

[2] See Memorandum, Myrta R. Christian, Designated Federal Official, FIFRA Scientific Advisory Panel, to Steven Bradbury, Acting Director, Office of Pesticide Programs, Transmittal of Meeting Minutes of the FIFRA Scientific Advisory Panel Meeting on the Draft Framework and Case Studies on Atrazine, Human Incidents, and the Agricultural Health Study: Incorporation of Epidemiology and Human Incident Data into Human Health Risk Assessment (April 22, 2010) (hereinafter cited as "SAP Meeting Minutes").

ED_002061_00044427-00001

In its petition, CLA argues that a guidance document on the use of epidemiological data in pesticide risk assessment would be inadequate. Instead, CLA requests that EPA establish by rule "firm criteria for quality assessment of epidemiological studies to be used in risk assessment" and not use any epidemiological studies in risk assessments prior to promulgation of that rule. CLA claims that a rule is necessary to address this issue because epidemiological data are "important" to pesticide risk assessment, the transparency of the rulemaking process is needed to produce defensible criteria on the acceptability of epidemiological studies, and criteria designated by a guidance document would be a "de facto rule" and thus invalid.

EPA's general practice is to address science issues through non-binding guidance documents rather than by mandatory regulations. There are several reasons for this approach. First, and probably most important, science questions usually cannot be reduced to a rigid decisional framework; rather, science questions invariably involve the weighing of multiple considerations and the use of scientific judgment. As the SAP report on EPA's Draft Framework noted in its recommendations on criteria to be used in EPA decision-making: "Inevitably, it will be necessary to exercise some degree of scientific judgment in this assessment."[3] Second, encasing science decision-making in a rigid rule structure is inconsistent with the fluid and developing nature of science. Thus, EPA is concerned that writing science decision-making rules will stultify or freeze the science underlying the rule making scientific advances less likely. Finally, the nature of science issues is not easily compatible with the timeframes associated with formal rulemaking. Given the extended time often required to promulgate or amend a rule, the science underlying science-based criteria may well have significantly advanced between the time of the proposal and the time of the final rule. EPA may then be forced into restarting the rulemaking process or may end up being locked into outdated science decision-making until a rule can be amended. There are numerous examples of EPA appropriately addressing important science questions through guidance, not rules, at both the Agency level and the program-specific (pesticide) level.[4]

CLA has offered no compelling reason to follow a different course with regard to epidemiological data. Epidemiological data are no more "important" to pesticide risk assessments than many other data inputs or science-related issues. Yet, as explained above, EPA invariably addresses pesticide risk assessment issues through guidance documents. For example, the Office of Pesticide Programs has issued almost two dozen science guidance documents on

---

[3] SAP Meeting Minutes at 9.

[4]. See, e.g., U.S. EPA, Framework for Metals Risk Assessment, (March 2007) (EPA 120/R-07/001); U.S. EPA, Guidance on Selecting Age Groups for Monitoring and Assessing Childhood Exposures to Environmental Contaminants (November 2005) (EPA/630/P-03/003F); U.S. EPA, Guidelines for Carcinogen Risk Assessment (March 2005) (EPA/630/P-03/001F); Office of Pesticide Programs, U.S. EPA, Office of Pesticide Programs' Policy on the Determination of the Appropriate FQPA Safety Factor(s) For Use in the Tolerance Setting Process (February 28, 2002); Office of Pesticide Programs, U.S. EPA, The Use of Data on Cholinesterase Inhibition for Risk Assessments of Organophosphorous and Carbamate Pesticides (August 18, 2000).

issues critical to pesticide risk assessment in the wake of the passage of the Food Quality Protection Act of 1996.[5] Further, as evidenced by the process followed to date with EPA's Draft Framework, there are many ways to insure a transparent process for science decision-making guidelines other than through rulemaking. Finally, there is nothing unique about evaluating epidemiological data that would indicate that EPA could not craft non-binding guidelines for incorporating epidemiological data in risk assessments, including non-binding guidance on specific criteria to be considered in weighing the value of particular epidemiological data.

EPA agrees that transparency is a critical part of its science decision making. Our decisions on important policies and guidance documents always follow a transparent process with numerous opportunities for public comment. Such a process was followed in the review of the Draft Framework by the SAP and will be followed as we further revise the guidance. EPA welcomes CLA's interest in its Draft Framework. As noted above, EPA plans to hold further public dialogue on the issues presented by the Draft Framework as it moves forward.

Sincerely,

Steven P. Bradbury, Ph.D., Director
Office of Pesticide Programs

[5] See U.S. EPA, Pesticides: Science and Policy, Science Policy Issues and Guidance Documents, available at http://www.epa.gov/oppfead1/trac/science/

PETITION FOR RULEMAKING TO ESTABLISH CRITERIA FOR ACCEPTANCE OF EPIDEMIOLOGICAL EVIDENCE INTO THE PESTICIDE RISK ASSESSMENT PROCESS FOR HUMAN HEALTH EFFECTS

CropLife America hereby petitions the United States Environmental Protection Agency ("EPA") to promulgate a rule establishing clear and scientifically-sound criteria for selection of epidemiological studies to be incorporated into the Office of Pesticide Programs ("OPP") risk assessment for a given pesticide product.

CropLife America is the national trade association for the plant science industry. Its member companies develop, produce, sell, and distribute virtually all of the agricultural crop protection technology products used by American farmers to provide consumers with safe, affordable, and abundant food and fiber.

I. Introduction

No pesticide product can be distributed or sold for use in the United States unless it has been registered by EPA under the Federal Insecticide, Fungicide and Rodenticide Act ("FIFRA"), 7 U.S.C. § 136 *et seq*. Through FIFRA, OPP receives extensive hazard and exposure information that is used to characterize the risks of pesticide products.

EPA at present uses a risk assessment process to evaluate the potential health and ecological effects of a pesticide to determine whether the product meets FIFRA's registration standard of no unreasonable adverse effects on human health or the environment. EPA must approve the use and registration of a new pesticide before it can enter the market. Existing pesticides must be re-evaluated periodically to ensure that they continue to meet the appropriate safety standard.

The decision process is part of a risk management process, which is conducted in registration for new pesticide chemicals or new uses of existing chemicals, or reregistration or registration review in the case of a general review of an existing chemical.

OPP's human risk assessment traditionally relies on toxicological studies using laboratory animals along with data to estimate the potential exposure based on the proposed use of the pesticide product. The process has not uniformly or consistently incorporated epidemiological studies of adverse effects in humans into the quantitative risk assessment process. Instead, OPP to date has sometimes utilized epidemiological evidence to support human risk assessments or generate new hypotheses about potential risk. OPP now seeks to change this process and incorporate epidemiological evidence directly into its pesticide risk assessment process through a proposed framework which utilizes a weight of evidence approach.

In January 2010, OPP published a *Draft Framework for Incorporating Human Epidemiologic & Incident Data in Health Risk Assessment* (the "Draft Framework"). The Draft Framework declares that "OPP intends to employ… epidemiology studies and human health incident data in its human health risk assessment" and that its "goal is to use such information in the most scientifically robust and transparent way." Draft Framework at 6. OPP based its decision to incorporate epidemiology into the risk assessment process on two reports issued by the National Research Council for the National Academy of Sciences, *Toxicity Testing in the 21st Century: A Vision and a Strategy* (2007) and *Science and*

ED_002061_00044428-00002

Decisions (2009). OPP states that these reports call for a "bold, new approach" and "advocate far reaching changes in how toxicity testing is performed, how such data are interpreted, and ultimately how regulatory decisions are made." Draft Framework at 6. This "new vision" involves incorporating data from new sources, specifically information found in epidemiology studies, human incident databases, and biomonitoring studies.

In the Draft Framework, OPP sets forth a general plan for incorporating epidemiology studies into its risk assessment process and for weighing that evidence alongside traditional mechanistic and toxicological evidence in a weight of the evidence analysis. The framework describes the major types of epidemiological studies, noting the strengths and limitations of each in terms of their applicability to the risk assessment process. OPP's framework is premised on a proposed weight of the evidence evaluation that uses the Bradford Hill Criteria as modified by a Mode of Action Human Relevance Framework as tools for organizing and evaluating diverse types of data to determine the evidence available on the potential human health consequences of pesticide exposure. In this sense, the proposed Framework attempts to explain *how* OPP will incorporate a given epidemiological study into a risk assessment. But OPP has not set forth any criteria for selecting the studies to be incorporated, or for evaluating the quality and validity of a particular epidemiological study to determine whether that study should be used in an EPA risk assessment in the first place. Toxicological and exposure studies, in contrast, generally must meet strict design and "good laboratory practice" quality criteria and disclose all analyses for

ED_002061_00044428-00003

consideration during registration or registration review processes. *See e.g.*, 40 C.F.R. §§ 152.50, 158.80, and Part 160.

The FIFRA Scientific Advisory Panel ("SAP") reviewed the Draft Framework at a meeting held in February 2010. While the SAP praised OPP for its use of the Bradford Hill criteria as the basis for *how* to incorporate epidemiology in a weight of the evidence analysis, the SAP strongly recommended that OPP establish a stringent set of quality-based criteria to determine *whether* to accept a given epidemiological study for use in risk assessment:

> An important issue is how the Agency decides whether to use particular sets of data. In the interests of transparency the Panel recommends that the Agency establish a set of criteria for determining the acceptability of epidemiologic studies. These criteria may be based on quantitative criteria, scientific judgment, or a combination of these. Inevitably, it will be necessary to exercise some degree of scientific judgment in this assessment. The Panel recommends that epidemiologists participate actively in the process.

FIFRA Scientific Advisory Panel Meeting Minutes No. 2010-03 at 10 (Feb. 2-4, 2010) ("SAP Minutes").

Epidemiological studies must be vetted through a credible process. To ensure that data from studies utilized in these risk assessments is accurate, reliable and unbiased, the process for vetting these studies must be transparent—and the formulation of that process requires public input. Not only are these steps required, but following them will help ensure the defensibility of future risk assessment decisions.

CropLife America urges OPP to (1) establish firm criteria for quality assessment of epidemiological studies to be used in risk assessment, in addition to

ED_002061_00044428-00004

procedures regulating the interpretation and use of selected studies, and (2) to do so through formal rulemaking. Formal rulemaking will permit the scientific and agricultural community a robust opportunity to comment on the proposal. Only through the evaluation of comments submitted by the scientific community at large can OPP ensure that it is meeting its goal to use epidemiological evidence only "in the most scientifically robust and transparent way."

II. Authority

This petition is filed under the Administrative Procedure Act, 5 U.S.C. § 553(e) ("Each agency shall give an interested person the right to petition for the issuance, amendment, or repeal of a rule."); the Federal Insecticide, Fungicide, and Rodenticide Act, FIFRA § 25, 7 U.S.C. § 136w ("The Administrator is authorized… to prescribe regulations to carry out the provisions of this subchapter."); and the United States Constitution, U.S. Const., amend. 1 ("Congress shall make no law… abridging… the right of the people… to petition the Government for a redress of grievances.").

III. Why a Rule is Necessary

A. Importance of Epidemiology Data in Risk Assessment

Data from properly conducted, high quality epidemiology studies may contain information that is useful in characterizing and evaluating human health risks. But not all epidemiological evidence is created equal. Bias, confounding factors, and in particular, unreliable and invalidated exposure assessments commonly occur in epidemiological studies, limiting the value of the researchers'

ED_002061_00044428-00005

conclusions for quantitative risk assessments. In addition, a study's probative value varies dramatically depending on design and approach – observational studies, for instance, such as case series or ecological "cluster" analyses, do not carry the strength of association assessment that prospective cohort and case control studies do and are often utilized (if at all) only for hypothesis generating purposes. *See* SAP Minutes at 20-21. "Weight of evidence," in this context, requires more than mere consideration of "all" forms of epidemiological studies. The weight of evidence approach considers all relevant information in an integrative assessment that takes into account the kinds of evidence available, the quality and quantity of the evidence, the strengths and limitations associated with each type of evidence and explains how the various types of evidence fit together. *See* U.S. Environmental Protection Agency, A Summary of General Assessment Factors for Evaluating the Quality of Scientific and Technical Information, EPA 100/B-03/001 June 2003. Epidemiology studies used in risk assessments should report all analyses, negative or positive and provide all underlying data.

While OPP has stated that epidemiological evidence, generically speaking, may provide important informative data for the risk assessment process, this determination does not mean that each and every published epidemiological study will provide equally important, valid or useful information. Like all information considered in a risk assessment, the quality and validity of the information provided by epidemiological studies needs to be closely scrutinized. EPA must establish clear, logical, enforceable, and scientifically-grounded principles for the quality assessment and selection of epidemiological studies to be used in human

ED_002061_00044428-00006

health risk assessment which will also comport with its obligations under the Information Quality Act guidelines.

B. EPA Must Ensure the Quality of Data in OPP Risk Assessments

The quality of scientific information forming the foundation for registration and permitting decisions is essential to all EPA risk assessments. For example, in 2002 the published the Guidelines for Ensuring and Maximizing the Quality, Objectivity, Utility, and Integrity of Information Disseminated by the Environmental Protection Agency, EPA/260R-02-008 (the Guidelines). EPA issued these Guidelines to formalize and maximize the quality of disseminated information, particularly with respect to the objectivity, utility, and integrity of scientific data. Under the Guidelines, information disseminated by EPA must be "presented in an accurate, clear, complete, and unbiased manner" with substance that "is accurate, reliable, and unbiased." *Id.* at 15. Objectivity of influential scientific information is judged against the quality principles in the Safe Drinking Water Act Amendments (SDWA) of 1996 to ensure the use of (i) the <u>best available science</u> and supporting studies conducted in accordance with sound and objective scientific practices, including, when available, peer reviewed science and supporting studies; and (ii) <u>data collected by accepted methods or best available methods</u> (if the reliability of the method and the nature of the decision justifies the use of the data). *Id.*at 22 (emphasis added).

Moreover, "influential" information, which is information that will have a clear and substantial impact on important public policies or private sector

ED_002061_00044428-00007

decisions, must "adhere to a rigorous standard of quality" and "should be subject to a higher degree of quality." *Id.* at 20. As noted in the Guidelines, information that can "adversely affect in a material way the economy, productivity, competition, jobs" or that addresses "precedent-setting or controversial scientific or economic issues" is considered influential. Because the overall economic impact on a currently registered pesticide of an OPP reassessment could be substantial, scientific data used to make those decisions would be influential. Unquestionably, epidemiological studies used in these risk assessments that impact registrations thus qualify as "influential" data subject to heightened quality standards under the Guidelines.

C. Transparency in the Selection of Studies is Essential

The SAP frames their recommendation to establish criteria with the phrase "in the interests of transparency" to ensure the appropriate application of epidemiological data to risk assessment. Without transparency in the process for selecting whether and which epidemiological studies are relied upon, the scientific basis of OPP risk assessments, and the resulting registration decisions, will be suspect. The importance of transparency is essential and cannot be overemphasized; testimony to which is shown by (1) the Agency's transparency criteria in their Risk Characterization Handbook, (2) President Obama's Memorandum on Transparency and Open Government and (3) Administrator Jackson's testimony before the U.S. Senate on scientific integrity

1. EPA's Risk Characterization Handbook

ED_002061_00044428-00008

EPA's Risk Characterization Handbook states that "risk characterization is therefore judged by the extent to which it achieves the principles of transparency, clarity, consistency, and reasonableness (TCCR)...Transparency is the principal value from among the four TCCR values, because, when followed, it leads to clarity, consistency, and reasonableness." *See* U.S. Environmental Protection Agency, Risk Characterization Handbook, EPA 100-B-00-002; December, 2000.

2. President Obama's Memorandum on Transparency and Open Government

This petition is fully within the spirit and intent of President Obama's memo on transparency and open government: "My administration is committed to creating an unprecedented level of openness in Government. We will work together to ensure the public trust and establish a system of transparency, public participation, and collaboration. Openness will strengthen our democracy and promote efficiency and effectiveness in Government." *See* Memorandum For The Heads of Executive Departments And Agencies on Transparency and Open Government, President Barack Obama, January 21, 2009.

3. Administrator Jackson's Testimony before the U.S. Senate Committee on Environment and Public Works

In testimony before the U.S. Senate Committee on Environment and Public Works, Administrator Jackson stated "The President's Memorandum stresses that 'scientific information ... developed and used by the Federal government should ... ordinarily be made available to the public' and that, where permitted by law, 'there should be transparency in the preparation, identification

ED_002061_00044428-00009

and use of scientific and technological information in policymaking.' Consistent with this principle and my commitment to transparency, I believe that the methodologies and guidelines that EPA uses for scientific analyses should be shared fully with the public. EPA's regulatory decisions should include a full explanation of the science issues addressed by the Agency, the data relevant to those issues, and the interpretations and judgments underlying the Agency's scientific findings and conclusions." *See* Hearing on Scientific Integrity, U.S. Senate Committee on Environment and Public Works, June 9, 2009

D. Importance of Establishing Criteria

A well-designed, robust epidemiological cohort or case control study has certain features, that OPP should look for before admitting a study into a risk assessment. These include, but are not limited to:

- well-characterized, quantitative exposure assessments that minimize measurement error and decrease the likelihood of inaccurate or biased information;
- a well-defined study population that includes persons with a wide range of exposures as well as unexposed persons;
- documented efforts to control for selection bias, information bias and confounding; and
- explicit, well-defined criteria for ascertainment of outcomes.

The SAP recommended a number of specific questions to be asked when evaluating each particular epidemiological study, for potential use in an OPP risk assessment. *See* SAP Minutes at 16-17:

ED_002061_00044428-00010

1. Was the epidemiological study conducted primarily in a hypothesis generating or a hypothesis testing mode? Studies with no specific a priori hypothesis are more likely to generate false positive results (Swaen 2001).

2. Was the method of assessing exposure reliable and adequate?

3. Were inclusion and exclusion criteria clearly stated and reasonable to provide a representative sample with regard to exposure and health outcome so as to provide a relatively unbiased and representative estimate of effect?

4. Was the method of assessing the criteria for determining health outcome clearly stated and valid and reliable; *e.g.* confirmed with histopathology; and were they designed to detect newly diagnosed (rather than prevalent) cases so that it was reasonably possible to determine that exposure preceded disease?

5. Was appropriate information on potentially confounding factors, such as socio-demographic, behavioral and dietary factors collected for both exposed and unexposed groups or for cases and controls in the same way, and were they appropriately controlled in the analyses of the data? Were data on co-morbid conditions collected? (*i.e.* factors that are associated with the health condition of interest as well as factors associated with exposure)

6. Did the study sample the population or individuals of interest? (*i.e.* was selection bias minimized and generalizability optimized?) How does the study population relate to the universe of potentially exposed populations?

7. Did the study examine individuals with a wide range of exposures? (*i.e.* ability to detect a dose-response and to generalize to other populations) Did the study include unexposed populations or individuals?

ED_002061_00044428-00011

8. Did the exposures examined in the study relate to past or current situations? (*i.e.* acute versus chronic exposures and the target health end points)

9. Did the study have adequate statistical power to detect meaningful differences for outcomes between the different groups of exposed and unexposed or less exposed individuals while controlling for important confounding factors? Does the sample size take into account the expected incidence of the target health effect in the study populations? Was the study powerful enough to detect statistically meaningful differences while adjusting for confounding variables and exposure measurement error that typically reduce statistical power?

See SAP Minutes at 16-17.

Other criteria could be added to this list, and would likely be the subject of additional comment during a rulemaking proceeding open to the participation and input of other interested entities. Criteria for assessing these factors, and any other relevant factors, should be explicitly set forth in a manner that can be applied to all pesticide chemicals under evaluation. While quantitative criteria are a critical first step in separating reliable epidemiological studies from less reliable studies, an element of scientific judgment is required to make a complete assessment. To ensure a well-informed process, OPP must utilize the expertise of qualified epidemiologists to review the body of epidemiology evidence for a given pesticide to determine whether each study satisfies the quality criteria set forth in the proposed rule.

E. Creating a Transparent Process Requires Public Input

ED_002061_00044428-00012

In establishing study-acceptance criteria, formal rulemaking – including a robust notice and comment process for public input – should be undertaken. *See Appalacian Power Company v. EPA*, 208 F.3d 1015 (DC Cir. 2000) (finding that an EPA guidance document outlining procedural steps in permit review process required formal rulemaking). As noted by the court in *Appalachian Power*, certain guidance documents are "binding" and subject to formal rulemaking:

> If an agency acts as if a document issued at headquarters is controlling in the field, if it treats the document in the same manner as it treats a legislative rule, if it bases enforcement actions on the policies or interpretations formulated in the document, if it leads private parties or State permitting authorities to believe that it will declare permits invalid unless they comply with the terms of the document, then the agency's document is for all practical purposes "binding."

Id. at 1021. Because the study acceptance criteria will create a *de facto* rule on the use of epidemiological studies in the Agency's pesticide registration process, a notice-and-comment period is mandatory under the Federal Administrative Procedures Act. Failure to follow these formalities could call the criteria into question, and cause the associated guidance document to be set aside in its entirety. *Id.* at 1028. That would benefit no one.

Inviting the public to comment on the proposed criteria and to propose additional or different criteria will maximize the quality of evidence considered in a pesticide risk assessment. President Obama has stressed from the very beginning of this administration that

> "[t]he public must be able to trust the science and scientific process informing public policy decisions. Political officials should not suppress or alter scientific or technological findings and conclusions. If scientific and technological information is developed and used by the Federal Government, it should

ED_002061_00044428-00013

> ordinarily be made available to the public. To the extent permitted by law, there should be transparency in the preparation, identification, and use of scientific and technological information in policymaking."

See Memorandum For The Heads of Executive Departments And Agencies on Scientific Integrity, President Barack Obama, March 9, 2009. A formal rule making process will ensure that OPP meets its, and the Obama Administration's, goal of ensuring epidemiological evidence is used "in the most scientifically robust and transparent way." Moreover, valuable comments will likely come from a variety of sources. Epidemiologists have a strong interest in how their research will be evaluated and used by the government in setting public policy. And pesticide registrants and America's farmers have a strong interest in ensuring that only legitimate, scientifically-sound studies will be used to inform the risk assessment of vital agricultural tools.

IV. Action Requested

CropLife America requests that EPA promulgate a new section in Title 40 of the Code of Federal Regulations that sets forth clear criteria and procedures for the selection of epidemiological evidence to be incorporated into the risk assessment process.

The new Section should include the following:

- Clear, scientifically-based criteria for determining the acceptability of epidemiological studies for use in risk assessment;
- A requirement that qualified epidemiologists review the available epidemiological studies for a pesticide under review and lead or

ED_002061_00044428-00014

participate in the determination of which studies will be accepted for use in the risk assessment; and

- An opportunity for stakeholders, including registrants, to comment on the acceptability of specific epidemiological studies for use in risk assessment and a commitment by OPP to consider and respond to such comments.

Finally, EPA should not incorporate epidemiological studies into risk assessment for pesticide products for the purposes of decision making under registration or registration review until the aforementioned criteria have been promulgated.

ED_002061_00044428-00015

November 29, 2016

Dr. Jack Housenger
United States Environmental Protection Agency
Director, Office of Pesticide Programs
2777 Crystal Drive
One Potomac Yard South
Room 12621
Arlington, VA 22202-3553

Sent via email

Re: Petition EPA to halt regulatory decisions that are highly influenced/determined by results of epidemiological studies that do not meet well-defined data quality standards, and that are not integrated into the health risk assessment in a transparent, well-defined manner

Dear Director Housenger:

Enclosed please find a CropLife America (CLA) Petition to the US Environmental Protection Agency (EPA) to suspend any regulatory decision making on human health risk assessment(s) for organophosphate and other pesticides, using an FQPA 10X Safety Factor supported principally by the use of epidemiologic studies that generally do not meet well-defined data quality standards, and specifically are heavily weighted on the Columbia Study outcomes.

CropLife America is the not-for-profit trade organization representing the nation's developers, manufacturers, formulators and distributors of plant science solutions for agriculture and pest management in the United States. Our member companies produce, sell and distribute virtually all the crop protection technology products used by American farmers and other consumers. We are committed to the safe and responsible use of the industry's products in order to provide safe and abundant food, as well as to control insect and plant disease vectors for the protection of human health, all providing valuable benefits to the agriculture, farmers and the consumer.

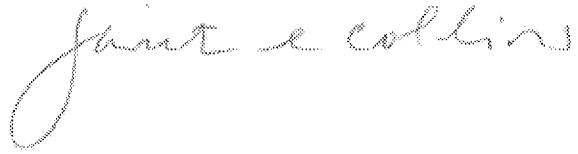
The Petition requests EPA to cease regulatory decision making, specifically with respect to the organophosphate pesticides, until EPA has transparently developed criteria for acceptance of epidemiologic studies in human health risk assessment, and guidance for integration of epidemiologic studies in pesticide risk assessment historically based on animal toxicological and *in vitro* studies.

Due to uncertainties within the regulatory environment created by the lack of such integration guidance, and the potential impact on the availability of crop protection products based on inappropriate selection and use of epidemiological studies by the EPA Office of Pesticide

Programs in its human risk assessments for pesticide registrations, CLA respectfully requests that you respond to our Petition within 45 days.

Should you have any questions regarding the CLA Petition, please contact me directly [jcollins@croplifeamerica.org (+1-202-833-4474)].

Respectfully submitted,



Janet E. Collins, Ph.D., R.D.
Executive Vice President
CropLife America

PETITION TO HALT REGULATORY DECISIONS DETERMINED BY RESULTS OF EPIDEMIOLOGICAL STUDIES THAT DO NOT MEET WELL-DEFINED DATA QUALITY STANDARDS AND THAT ARE NOT INTEGRATED INTO THE RISK ASSESSMENT IN A TRANSPARENT, WELL-DEFINED MANNER

CropLife America (CLA) petitions the Environmental Protection Agency (EPA or the Agency) to halt any regulatory decisions on any organophosphate pesticide (OP), where that action is based primarily on results from highly influential studies that do not meet well-defined data quality standards, where the use of such a study is a determinative factor, yet the public has no means of knowing how EPA is determining the data quality of such a study and how it is being integrated into the risk assessment when its conclusions are not consistent with results from more traditional, and required testing. EPA's reliance on the Columbia Center for Children's Environmental Health epidemiology cohort studies of inner-city mothers and children (the Columbia Study) concerning pre- -natal pesticide exposure to chlorpyrifos to drive regulatory decisions is an example of this.

Established in 1933, CLA represents the developers, manufacturers, formulators and distributors of plant science solutions for agriculture and pest management in the United States. CLA's member companies produce, sell and distribute virtually all the vital and necessary crop protection and biotechnology products used by farmers, ranchers and landowners. Crop protection products are necessary to ensure safe, predictable and adequate supplies of food, fiber and fuel. CLA members support science-based regulation of pesticides to ensure that these products can be used without causing unreasonable adverse effects to either human health or the environment.

STATUTORY AND REGULATORY BACKGROUND

No pesticide product can be distributed or sold for use in the United States unless it has been registered by EPA under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA),

7 U.S.C. § 136 *et seq.* Through FIFRA, the EPA receives extensive hazard and exposure information that is used to evaluate the risks of pesticide products.

The 1996 Food Quality Protection Act (FQPA) amended FIFRA and the Federal Food Drug and Cosmetic Act (FFDCA), requiring EPA, when setting tolerances for pesticide residues on food, to make a safety finding that the pesticide can be used with "a reasonable certainty of no harm" to human health. 21 U.S.C. § 346a(b)(2)(A). To meet this standard, EPA must consider (among other things) the special susceptibility of children to pesticides by using an additional tenfold (10X) safety factor (the Safety Factor) when setting and reassessing tolerances unless adequate data are available to support a different factor. *Id.*

EPA's human health pesticide risk assessment has traditionally relied on validated toxicological studies using laboratory animals along with data to estimate the potential exposure based on the proposed use of the pesticide product. Epidemiological studies of adverse effects in humans have not been uniformly or consistently incorporated into this quantitative risk assessment process, due to the observational nature of epidemiologic research, primarily due to questions related to study design, population studied, and lack of clear evidence of the magnitude and duration of exposure during critical phases of development and/or evidence of a dose-response relationship that would more clearly support a conclusion that exposure to a particular chemical may have caused an observable/measurable toxic effect, among other factors that would impact the use of epidemiologic study outcomes in quantitative risk assessment. While EPA has stated that epidemiological evidence, generically speaking, may provide important informative data for the risk assessment process, this determination does not mean that each and every published epidemiological study will provide equally important, valid or useful information. Like all information considered in a risk assessment, the quality and validity of the

information provided by epidemiological studies needs to be closely scrutinized before use in a regulatory risk assessment.

Through recent statements made by EPA in OP registration review dockets, EPA now seeks to change this process and incorporate unreliable and unverifiable epidemiological evidence directly into its pesticide risk assessment process through a framework that utilizes a weight of evidence approach, without explaining to the public how these studies, of varying quality, will be weighted when EPA does not have access to the raw data, and no criteria exist for either choice of study or integration of such data. EPA should cease further action on regulatory decision-making that finds epidemiological study results to be determinative until the Agency can establish criteria against which such study results can be evaluated, and clearly articulate how the Agency will determine the value added within the risk assessment in the context of the traditional empirical data.

EPA'S SHIFT IN APPROACH TO RISK ASSESSMENT

In January 2010, EPA published a Draft Framework for Incorporating Human Epidemiologic & Incident Data in Health Risk Assessment (the Draft Framework). The Draft Framework declares that "[EPA] intends to employ…epidemiology studies and human health incident data in its human health risk assessment" and that its "goal is to use such information in the most scientifically robust and transparent way." Draft Framework at 6. EPA based its decision to incorporate epidemiology into the risk assessment process on two reports issued by the National Research Council for the National Academy of Sciences, *Toxicity Testing in the 21st Century: A Vision and a Strategy* (2007) and *Science and Decisions* (2009). Per the Draft Framework, EPA expressed its intent to conduct human health risk assessments

that incorporate data from new sources, specifically information found in epidemiology studies, human incident databases, and biomonitoring studies.

In the Draft Framework, EPA sets forth a general plan for incorporating epidemiological studies into its risk assessment process and for weighing that evidence alongside traditional mechanistic and toxicological evidence in a weight of the evidence analysis. The Draft Framework describes the major types of epidemiological studies, noting the strengths and limitations of each in terms of their applicability to the risk assessment process. EPA's Draft Framework is premised on a proposed weight of the evidence evaluation that uses the modified Bradford Hill criteria as established in the Mode of Action Human Relevance Framework[1]; the framework serves as an approach for organizing and evaluating diverse types of data to determine the evidence available on the potential human health consequences of pesticide exposure.

In a critical failing, the Draft Framework did not set forth any criteria for selecting the epidemiological studies to be incorporated, or for evaluating the quality and validity of a particular epidemiological study to determine whether that study should be used in an EPA risk assessment in the first place, in spite of the fact that in its own guidance on use of open literature data for human health risk assessment, and the data quality guidance, EPA stated that it would work with the public (including the regulated community) to develop these criteria. Toxicological and exposure studies, in contrast, generally must meet strict design and "good laboratory practice" quality criteria and disclose all analyses for consideration during registration or registration review processes. *See e.g.*, 40 C.F.R. §§ 152.50, 158.80, and Part 160.

---

[1] A summary of EPA's Motion of Action Human Relevance Framework can be found at: https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=246035.

As discussed in more detail below, a FIFRA Scientific Advisory Panel (2010 SAP) reviewed the Draft Framework at a meeting held in February 2010. While the 2010 SAP praised EPA for its use of the Bradford Hill criteria as the basis for how to incorporate epidemiology in a weight of the evidence analysis, the SAP strongly recommended that EPA establish a stringent set of quality-based criteria to determine whether to accept a given epidemiological study for use in risk assessment. FIFRA Scientific Advisory Panel Meeting Minutes No. 2010-03 at 10 (Feb. 2-4, 2010) (2010 SAP Minutes). Since the 2010 SAP meeting, CLA and its members have repeatedly requested an opportunity for input into such criteria; the Agency has continued to deny these requests, and has not issued the 2010 SAP-recommended criteria, despite have stated that it would work with the public to develop such criteria.

On September 25, 2015, EPA made available for public comment its "Pesticide Registration: Draft Human Health and Ecological Risk Assessments for Sulfonylureas and Certain Other Pesticides" (Draft Risk Assessments).[2] The Draft Risk Assessments were intended to support the registration review of (1) a group of 22 pesticides known as sulfonylureas (not at issue here) and (2) seven OPs. CLA provided comments to each of the dockets contained within the overarching docket.[3] At that time, CLA also commented on the "Literature Review on Neurodevelopmental Effects and FQPA Safety Factor Determination for the Organophosphates" (the Literature Review) that was part of each of the seven OP dockets.[4] In the Literature Review EPA stated (buried on page 80 of 101) that it will be retaining the full 10X Safety Factor for *all*

---

[2] "Pesticide "Pesticide Registration Review; Draft Human Health and Ecological Risk Assessments for Sulfonylureas and Certain Other Pesticides; Notice of Availability and Request for Comment," 80 Fed. Reg. 57812 (Sept. 25, 2015), Docket No. EPA-HQ-OPP-2015-0386, available at: https://www.regulations.gov/searchResults?rpp=25&po=0&s=EPA-HQ-OPP-2015-0386&fp=true&ns=true.

[3] *See* Docket No. EPA-HQ-OPP-2012-0372-0066 (Sept. 12, 2016), available at: https://www.regulations.gov/docket?D=EPA-HQ-OPP-2012-0372; Docket No. EPA-HQ-OPP-2008-0345-0046 (March 3, 2016), available at: https://www.regulations.gov/document?D=EPA-HQ-OPP-2008-0345-0046.

[4] *See* EPA-HQ-OPP-2010-0119-0039 (February 22, 2016), available at: https://www.regulations.gov/document?D=EPA-HQ-OPP-2010-0119-0039.

OP pesticides. The Agency's stated rationale for retaining the full Safety Factor was based primarily on conclusions drawn from the Columbia Study, despite the fact that EPA has not evaluated, or even reviewed, the data underlying the Columbia Study's conclusions.

More recently, on November 10, 2016, the Agency issued a prepublication version of "Chlorpyrifos; Tolerance Revocations; Notice of Data Availability and Request for Comment."[5] In that document EPA states that it intends to revoke all food tolerances for the OP. That decision is based, at least in part, on applying the full 10X Safety Factor using the Columbia Study as the comparator for exposure levels considered to cause harm.

INTEGRATION OF DATA SOURCES IN HUMAN HEALTH RISK ASSESSMENT

Importance of Epidemiological Data in Risk Assessment

While EPA has stated that epidemiological evidence, generically speaking, may provide important informative data for the risk assessment process, this determination does not mean that each and every published epidemiological study will provide equally important, valid or useful information. Like all information considered in a risk assessment, the quality and validity of the information provided by epidemiological studies needs to be closely scrutinized.

Findings from properly conducted, high quality epidemiological studies may prove useful in characterizing and evaluating human health risks. But not all epidemiological evidence is created equal. Bias, confounding factors, and in particular, unreliable and invalidated exposure assessments commonly occur in epidemiological studies, limiting the value of the researchers' conclusions for quantitative risk assessments. In addition, a study's probative value varies dramatically depending on design and approach – observational studies, for instance, such as case series or ecological "cluster" analyses, do not carry the strength of association assessment

[5] https://www3.epa.gov/pesticides/PrePublicationCopy_16P-0280_2016-11-10.pdf

that prospective cohort and case control studies do and are often utilized only (if at all) for hypothesis generating purposes. *See* 2010 SAP Minutes at 20-21.

"Weight of evidence," in this context, requires more than mere consideration of "all" forms of epidemiological studies. The weight of evidence approach considers all relevant information in an integrative assessment that takes into account the kinds of evidence available, the quality and quantity of the evidence, the strengths and limitations associated with each type of evidence and explains how the various types of evidence fit together. *See* U.S. Environmental Protection Agency, A Summary of General Assessment Factors for Evaluating the Quality of Scientific and Technical Information, EPA 100/B-03/001 June 2003. Epidemiology studies used in risk assessments, thus, should report all analyses, negative or positive and provide all underlying data.

To date, EPA has provided no transparency regarding how it is judging the quality of epidemiological studies or weighing them against its traditional data requirements. Additional transparency and structure is needed before decisions are made based on this inscrutable evaluation.

EPA MUST ENSURE THE QUALITY OF DATA USED IN RISK ASSESSMENTS

The quality of scientific information forming the foundation for pesticide registration and use decisions is essential to all EPA risk assessments. For example, in 2002 EPA published "Guidelines for Ensuring and Maximizing the Quality, Objectivity, Utility, and Integrity of Information Disseminated by the Environmental Protection Agency," EPA/260R-02-008 (the Guidelines). EPA issued these Guidelines to formalize and maximize the quality of disseminated information, particularly with respect to the objectivity, utility, and integrity of scientific data. Under the Guidelines, information disseminated by EPA must be "presented in an accurate,

clear, complete, and unbiased manner" with substance that "is accurate, reliable, and unbiased." *Id.* at 15. Objectivity of influential scientific information is judged against the quality principles in the Safe Drinking Water Act Amendments (SDWA) of 1996 to ensure the use of (i) the best available science and supporting studies conducted in accordance with sound and objective scientific practices, including, when available, peer reviewed science and supporting studies; and (ii) data collected by accepted methods or best available methods (if the reliability of the method and the nature of the decision justifies the use of the data). *Id.* at 22.

Moreover, "influential" information, which is information that will have a clear and substantial impact on important public policies or private sector decisions, must "adhere to a rigorous standard of quality" and "should be subject to a higher degree of quality." *Id.* at 20. As noted in the Guidelines, information that can "adversely affect in a material way the economy, productivity, competition, jobs" or that addresses "precedent-setting or controversial scientific or economic issues" is considered influential. Because the overall economic impact on a currently registered pesticide of an EPA reassessment could be substantial, scientific data used to make those decisions would be influential. Unquestionably, epidemiological studies used in these risk assessments that impact registrations thus qualify as "influential" data subject to heightened quality standards under the Guidelines.

Requirements set out in the FQPA for setting tolerances mirror these guidelines. Under the FQPA, EPA must consider, among other relevant factors, the validity, completeness, and reliability of the available data from studies of the pesticide chemical. 21 U.S.C. § 346a.

CRITERIA FOR A WELL-DESIGNED, ROBUST EPIDEMIOLOGICAL STUDY

A well-designed, robust epidemiological cohort or case control study has certain features that OPP should look for before admitting a study into a risk assessment. These include, but are not limited to:

- well-characterized, quantitative exposure assessments that minimize measurement error, evaluates exposure during an etiologically relevant period, and decrease the likelihood of inaccurate or biased information;
- well-characterized, documented identification of disease and/or health status.
- a well-defined study population that includes persons with a wide range of exposures as well as unexposed persons; and
- documented efforts to control for selection bias, information bias and confounding; and
- full disclosure of the results of all statistical analyses conducted and models evaluated.

While quantitative criteria are a critical first step in separating reliable epidemiological studies from less reliable studies, an element of scientific judgment is required to make a complete assessment. To ensure a well-informed process, EPA must utilize the expertise of qualified epidemiologists to review the body of epidemiology evidence for a given pesticide to determine whether each study satisfies quality criteria. The 2010 SAP recommended several specific questions to be asked when evaluating each specific epidemiological study for potential use in a risk assessment. Those include:

1. Was the epidemiological study conducted primarily in a hypothesis- generating or a hypothesis-testing mode? Studies with no specific *a priori* hypothesis are more likely to generate false positive results.
2. Was the method of assessing exposure reliable and adequate?

3. Were inclusion and exclusion criteria clearly stated and reasonable to provide a representative sample with regard to exposure and health outcome so as to provide a relatively unbiased and representative estimate of effect?
4. Was the method of assessing the criteria for determining health outcome clearly stated and valid and reliable; e.g. confirmed with histopathology; and were they designed to detect newly diagnosed (rather than prevalent) cases so that it was reasonably possible to determine that exposure preceded disease?
5. Was appropriate information on potentially confounding factors, such as socio-demographic, behavioral and dietary factors collected for both exposed and unexposed groups or for cases and controls in the same way, and were they appropriately controlled in the analyses of the data? Were data on co-morbid conditions collected? (i.e. factors that are associated with the health condition of interest as well as factors associated with exposure)
6. Did the study sample the population or individuals of interest? (i.e. was selection bias minimized and generalizability optimized?) How does the study population relate to the universe of potentially exposed populations?
7. Did the study examine individuals with a wide range of exposures? (i.e. ability to detect a dose-response and to generalize to other populations). Did the study include unexposed populations or individuals?
8. Did the exposures examined in the study relate to past or current situations? (i.e. acute versus chronic exposures and the target health end points)
9. Did the study have adequate statistical power to detect meaningful differences for outcomes between the different groups of exposed and unexposed or less exposed

individuals while controlling for important confounding factors? Does the sample size take into account the expected incidence of the target health effect in the study populations? Was the study powerful enough to detect statistically meaningful differences while adjusting for confounding variables and exposure measurement error that typically reduce statistical power?

See 2010 SAP Minutes at 16-17.

NECESSITY OF THIS PETITION

It is undisputed that numerous high-quality animal toxicity studies on OPs have been conducted according to scientifically validated test methods and reviewed by the Agency in support of their registrations under FIFRA. The weight of evidence from these studies currently supports a highly conservative regulatory point of departure based on replicated Good Laboratory Practice studies in multiple species, durations and routes of exposure. Furthermore, the outcomes from these animal studies are aligned with and corroborated by standard physicochemical and mechanistic principles that have withstood the test of time. Thus, based on extensive laboratory data, EPA has moved away from the full 10X Safety Factor for OPs.

EPA has made a significant and fundamental change in the way it is applying the Safety Factor. As stated in the Literature Review and evidenced in the current OP draft risk assessments, EPA now intends to apply the full 10X Safety Factor to all OP pesticides, based primarily on conclusions drawn from the Columbia Study, although neither EPA nor the registrant have access to the data upon which this decision is based. Significant data quality inadequacies in the Columbia Study 'file' exist; and there is no indication of how EPA 'weighted' the Columbia Study results in any type of weight-of-evidence process, with no criteria for such approach. EPA's reliance on this study, in spite of significant and negative

comment and direction from three separate FIFRA SAPs is without precedent. Where are the data; what is the EPA response to the three SAP reviews and recommendations; and, most importantly, what is new in assessment of the Columbia Study publications since EPA began its assessment of chlorpyrifos that would support EPA changing its approach to assessment long after the Columbia Studies were published?

Such reliance violates both FIFRA and the FQPA and flies in the face of the Guidelines and 2010 SAP recommendations, discussed above. Neither EPA nor interested stakeholders, of which OP registrants are included, have been granted access to the Columbia Study's underlying data. Thus, EPA could not have adequately evaluated the data to determine its validity, completeness, and reliability, as required by the FQPA. The Agency has not confirmed that the data meets the quality criteria as recommended by 2010 SAP and it certainly does not meet the Guidelines' standard that information disseminated by EPA be "presented in an accurate, clear, complete, and unbiased manner" with substance that "is accurate, reliable, and unbiased." Because EPA's intended use of the Columbia Study meets none of the quality criteria required for epidemiological data use in pesticide risk assessment, EPA should not be allowed to make decisions based upon it.

For the reasons set forth here, CLA petitions EPA to cease further action on any regulatory decision-making based primarily on epidemiological studies until the Agency develops, and sets forth for public comment, data quality criteria for how these study results will be evaluated, weighed and integrated into the traditional data set required for pesticide risk assessment, consistent with the quality standards required by both Congress and EPA, itself.

Message

From: Janet Collins [jcollins@croplifeamerica.org]
Sent: 4/13/2018 6:12:42 PM
To: Beck, Nancy [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=168ecb5184ac44de95a913297f353745-Beck, Nancy]; Keigwin, Richard [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=151baabb6a2246a3a312f12a706c0a05-Richard P Keigwin Jr]
CC: csmith@gowanco.com [/o=ExchangeLabs/ou=Exchange Administrative Group (FYDIBOHF23SPDLT)/cn=Recipients/cn=85a93dee627e495997f325593ed303eb-csmith@gowa]
Attachments: martyetal oxon paper 2012.pdf

Dear Nancy and Rick- thank you for the time you dedicated to meeting with us on Wednesday morning.

During the meeting, we discussed the EPA consideration of the exposure information from the CHAMACOS study. We discussed that CHAMACOS did not report chlorpyrifos but did report on the oxons of chlorpyrifos. Attached please find a paper published in 2012 wherein you will note the authors statement (see last sentence in abstract) that oxons would not be in the peripheral tissues- thus, would not be present in the brain- brain function would not then be affected by oxons in the blood samples.

We welcome the opportunity to discuss this further, and likely will address that specific point when we provide the final study report that we have conducted to plot the data from the Columbia University study.

Thank you again.

My best

Janet E Collins, Ph.D., R.D.
Executive Vice President, Science and Regulatory Affairs
CropLife America
1156 15th Street, NW; Suite 400
Washington DC 20001

Ex. 6

Regulatory Toxicology and Pharmacology 63 (2012) 209–224

Contents lists available at SciVerse ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Cholinesterase inhibition and toxicokinetics in immature and adult rats after acute or repeated exposures to chlorpyrifos or chlorpyrifos–oxon

M.S. Marty [a,*], A.K. Andrus [a], M.P. Bell [a], J.K. Passage [a], A.W. Perala [a], K.A. Brzak [a], M.J. Bartels [a], M.J. Beck [b], D.R. Juberg [c]

[a] Toxicology & Environmental Research and Consulting, The Dow Chemical Company, 1803 Building, Midland, MI 48674, United States
[b] WIL Research Laboratories LLC, 1407 George Road, Ashland, OH 44805, United States
[c] Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, IN 46268, United States

ARTICLE INFO

Article history:
Received 10 December 2011
Available online 7 April 2012

Keywords:
Chlorpyrifos
Chlorpyrifos–oxon
Cholinesterase inhibition
Comparative cholinesterase inhibition
Age-related sensitivity

ABSTRACT

The effect of age or dose regimen on cholinesterase inhibition (ChEI) from chlorpyrifos (CPF) or CPF–oxon (CPFO) was studied in Crl:CD(SD) rats. Rats were exposed to CPF by gavage in corn oil, rat milk (pups), or in the diet (adults) or to CPFO by gavage in corn oil. Blood CPF/CPFO levels were measured. With acute exposure, ChEI NOELs were 2 mg/kg CPF for brain and 0.5 mg/kg CPF for red blood cells (RBCs) in both age groups. In pups, ChEI and blood CPF levels were similar using either milk or corn oil vehicles. Compared to gavage, adults given dietary CPF (12 h exposure) had greater RBC ChEI, but lower brain ChEI at corresponding CPF doses, indicating an effect of dose rate. With repeated CPF exposures, ChEI NOELs were the same across ages (0.5 and 0.1 mg/kg/day for brain and RBCs, respectively). With CPFO dosing, the ChEI NOELs were 0.1 mg/kg (acute) and 0.01 mg/kg/day (repeated doses) for RBCs with no ChEI in brain at CPFO doses up to 0.5 (pup) or 10 mg/kg (adult) for acute dosing or 0.5 mg/kg/day for both ages with repeat dosing. Thus, there were no age-dependent differences in CPF ChEI via acute or repeated exposures. Pups had less ChEI than adults at comparable blood CPF levels. Oral CPFO resulted in substantial RBC ChEI, but no brain ChEI, indicating no CPFO systemic bioavailability to peripheral tissues.

© 2012 Published by Elsevier Inc.

1. Introduction

Chlorpyrifos (CPF) is a broad spectrum organophosphate insecticide that inhibits acetylcholinesterase (AChE), which can result in accumulation of synaptic acetylcholine, neuronal overstimulation, and subsequently, cholinergic signs of toxicity (e.g., excessive salivation, lacrimation, urination, defecation, incoordination, tremors, etc.). Previous studies have demonstrated that young animals are more sensitive to lethality, some neurobehavioral effects and cholinesterase (ChE) inhibition following acute exposures to high doses of CPF (e.g., Pope et al., 1991). At 15–20 mg/kg, PND 17 rats exhibited the same magnitude of brain ChE inhibition as adult rats given 80 mg/kg CPF (Moser and Padilla, 1998; Moser et al., 1998). Functional neurobehavioral changes following acute CPF exposure have been correlated with ChE inhibition (Moser, 1995; Nostrandt et al., 1997). Generally, signs of cholinergic toxicity were seen when brain ChE was inhibited >60% (Nostrandt et al., 1997; Moser et al., 1998). ChE inhibition is considered the most sensitive endpoint for CPF toxicity based on previous dose–response studies and provides the most useful data for determining point of departure (US EPA, 2011). These differences in sensitivity are partially related to toxicokinetic differences between young animals and adults (i.e., young animals have a lower capacity to detoxify chlorpyrifos–oxon (CPFO), the active metabolite; Timchalk et al., 2006).

Investigators have demonstrated that age-related differences in sensitivity to CPF exist with acute, high-dose exposures and are proportional to the magnitude of ChE inhibition. Pope and Chakraborti (1992) demonstrated that there was a high correlation between brain and plasma ChE inhibitory potency (ED_{50} values) and sensitivity to acute toxicity at the maximum-tolerated dose across age groups with acute *in vivo* CPF exposure. Across three organophosphates (OPs), maximum-tolerated doses given to immature and adult rats produced similar degrees of brain ChE inhibition. With intermittent repeated exposures (i.e., 40 mg/kg CPF once every 4 days, total of four doses), adult rats were more sensitive to neurochemical changes, including brain ChE inhibition, than immature rats (PND 7–20) (Chakraborti et al., 1993). The paucity of data on age-related sensitivity at low dose levels was acknowledged by the US Environmental Protection Agency (US EPA) Scientific Advisory Panel (2008), which noted that there was uncertainty regarding the relative sensitivity of young animals to ChE inhibition after exposure to low doses of CPF.

* Corresponding author. Fax: +1 989 638 9863.
E-mail address: mmarty@dow.com (M.S. Marty).

0273-2300/$ - see front matter © 2012 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.yrtph.2012.03.015

Aside from evaluating age-related sensitivity to CPF-induced ChE inhibition, there also were concerns for potential ChE inhibition due to exposures to CPFO. The US EPA's Environmental Fate and Effects Division has proposed that drinking water exposures to CPFO are possible. Estimated drinking water concentrations (EDWCs) of CPFO were based on Tier II surface water and Tier I groundwater model simulations for currently registered uses of CPF, because CPF is expected to transform to CPFO during drinking water treatment and there are limited environmental fate data available for CPFO, in part due to the short half-life of the oxon. Thus, due to the concern for potential drinking water exposures, acute and repeated-dose inhibition of ChE following CPFO exposure also was examined.

This study was derived from a standard US EPA Comparative Cholinesterase Study design to examine the relative sensitivity of adult rats and pups to ChE inhibition with the goal to better characterize age-dependent toxicity of CPF over lower portions of the dose–response curve. These data would establish whether previously identified age-related differences in ChE inhibition at high CPF doses apply at lower dose levels that are more relevant for human exposures. The study design also was expanded to examine the impact of dose vehicle, dose rate, and the effect of acute and repeated CPFO dosing on ChE inhibition with measurements of internal dose to provide context for these results. First, immature (postnatal day (PND) 11) and young adult rats were given acute (single bolus) or repeated (11 day) exposures to CPF or CPFO in corn oil. The impact of vehicle and dosing regimen was evaluated after bolus dosing in rat milk vehicle (pups) or 12 h dietary exposure (adults). The highest dose levels were selected to induce approximately 50–60% inhibition of brain ChE in the CPF studies and marked inhibition of RBC ChE in the CPFO studies. To examine behavioral neurotoxicity, clinical observations were included in the acute studies, whereas a functional observational battery (FOB) and motor activity were included after 10 daily exposures in the repeated dose study. Brain, RBC and plasma ChE inhibition were measured at the time-of-peak inhibition following both acute and repeated exposures to CPF or CPFO. Blood levels of CPF, CPFO, and/or trichlorpyridinol (TCP) were measured to examine the relationship between ChE activity and systemic exposure levels and to improve the existing physiologically-based pharmacokinetic/pharmacodynamic (PBPK/PD) model for CPF (Timchalk et al., 2002, 2006). Data from this study were modeled using benchmark dose analysis in the accompanying paper by Reiss et al. (2012).

2. Materials and methods

2.1. Materials and animal husbandry

Chlorpyrifos Technical (CPF; *O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridinyl)ester phosphorothioic acid; 99.8% pure) and chlopyrifos–oxon (CPFO; diethyl 3,5,6-trichloro-2-pyridinyl ester phosphoric acid, 94.9% pure), a CPF metabolite that inhibits cholinesterase (ChE), were supplied by Dow AgroSciences LLC (Indianapolis, Indiana). Rat milk used as a vehicle in some PND 11 pup experiments was collected from untreated lactating dams or was purchased from Bioreclamation, Inc. (Hicksville, New York). Stability of CPF and CPFO in corn oil and milk was confirmed prior to the start of the pilot studies; stability of CPF in rodent diet was previously established for at least 30 days at concentrations spanning those used in this study. Concentration verification and homogeneity analyses were conducted for all dose solutions, and for the premix and all test diets.

Adult female Crl:CD(SD) rats were approximately 63 days of age at receipt. Females were selected for study, because adult females were either slightly more sensitive (e.g., Moser, 2000) or equally sensitive to adult males (Betancourt and Carr, 2004); in other studies, gender-related differences were not reported (Timchalk et al., 2006; Zheng et al., 2000). Male and female Crl:CD(SD) rat pups were 4 or 5 days of age at the time of arrival in the laboratory. Animals were allowed to acclimate under standard environmental conditions for approximately one week prior to study initiation.[1] Adults were singly housed and pups (8/litter) were housed with lactating dams. Unless otherwise stated, LabDiet Certified Rodent Diet #5002 (PMI Nutrition International, St. Louis, Missouri) and municipal tap water were provided *ad libitum* and pups were given free access to lactating dams. Adult female rats were assigned randomly to treatment groups using a computer program designed to increase the probability of uniform group mean weights and standard deviations at the start of the study. Within litters, pups were randomly assigned to treatment groups with a preference for using pups of intermediate body weights when possible (i.e., avoiding unusually large or small pups). For all studies, pups from the same litter were assigned to different treatment groups to control for litter effects at each time point. The study was approved by the Institutional Animal Care and Use Committee and complied with Good Laboratory Practice regulations.

2.2. Experimental design

The primary objective of this study was to provide data for a comparison of CPF- and CPFO-induced ChE inhibition in pre-weanling and young adult rats to determine whether age-related differences in sensitivity exist. Data were also collected to evaluate how differences in dose vehicle (corn oil vs. milk) or dose regimen (gavage vs. diet) affected the toxicokinetics and pharmacological effects of CPF. The study was conducted in four phases (Table 1): *phase 1*: a range-finding study; *phase 2*: an acute pilot; *phase 3*: a definitive acute study; and *phase 4*: a definitive repeat-dose study.

For each study phase, oral gavage doses were administered at a volume of 3 ml/kg based upon body weights collected just prior to dosing. Pups were removed from the dams approximately 1 h prior to dosing and returned to the dams after dose administration until the time of euthanasia. For gavage experiments, adult females were fasted overnight prior to dose administration, then were given *ad libitum* access to feed during the day. At termination, blood and brain samples were collected at each phase for the assessment of plasma, RBC and brain ChE levels.

2.2.1. Phase 1: range-finding study

A preliminary range-finding study was conducted to determine the approximate dose of CPF and CPFO necessary to produce marked (~50–60%) brain ChE inhibition in young adult female rats in order to anchor the dose–response curves with an effective dose. For this study, ~70-day-old female rats (3/dose group) were treated as shown in Table 1. There was a shared vehicle (corn oil) control group. All females were weighed and dosed by gavage in the morning and ChE samples were collected at 5 h following dose administration. Sample collection is described below. With CPFO, significant brain ChE inhibition was not seen, so RBC ChE inhibition was used for CPFO dose selection in subsequent study phases (Table 2).

2.2.2. Phase 2: acute pilot study

The pilot study was conducted to determine the time-of-peak ChE inhibition with the different dosing scenarios (Table 1). PND 11 rats (female) and young adult females (~70 days of age) were euthanized at numerous time points after administration of CPF (3 mg/kg in PND 11 pups; 10 mg/kg in adults) or CPFO (0.5 mg/kg in PND 11 pups; 0.3 mg/kg in adults) and ChE activity was

[1] Laboratory fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International).

Table 1
Chlorpyrifos comparative cholinesterase study design.

Age	Test compound	Vehicle	Dose level(s)[a] (mg/kg/day)	Duration	ChE sample collection time[b]	TK samples?
Phase 1: range-finding study						
Adult (~70 days of age)	CPF	Corn oil	0, 5, 10, 20	Single dose	5 h	N
	CPFO	Corn oil	0, 1, 5, 10	Single dose	5 h	N
Phase 2: time-of-peak inhibition study						
PND 11 (F)	CPF	Corn oil	0, 3	Single dose	2, 4, 6, 8, 12, 24 and 48 h	N
	CPF	Milk	0, 3	Single dose	2, 4, 6, 8, 12, 24 and 48 h	N
	CPFO	Corn oil	0, 0.5	Single dose	2, 4, 6, 8, 12 and 24 h	N
Adult (~70 days of age)	CPF	Corn oil	0, 10	Single dose	2, 4, 6, 8, 12 and 24 h	N
	CPF	Diet	0, 10	12-h Dietary exposure	2, 4, 6, 8, 12 and 24 h	N
	CPFO	Corn oil	0, 0.3	Single dose	2, 4, 6, 8, 12 and 24 h	N
Phase 3: acute dose–response study						
PND 11 (M and F)	CPF	Corn oil	0, 0.05, 0.1, 0.5, 2, 5	Single dose	6 h	Y
	CPF	Milk	0, 0.05, 0.1, 0.5, 2, 5	Single dose	8 h	Y
	CPFO	Corn oil	0, 0.005, 0.01, 0.05, 0.1, 0.5	Single dose	4 h	Y
Adult (~ 70 days of age)	CPF	Corn oil	0, 0.05, 0.1, 0.5, 2, 10	Single dose	8 h	Y
	CPF	Diet	0, 0.05, 0.1, 0.5, 2, 10	12-h Dietary exposure	8 h after removal of diet	Y
	CPFO	Corn oil	0, 0.01, 0.05, 0.1, 0.5, 1	Single dose	4 h	Y
Phase 4: repeat dose study						
PND 11 (M and F)	CPF	Corn oil	0, 0.05, 0.1, 0.5, 1, 3.5	11 doses; PND 11–21	6 h	Y
	CPFO	Corn oil	0, 0.01, 0.5	11 doses; PND 11–21	4 h	Y
Adult (~70 days of age)	CPF	Corn oil	0, 0.05, 0.1, 0.5, 1, 3.5	11 doses; ~70–80 days of age	8 h	Y
	CPFO	Corn oil	0, 0.01, 0.5	11 doses; ~70–80 days of age	4 h	Y

F, females; M, males.
[a] Whenever possible, a shared control group was used.
[b] Hours after administration of the last dose.

Table 2
Phase 1: range-finding study – summary of CPF and CPFO ChE inhibition in adult female CD rats after single gavage exposure in corn oil (samples collected 5 h post-exposure).

Dose group (mg/kg)	RBC ChE (U/L) (100 μl sample)	RBC ChE (U/L) (50 μl sample)	Mean RBC ChE (U/L)	Mean plasma ChE (U/L)[a]	Brain ChE (U/L)	Mean brain ChE (U/L)
0	4296	4028	4247 ± 298	2377	48,198	50,400 ± 2812
	4650	4014			53,568	
					49,434	
5 chlorpyrifos	2614	2020	2293 ± 448	737	53,505	48,840 ± 6653 (3% decrease)
	2728	1808	(46% decrease)		51,794	
					41,222	
10 chlorpyrifos	636	324	434 ± 264	433	29,675	31,400 ± 4216 (38% decrease)
	112	664	(90% decrease)		28,319	
					36,205	
20 chlorpyrifos	UR	UR	UR	354	14,440	12,669 ± 1555 (75% decrease)
	UR	UR			12,038	
					11,529	
1 chlorpyrifos–oxon	396	UR	212 (95% decrease)	633	53,257	51,422 ± 3160
	28	UR			47,773	
					53,236	
5 chlorpyrifos–oxon	UR	UR	UR	295	52,353	51,839 ± 1221
	UR	UR			52,720	
					50,445	
10 chlorpyrifos–oxon	UR	UR	UR	362	50,331	52,183 ± 3574
	UR	UR			49,915	
					56,303	

U/L, international units/L; an international enzyme unit per liter (U/L) is defined as the activity of enzyme which converts 1 μmol/L of acetylthiocholine in one minute at standard conditions.
UR, under range (lowest range of standard curve = 20).
[a] Hemolysis was noted in some plasma samples (subsequent procedural change made to accommodate analytical sampling).

measured in various tissues (plasma, RBCs, brain). Time-of-peak ChE inhibition was determined experimentally using one dose level in the pilot study, then modeled across dose levels using the CPF PBPK/PD model (Timchalk et al., 2002, 2006; Supplemental data 1).

2.2.3. Phase 3: definitive acute dose–response study

The experimental design for the acute study examining the dose–response for ChE inhibition in PND 11 pups after acute CPF or CPFO administration is outlined in Table 1. Male and female pups (8/sex/dose; PND 11) and adult female rats (8/dose; ~70 days of age) were given a single gavage dose of 0, 0.05, 0.1, 0.5, 2, 5 (pups only) or 10 (adults only) mg/kg CPF in corn oil or 0, 0.005 (pups only), 0.01, 0.05, 0.1, 0.5, or 1 (adults only) mg/kg CPFO in corn oil. Prior to euthanasia, pups and adult female rats were given clinical observations to determine whether signs of cholinergic toxicity were present.

Samples (blood and brain) were collected at the time-of-peak ChE inhibition (CPF in corn oil: 6 h post-dosing in pups and 8 h post-dosing in adults; CPFO in corn oil: 4 h post-dosing in pups and adults).

A separate group of PND 11 male and female pups (8/sex/dose) were given a single gavage dose of 0, 0.05, 0.1, 0.5, 2, or 5 mg/kg

CPF in rat milk. This dosing scenario was designed to evaluate the effect of vehicle on ChE inhibition and blood levels of CPF, CPFO and TCP. Pups were terminated at the time-of-peak ChE inhibition (8 h after dosing as determined in the pilot study and by PBPK/PD modeling).

A separate group of females (8/dose; ~70 days of age) was exposed to CPF in the diet for 12 h at concentrations designed to achieve similar mg/kg doses to gavage CPF (0, 0.05, 0.1, 0.5, 2 or 10 mg/kg). This dosing scenario is more consistent with acute dietary exposures in humans and can be compared with adults exposed via CPF gavage in corn oil. Females in the dietary group were given CPF-supplemented diet at the start of the dark cycle for 12 h, and the amount of feed consumed was measured. Females were terminated at the time-of-peak ChE inhibition (8 h after removal of the CPF-containing diets as determined in the pilot study and by PBPK/PD modeling). Test material intake (mg/kg body weight/day) was calculated for the 12 h dietary exposures.

2.2.4. Phase 4: definitive repeat-dose study

ChE inhibition in plasma, RBCs and brain was examined in preweanling (male and female) and adult female rats after repeated gavage exposures (11 days) to CPF or CPFO in corn oil (Table 1). The experimental design used litters with all male or all female pups, and assigned pups within each litter to a CPF- or CPFO-exposure group. PND 11 male and female pups (8/sex/dose) and adult female rats (8/dose) were given daily gavage doses of CPF in corn oil at doses of 0, 0.05, 0.1, 0.5, 1, 3.5 mg/kg/day or CPFO in corn oil at doses of 0, 0.01 or 0.5 mg/kg/day for 11 days. CPF doses were selected to produce substantial brain ChE inhibition at the highest dose level. CPFO, which had only two treatment groups per age, was designed to include a high dose that caused RBC ChE inhibition, because brain inhibition was not seen with CPFO exposure. Pups and adults received daily clinical observations on PND 11–19 and 21 for pups and approximately 70–78 and 80 days of age for adults. A functional observational battery (FOB) was conducted at the approximate time-of-peak inhibition on PND 20 (preweanlings) or at ~79 days of age (adults) to determine whether signs of cholinergic toxicity were present. The FOB, which was conducted according to previously described procedures (Mattsson et al., 1986, 1997), included cage-side, hand-held and open-field observations, and measurements of body weight, rectal temperature, fore- and hindlimb grip performance, and motor activity. The FOB was conducted by an observer who was blind to the treatment status of the animal. The same observer was used for all rats. Adult and immature rats were euthanized at 80 and 21 days of age, respectively. Samples (blood and brain) were collected at the time-of-peak ChE inhibition determined in the acute study (6 h and 8 h after the last dose of CPF in pups and adults, respectively, and 4 h after the last dose of CPFO). In addition, an aliquot of the terminal blood sample was used to determine internal dosimetry for CPF, CPFO and/or TCP.

2.3. Sample collection

Rats were anesthetized with isoflurane. Blood samples were collected from the inferior vena cava (adults) or by heart nick with blood collection into capillary tubes (pups). Rats were euthanized by exsanguination followed by decapitation. An aliquot of blood (4/dose) was analyzed for CPF, CPFO, and TCP using analytical methods described in Brzak et al. (1998) and Mattsson et al. (2000). Remaining blood samples were placed into heparinized tubes and kept on ice. Brain tissue also was collected from each rat, weighed and quick frozen in liquid nitrogen. Blood was centrifuged to separate plasma and packed RBCs. RBCs were diluted in 1% Triton X-100. Samples were stored frozen (−80 °C) until shipped on dry ice to WIL Research Laboratories LLC (Ashland, OH) for analysis of ChE activity using a modified Ellman method (Ellman et al., 1961; Hunter et al., 1997). Frozen brain samples were diluted in 10 volumes of 1% Triton X-100 (based on brain weight), immediately homogenized, centrifuged, and analyzed for ChE activity.

2.4. Statistics

ChE activity was analyzed separately for adults, male and female pups to determine whether a significant, dose-related difference in ChE inhibition exists. These analyses involved a one-way analysis of variance (ANOVA) using dose as a factor. Descriptive statistics (mean and standard deviation) were reported for blood levels of CPF, CPFO and TCP. Statistical analyses were conducted on body weights (collected at FOB time points), grip performance, rectal temperature, motor activity and FOB observations, with analyses being conducted separately for each age, sex and test material. These analyses are described in Supplemental data 2.

3. Results

3.1. Phase 1: range-finding study

Adult females were exposed to 0, 5, 10 or 20 mg/kg CPF or 1, 5 or 10 mg/kg CPFO to identify dose levels that produced marked inhibition of brain ChE without maximum inhibition of RBC ChE activity. Samples were collected at 5 h post-dosing. The high dose level (20 mg/kg CPF) produced 75% brain ChE inhibition, with >99% inhibition of RBC ChE activity (Table 2). At 10 mg/kg CPF, brain and RBC ChE activity were decreased by 38% and 90%, respectively. The highest dose of CPFO (10 mg/kg) produced >99% RBC ChE inhibition, but did not alter brain ChE activity in adult female rats. The lowest CPFO dose (1 mg/kg) decreased RBC ChE activity by 95%. Based on these data, 0 and 10 mg/kg CPF and 0 and 0.3 mg/kg CPFO were selected for the time-of-peak inhibition studies in adults. Dose levels in the time-of-peak inhibition studies in PND 11 pups were adjusted to 0 and 3 mg/kg CPF and 0 and 0.5 mg/kg CPFO based on the magnitude of ChE inhibition seen in adult female rats.

3.2. Phase 2: time-of-peak inhibition studies

Studies were conducted to determine the time-of-peak inhibition for RBC, brain and plasma ChE in pups and adults using the different acute dosing scenarios. Time-of-peak inhibition for brain ChE was used for subsequent experiments. For PND 11 pups, the times-to-peak inhibition were 6 h post-dosing for CPF in corn oil, 4 h post-dosing for CPFO in corn oil and 8 h post-dosing for CPF in milk. For adult female rats, the times-to peak inhibition were 8 h post-dosing for CPF in corn oil, 4 h post-dosing for CPFO in corn oil, and 8 h post-exposure for CPF in diet (i.e., after conclusion of the 12-h dietary exposure to CPF-containing diet). Using the PBPK/PD model, the time-of-peak brain ChE inhibition was shown to be similar across dose levels within each age group; therefore, the same sample collection times could be used. The results of the time-of-peak inhibition studies and subsequent modeling appear in Supplemental data 1.

3.3. Phase 3: definitive acute dose–response study

The definitive acute comparative ChE study examined age-related differential sensitivity across multiple dose levels of CPF or CPFO after a single exposure. Samples were collected at the time-of-peak ChE inhibition. Blood levels of CPF, CPFO and TCP also were determined at these time points. There were no treatment-related clinical observations during any phase of the acute studies.

(a) CPF in corn oil: data for ChE activity measured in PND 11 male and female CD rat pups (8/sex/dose; 0.05–5 mg/kg sampled 6 h post-dosing) and adult female CD rats (8/dose; 0.05–10 mg/kg sampled 8 h post-dosing) exposed to a single gavage dose of CPF (corn oil vehicle) are shown in Table 3. At both ages, the highest dose level was designed to provide an anchoring point for the dose–response curve, causing consistent, measurable brain ChE inhibition. There were no discernable gender differences in ChE activity in male or female PND 11 pups in response to CPF treatment. At the highest dose of CPF (5 mg/kg in PND 11 pups and 10 mg/kg in adults), both pups and adults had significant and comparable decreases in brain ChE activity (42.5–49% of control brain ChE). Brain ChE was not inhibited in pups or adults at 2.0 mg/kg CPF. Both ages also showed significant RBC and plasma ChE inhibition at doses ⩾2 mg/kg. The 0.5 mg/kg CPF dose was considered a no-observed-effect-level (NOEL) for ChE inhibition across all tissues in both age groups with acute exposure.

Figs. 1A and 2A show blood levels of CPF and CPFO relative to ChE inhibition across tissues with samples collected at the time-of-peak ChE inhibition. Consistent with the ChE inhibition data, there were no apparent gender-related differences in blood levels of CPF or its metabolites in PND 11 pups; therefore, the figures show mean pup values. In PND 11 pups, CPF and TCP were detectable in blood at all doses of CPF, whereas CPFO was below the level of quantitation (LLQ) at ⩽0.1 mg/kg CPF and only had one value from four samples that was above the LLQ at 0.5 mg/kg CPF (Fig. 1A). Increases in blood CPF levels were approximately dose proportional at doses ⩽2 mg/kg, whereas at 5 mg/kg, CPF blood levels were 466× greater than blood levels at 0.05 mg/kg CPF with only a 100× increase in dose. This may suggest different kinetics at the high dose of 5 mg/kg than at lower dose levels. Furthermore, the [TCP]:[CPF] ratio was lower at 5 mg/kg than 0.05 mg/kg (21 vs. 300, respectively), suggesting that the relative amount of CPF metabolized to TCP is inversely related to CPF dose. This interpretation is consistent with predictions by the PBPK/PD model for CPF (Timchalk et al., 2002). In adult females, blood CPF was detectable at doses ⩾0.5 mg/kg and TCP was detectable at all dose levels in adult females; whereas CPFO was at or below the LLQ at all doses of CPF (Fig. 2A). It was difficult to fully evaluate dose proportionality (i.e., [TCP]:[CPF] ratio) in adult females with so few data points for CPF, but TCP levels were approximately dose proportional.

When comparing TK data with ChE inhibition data, it is important to note that samples were collected at 6 h post-dosing in PND 11 pups compared with 8 h post-dosing in adults. Furthermore, the time-of-peak ChE inhibition may not correspond with the time-of-peak blood levels of CPF or its metabolites, particularly given the slow recovery of ChE activity. The peak metabolite levels may occur earlier than the sampling times chosen for ChE measurements. However, these TK data were collected to aid in current understanding of metabolite ratios between dose levels, vehicle and age. These ratios provide useful information on relative metabolism of CPF between the experimental groups and are useful to refine PB/PK/PD models of chlorpyrifos in the adult and neonatal rat.

The TK data indicate that pups were exposed to higher blood concentrations of CPF than adults at high doses (e.g., at 5 mg/kg, pups had 3× higher blood CPF levels than adults given 10 mg/kg). In addition, pups had detectable levels of CPFO at these dose levels (5 vs. 10 mg/kg in adults), whereas pups had lower levels of TCP (67% of adult levels at these doses). At 2 mg/kg, PND 11 pups had 10× higher levels of blood CPF than adults; however, unlike the profile seen at the high dose, pups also had 2× higher blood TCP levels than adults, indicating that pups were metabolizing proportionately more CPF at this lower dose. There was no brain inhibition in either pups or adults at 2 mg/kg CPF.

Thus, the overall profile of effects was that pups were more sensitive to ChE inhibition at higher doses of CPF (e.g., 5 mg/kg); however, at lower CPF levels, pups had lower ChE inhibition than adults at comparable blood levels of CPF. There was no evidence of age-related differential sensitivity to acute CPF exposure over the lower portion of the dose–response curve.

(b) CPF in rat milk to PND 11 pups: data for ChE activity measured in PND 11 male and female CD rat pups (8/sex/dose; 0.05–5 mg/kg sampled 8 h post-dosing) exposed to a single gavage dose of CPF (rat milk vehicle) are shown in Table 4. Similar to the experiments using corn oil vehicle, there were no discernable gender differences in ChE activity in male or female PND 11 pups in response to CPF

Table 3
ChE inhibition in PND 11 pups and adult females following an acute dose of CPF in corn oil.

Dose (mg/kg)	RBC ChE (U/L)	RBC ChE (% control)	Brain ChE (U/L)	Brain ChE (% control)	Plasma ChE (U/L)	Plasma ChE (% control)
Adults – CPF in corn oil						
0	5409 ± 351	100.0	54,728 ± 1283	100.0	2143 ± 803	100.0
0.05	5146 ± 533	95.1	54,461 ± 2301	99.5	2317 ± 635	108.2
0.1	5494 ± 357	101.6	51,116 ± 2484	93.4	1944 ± 684	90.7
0.5	5975 ± 1150	110.4	52,243 ± 1586	95.5	1874 ± 262	87.5
2.0	**4360 ± 536***	80.6	52,194 ± 2580	95.4	**981 ± 284***	45.8
10.0	**851 ± 622***	15.7	**23,276 ± 11,567***	42.5	**283 ± 80***	13.2
PND 11 male pups – CPF in corn oil						
0	6891 ± 782	100.0	22,765 ± 3336	100.0	1407 ± 111[a]	100.0
0.05	7034 ± 923	102.1	25,891 ± 2664	113.7	1440 ± 221	102.3
0.1	7195 ± 861	104.4	24,339 ± 1508	106.9	1494 ± 177	106.2
0.5	6538 ± 544	94.9	25,202 ± 1847	110.7	1301 ± 89	92.4
2.0	**4434 ± 590***	64.3	22,346 ± 2910	98.2	**690 ± 63***	49.0
5.0	**804 ± 249***	11.7	**11,163 ± 2821***	49.0	**321 ± 54***	22.8
PND 11 female pups – CPF in corn oil						
0	6432 ± 776	100.0	24,629 ± 3768	100.0	1421 ± 106	100.0
0.05	6674 ± 634	103.8	25,125 ± 2987	102.0	1409 ± 150	99.1
0.1	6518 ± 677	101.3	24,910 ± 3080	101.1	1374 ± 200	96.7
0.5	6345 ± 1308	98.7	25,372 ± 2069	103.0	1283 ± 136	90.3
2.0	**4441 ± 392***	69.0	22,933 ± 3188	93.1	**752 ± 141*,a**	53.0
5.0	**873 ± 282***	13.6	**10,955 ± 1517***	44.5	**313 ± 69***	22.0

$n = 8$ pups/sex/dose level or 8 adult females/dose level unless otherwise indicated.
U/L, international units/L.
[a] $n = 7$ in the male pup plasma control group and the female pup plasma 2.0 mg/kg dose group.
* Significantly different from controls at alpha = 0.05 using Dunnett's test when raw ChE data were analyzed (Treatment-related effects in bold text).

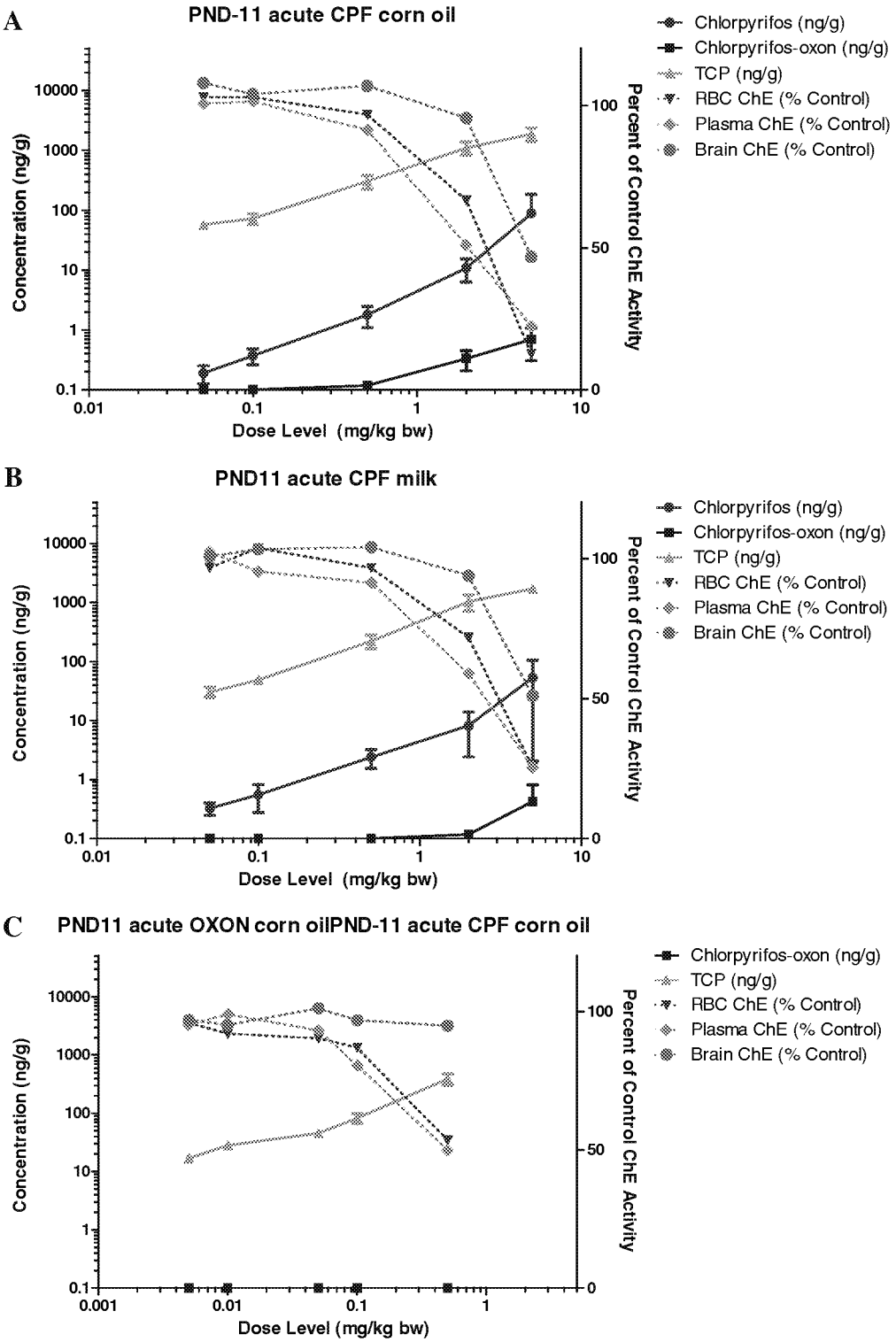


Fig. 1. Mean RBC, brain and plasma ChE inhibition relative to mean blood CPF, CPFO and TCP levels in PND 11 pups following acute gavage exposure at multiple dose levels to CPF in corn oil vehicle (A; sampled at 6 h post-dosing), milk vehicle (B; sampled at 8 h post-dosing), or CPFO in corn oil vehicle (C; sampled at 4 h post-dosing). Data show mean values of combined male and female pup data as there were no gender-related differences in either ChE inhibition or blood CPF/CPFO levels. (n = 7–8 pups/sex/dose level for ChE measurements; n = 4 pups/dose level for determination of blood CPF, CPFO and TCP levels).

treatment. ChE inhibition across tissues was comparable in PND 11 pups using either milk or corn oil vehicle. At 5 mg/kg CPF, brain ChE was inhibited to approximately 47% of control in corn oil compared with 51% of control in rat milk. As with corn oil, brain ChE was not inhibited at 2.0 mg/kg CPF in milk. Similarly, RBC and plasma ChE at 2.0 mg/kg CPF were inhibited to approximately 67% and 51% of control values using corn oil vehicle compared with 72% and 59% of control values using milk vehicle. There were no significant effects in ChE values at 0.5 mg/kg, which was the NOEL for ChE inhibition across all tissues regardless of vehicle.

Fig. 1B shows blood levels of CPF and CPFO relative to ChE inhibition across tissues with samples collected at the time-of-peak

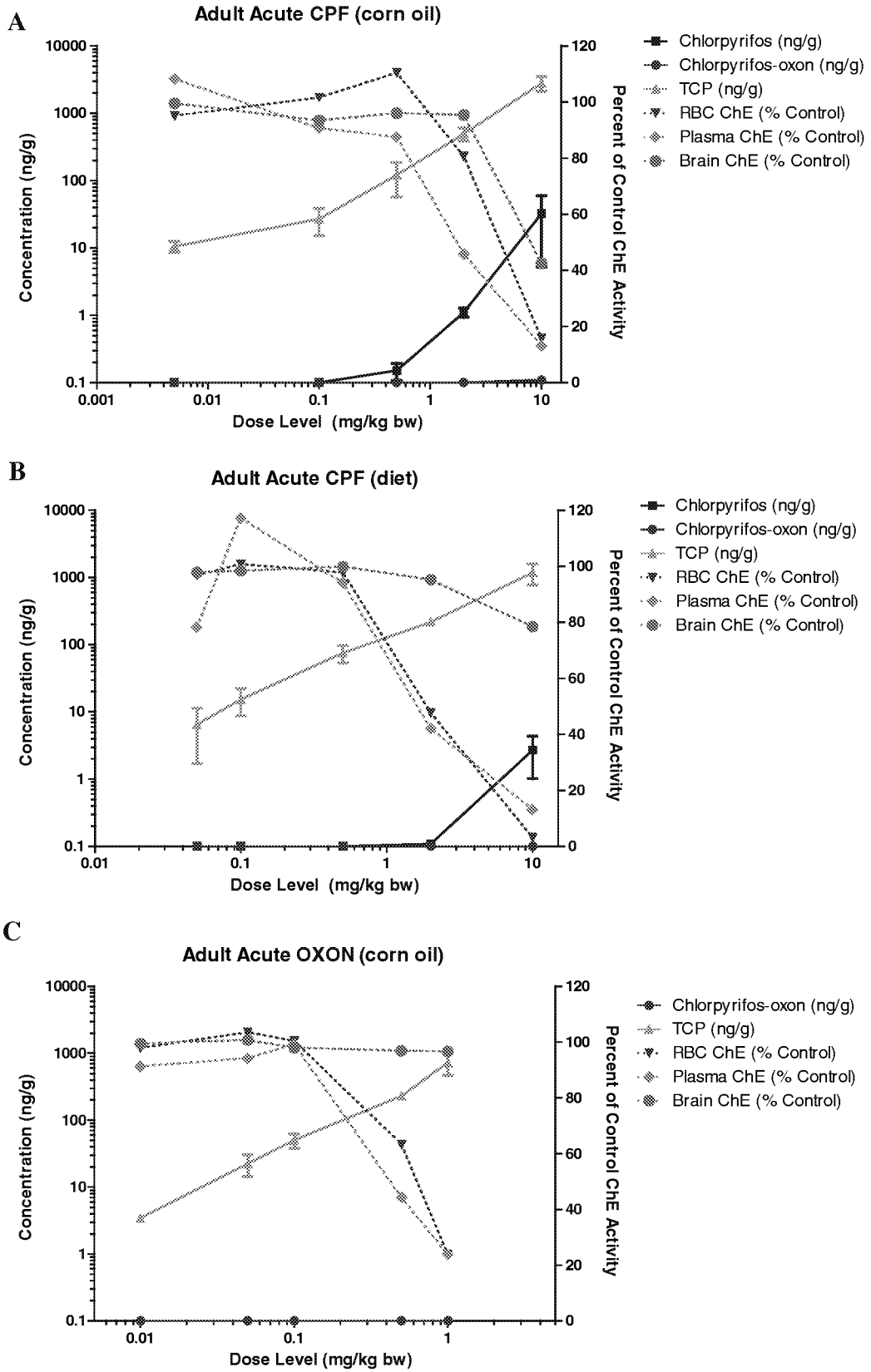


Fig. 2. Mean RBC, brain and plasma ChE inhibition relative to mean blood CPF, CPFO and TCP levels in adult female rats following acute exposure to CPF at multiple dose levels in corn oil vehicle (gavage) (A; sampled at 8 h post-dosing), 12-h dietary exposure (B; sampled 8 h after removal of test diet), or CPFO in corn oil vehicle (gavage) (C; sampled at 4 h post-dosing). ($n = 8$ rats/dose level for ChE measurements; $n = 4$ rats/dose level for determination of blood CPF, CPFO and TCP levels).

ChE inhibition. Consistent with the ChE inhibition data, there were no apparent gender-related differences in blood levels of CPF or its metabolites in PND 11 pups; therefore, the figure shows mean pup values. At the time-of-peak inhibition, the blood CPF and CPFO

Table 4
ChE inhibition in PND 11 pups following an acute dose of CPF in rat milk.

Dose (mg/kg)	RBC ChE (U/L)	RBC ChE (% control)	Brain ChE (U/L)	Brain ChE (% control)	Plasma ChE (U/L)	Plasma ChE (% control)
PND 11 male pups – CPF in milk						
0	6305 ± 1460	100.0	23,911 ± 1873	100.0	1613 ± 185	100.0
0.05	6017 ± 1385	95.4	24,489 ± 3432	102.4	1629 ± 181	101.0
0.1	6591 ± 924	104.5	25,279 ± 2155	105.7	1573 ± 239	97.5
0.5	6320 ± 1028	100.2	25,325 ± 2815	105.9	1510 ± 142	93.6
2.0	4459 ± 1916	70.7	24,760 ± 2744	103.6	**985 ± 198***	61.1
5.0	**1805 ± 1706***	28.6	**13,918 ± 4867***	58.2	**472 ± 508***	29.2
PND 11 female pups – CPF in milk						
0	6324 ± 648	100.0	26,410 ± 3026	100.0	1586 ± 137	100.0
0.05	6197 ± 614	98.0	26,113 ± 2979	98.9	1646 ± 171	103.7
0.1	6501 ± 979	102.8	26,609 ± 2104	100.8	1476 ± 179	93.0
0.5	5877 ± 1505	92.9	26,969 ± 1413	102.1	1410 ± 160	88.9
2.0	**4600 ± 1759***	72.7	22,201 ± 4811	84.1	**896 ± 220***	56.5
5.0	**1375 ± 1634***	21.7	**11,511 ± 5332***	43.6	**341 ± 239***	21.5

$n = 8$ pups/sex/dose level.
U/L, international units/L.
* Significantly different from controls at alpha = 0.05 using Dunnett's test when raw ChE data were analyzed (treatment-related effects in bold text).

levels, as well as the magnitude of ChE inhibition across tissues, were similar with both milk and corn oil vehicles. As with CPF in corn oil, CPF and TCP were detectable in blood at all doses of CPF in rat milk, whereas CPFO was below the LLQ at ⩽0.5 mg/kg CPF (vs. the 0.1 mg/kg dose with CPF in corn oil) and only had one value from four samples that was above the LLQ at 2.0 mg/kg CPF. Increases in blood CPF levels were approximately dose proportional at doses ⩽2 mg/kg, whereas at 5 mg/kg, CPF blood levels were 165× greater than blood levels at 0.05 mg/kg CPF with only a 100× increase in dose. This value was not as great as the 466× differential seen with CPF in corn oil; however, there may have been an impact of collecting samples 2 h later with CPF in milk (8 h post-dosing vs. 6 h with corn oil). Again, these data suggest different kinetics at the high dose of 5 mg/kg than lower dose levels, regardless of milk or corn oil vehicle. Furthermore, the [TCP]/[CPF] ratio was lower at 5 mg/kg than 0.05 mg/kg (32 vs. 95, respectively), again suggesting that the relative amount of CPF metabolized to TCP was decreased when the CPF dose reached 5 mg/kg in milk. This interpretation is consistent with predictions by the PBPK/PD model for CPF.

At the time-of-peak inhibition, blood CPF and CPFO levels, as well as the magnitude of ChE inhibition across tissues, were similar with both milk and corn oil vehicles. Thus, PND 11 pups appeared to be equally sensitive to ChE inhibition following acute CPF exposure in either corn oil or rat milk vehicle.

(c) CPF in diet to adult females: data for ChE activity measured in adult female rats (8/sex/dose; 0.05–10 mg/kg) exposed for 12-h to dietary CPF are shown in Table 5. The dietary route was included as it was considered a more relevant dosing scenario. Based on body weights and feed consumption over the 12-h period, dietary test material intake for these females was 0.05, 0.10, 0.53, 2.06, or 9.59 mg/kg for the nominal doses of 0, 0.05, 0.1, 0.5, 2 or 10 mg/kg. Samples were collected at 8 h post-dosing as determined by a limited probe study (data in Supplemental data 1) and modeled using the existing PBPK/PD model. Results of these experiments are shown in Table 5. With a 12 h dietary exposure to CPF, adult females exhibited significant brain ChE inhibition at the same concentration as CPF given via gavage in corn oil (10 mg/kg); however, the magnitude of brain ChE inhibition was less by the dietary route (76.3% of control values compared with 42.5% of control in the oral gavage group). Similar to corn oil gavage, brain ChE was not inhibited at dietary doses ⩽2 mg/kg CPF. Conversely, RBC ChE activity appeared to be slightly more sensitive to dietary ChE with inhibition to 47.7% of control values at 2 mg/kg CPF (compared with 80.6% of control with gavage exposure at this dose). Again, RBC ChE was not inhibited at dietary doses ⩽0.5 mg/kg CPF as was seen with gavage dosing. Plasma ChE showed a similar response to RBC ChE activity with similar sensitivity via the dietary and gavage routes. As with corn oil gavage, 0.5 mg/kg CPF was the NOEL for ChE inhibition across all tissues.

Fig. 2B shows blood levels of CPF and CPFO relative to ChE inhibition across tissues with samples collected at the time-of-peak ChE inhibition. At the time-of-peak inhibition, the blood CPF and CPFO levels, as well as the magnitude of ChE inhibition across tissues, were different from the curves seen with corn oil vehicle. At 8 h post-exposure, blood CPF was >LLQ in all animals at 10 mg/kg and in one of four animals at 2 mg/kg and CPFO was below the LLQ in all samples, making the TK data somewhat limited for these analytes. However, TCP was detectable at all doses of CPF and increased in a dose-proportional manner. With only limited determinations, [TCP]:[CPF] ratio was similar to other dosing scenarios with a decrease in the proportion of CPF metabolized to

Table 5
ChE inhibition in adult females following an acute 12-h exposure to CPF in the diet.

Dose (mg/kg)	RBC ChE (U/L)	RBC ChE (% control)	Brain ChE (U/L)	Brain ChE (% control)	Plasma ChE (U/L)	Plasma ChE (% control)
Adults – CPF in diet						
0	5337 ± 305	100.0	53,888 ± 1242	100.0	1754 ± 395	100.0
0.05	5164 ± 575	96.8	52,700 ± 2265	97.8	1373 ± 249	78.3
0.1	5380 ± 525	100.8	53,020 ± 1698	98.4	2058 ± 458	117.3
0.5	5219 ± 575	97.8	53,844 ± 1737	99.9	1648 ± 364	94.0
2.0	**2548 ± 446***	47.7	51,294 ± 1639	95.2	**737 ± 190***	42.0
10.0	**167 ± 146***	3.1	**41,125 ± 7650***	76.3	**230 ± 82***	13.1

$n = 8$ adult females/dose level.
U/L = international units/L.
* Significantly different from controls at alpha = 0.05 using Dunnett's test when raw ChE data were analyzed (treatment-related effects in bold text).

TCP at the highest dose (10 mg/kg). Furthermore, blood CPF and TCP levels were 7.4× and 2.4× lower, respectively, by the dietary route than via corn oil gavage. This result suggests that dose rate impacts blood levels of CPF and its metabolites and subsequently, the magnitude of brain ChE inhibition.

While plasma ChE was similarly inhibited by both corn oil gavage and diet, RBC ChE was more inhibited by the dietary route at 2 mg/kg (47.7% compared with 80.6% by gavage), despite having approximately 10× and 2.3× lower blood levels of CPF and TCP, respectively. Thus, dose rate impacts blood levels of CPF and its metabolites and shifts the amount of ChE inhibition across tissues.

(d) CPFO in corn oil: data for ChE activity measured in PND 11 male and female CD rat pups (8/sex/dose; 0.005–1 mg/kg sampled 4 h post-dosing) and adult female CD rats (8/dose; 0.005–1 mg/kg sampled 4 h post-dosing) exposed to a single gavage dose of CPFO (corn oil vehicle) are shown in Table 6. At both ages, the highest dose level was designed to provide an anchoring point for the dose–response curve, causing consistent, measurable RBC ChE inhibition. There were no discernable gender differences in ChE activity in male or female PND 11 pups in response to CPFO treatment. There was no significant inhibition of brain ChE in either pups or adults at any dose level of CPFO. This result was consistent with results seen in the range-finding study with adult rats, in which no brain inhibition was seen at 10 mg/kg CPFO in corn oil, despite near complete inhibition of RBC ChE (Table 2). RBC ChE inhibition was seen at the same dose level (0.5 mg/kg) in PND 11 pups and adults (approximately 53% and 63% of control RBC ChE in pups and adults, respectively); however, PND 11 pups exhibited significant plasma ChE inhibition at a slightly lower dose than adults (0.1 mg/kg, where pup plasma ChE was approximately 80.5% of control compared with 99% in adult females).

Figs. 1C and 2C show blood levels of CPFO relative to ChE inhibition across tissues with samples collected at the time-of-peak ChE inhibition. Consistent with the ChE inhibition data, there were no apparent gender-related differences in blood levels of CPFO in PND 11 pups; therefore, the figures show mean pup values. Plasma ChE inhibition in pups at 0.1 mg/kg CPFO may be related to higher CPFO blood levels as suggested by the 1.6× higher blood TCP levels in pups at this dose level (CPFO was below the LLQ in pups and adults). In adults, CPFO was below the LLQ at all dose levels, whereas TCP was detectable at all doses of CPFO. In both pups and adults, blood TCP levels increased in a dose-related fashion, although the increase was lower than dose proportional in PND 11 pups at CPFO doses ⩾0.05 mg/kg (i.e., with a 100× increase in CPFO dose from 0.005 to 0.5 mg/kg, TCP levels increased only 23×) and at or slightly greater than dose proportional in adults.

With samples collected at the same time post-dosing (4 h), pups and adults had similar levels of ChE inhibition in RBCs and plasma at 0.5 mg/kg CPFO, although pups had 1.7× higher levels of blood TCP. At 0.1 mg/kg CPFO, neither pups nor adults had significant inhibition of RBC ChE, but pups had significant inhibition of plasma ChE. Pup blood TCP was again 1.6× higher than blood TCP levels in adults; thus, it was possible that higher CPFO dosimetry in pups explained this difference. At 0.05 mg/kg CPFO, pups had similar levels of TCP as adults at 0.1 mg/kg CPFO. At this internal dose, there was no inhibition of ChE in any tissue. Brain ChE was not inhibited at any dose of CPFO in either pups or adults when the highest oxon dose (0.5 mg/kg) yielded TCP levels of 387.5 ng/g and 724.8 ng/g, respectively.

Thus, the 0.1 mg/kg CPFO dose was considered a NOEL for ChE inhibition in RBC in PND 11 pups and a NOEL in both RBC and plasma for adult females with acute exposure. Due to plasma ChE inhibition at 0.1 mg/kg CPFO, 0.05 mg/kg CPFO was the NOEL across all tissues in PND 11 pups with acute CPFO exposure.

3.4. Phase 4: definitive repeat-dose study

The definitive repeat-dose study examined age-related differential sensitivity across multiple dose levels of CPF or CPFO after 11 daily doses administered by gavage. Blood levels of CPF, CPFO and TCP also were determined in terminal samples collected at the time-of-peak inhibition determined in the acute studies.

(a) CPF in corn oil: in the repeated dose study, there were no treatment-related clinical observations in male or female rat pups (8/sex/dose; dosed PND 11–21) or adult female rats (8/dose; dosed PND 70–80) following exposure to eleven daily gavage doses (corn oil vehicle) of 0, 0.05, 0.1, 0.5, 1, or 3.5 mg/kg/day CPF (data not shown). Neither pup nor adult female body weights were affected

Table 6
ChE inhibition in PND 11 pups and adult females following an acute dose of CPFO in corn oil.

Dose (mg/kg)	RBC ChE (U/L)	RBC ChE (% control)	Brain ChE (U/L)	Brain ChE (% control)	Plasma ChE (U/L)	Plasma ChE (% control)
Adults – CPFO in corn oil						
0	5630 ± 906	100.0	52,826 ± 2036	100.0	2106 ± 562	100.0
0.01	5510 ± 662	97.9	52,517 ± 1503	99.4	1922 ± 369	91.3
0.05	5830 ± 402	103.5	53,203 ± 2367	100.7	1984 ± 374	94.2
0.1	5655 ± 210	100.4	51,751 ± 991	98.0	2088 ± 736	99.2
0.5	**3572 ± 905***	63.4	51,169 ± 1299	96.9	**931 ± 369***	44.2
1.0	**1338 ± 497***	23.8	51,046 ± 1830	96.6	**502 ± 141***	23.8
PND 11 male pups – CPFO in corn oil						
0	6564 ± 513	100.0	25,539 ± 1711	100.0	1445 ± 118	100.0
0.005	6174 ± 828	94.1	24,558 ± 2114	96.2	1362 ± 115	94.2
0.01	5565 ± 1281	84.8	23,709 ± 2580	92.8	1466 ± 127	101.4
0.05	6159 ± 807	93.8	24,800 ± 3116	97.1	1378 ± 210	95.4
0.1	5506 ± 977	83.9	24,798 ± 2812	97.1	**1182 ± 190***	81.8
0.5	**3534 ± 489***	53.8	23,489 ± 3133	92.0	**738 ± 72***	51.1
PND 11 female pups – CPFO in corn oil						
0	6287 ± 856	100.0	22,994 ± 4168	100.0	1487 ± 210	100.0
0.005	6146 ± 1557	97.7	22,436 ± 1790	97.6	1428 ± 125	96.0
0.01	6227 ± 1234	99.0	22,358 ± 2620	97.2	1433 ± 172	96.4
0.05	5444 ± 1025	86.6	24,178 ± 1236	105.2	1352 ± 200	90.9
0.1	5651 ± 1274	89.9	22,202 ± 3687	96.6	**1179 ± 124***	79.3
0.5	**3329 ± 772***	52.9	22,462 ± 2964	97.7	**723 ± 62***	48.6

n = 8 pups/sex/dose level or 8 adult females/dose level.
U/L, international units/L.
* Significantly different from controls at alpha = 0.05 using Dunnett's test when raw ChE data were analyzed (treatment-related effects in bold text).

by repeated CPF exposure (data not shown except FOB body weights in Supplemental data 2). There were no treatment-related effects on neurobehavioral endpoints, which included a FOB and motor activity, evaluated at the time-of-peak ChE inhibition after the 10th dose of CPF (Supplemental data 2).

Data for ChE activity measured in PND 21 male and female rat pups and 80-day-old female rats exposed to various doses of CPF for eleven days are shown in Table 7. At both ages, the highest dose levels were designed to provide an anchoring point for the dose–response curves, causing consistent, measurable brain ChE inhibition. Samples for ChE inhibition were collected at the time-of-peak inhibition determined in the acute studies – 6 h post-dosing for the pups and 8 h post-dosing in the adults. At the high dose of CPF (3.5 mg/kg/day), pups had similar ChE inhibition to adults across all tissues. At 1 mg/kg/day, ChE was significantly decreased in both pups and adults across all tissues, although adult brain ChE activity was 91.1% of control, a change that was not considered biologically meaningful. At 0.5 mg/kg/day, there was no significant brain ChE inhibition in either adults or pups, but both age groups had significant decreases in RBC ChE activity and plasma ChE activity with no apparent difference in sensitivity. RBC ChE was significantly decreased in adults at 0.1 mg/kg/day, but this result was considered spurious because plasma ChE, which has similar or greater sensitivity to ChE inhibition by CPF (Lotti, 1995; Garabrant et al., 2009; UE EPA, 2008; Timchalk et al., 2006) was not inhibited at this dose. Thus, for a given CPF dose level, pups were equally sensitive to plasma ChE inhibition as adults. The 0.1 mg/kg/day CPF dose was considered a no-observed-effect-level (NOEL) for ChE inhibition across all tissues in both age groups with repeated exposure.

Fig. 3 shows blood levels of CPF and CPFO relative to ChE inhibition across tissues with samples collected at the time-of-peak ChE inhibition as determined in the acute CPF experiments. In PND 21 pups, CPF and TCP were detected in blood at all doses of CPF, whereas CPFO was below the LLQ (Fig. 3A). Overall, there were dose proportional increases in blood CPF and TCP levels; that is, as the doses increased from 0.05 to 3.5 (70× from low to high dose), blood CPF and TCP levels increased approximately 74× and 82×, respectively. There was no consistent pattern in the ratio of blood [TCP]:[CPF] across dose groups. For adults, CPF was detected in blood samples at doses ⩾0.5 mg/kg/day CPF, whereas CPFO was below the LLQ at all doses (Fig. 3B). TCP was detected in blood at all dose levels. There was a dose proportional increase in blood TCP levels; that is, as the doses increased from 0.05 to 3.5 (70× from low to high dose), blood TCP levels increased 78×. With limited data on CPF levels in blood, a pattern in the ratio of blood [TCP]:[CPF] could not be evaluated.

Blood levels of CPF and its metabolites facilitated interpretation of repeat-dose data. For example, at 0.5–1.0 mg/kg/day CPF, male pup RBC ChE was 63.2% and 38.7% of control compared with 81.8% and 56.0% of control in female pups at these dose levels. Based on blood values, there were no clear gender-related differences in CPF and TCP levels to support a differential sensitivity of male vs. female RBC ChE activity. Furthermore, plasma ChE inhibition was similar in both sexes at these doses. Therefore, it was concluded that this apparent gender-related difference in RBC ChE sensitivity was spurious. Gender-related differences in sensitivity were not seen in preweanling rats in other CPF studies (e.g., Moser and Padilla, 1998). As with the acute studies, pups had lower ChE inhibition across tissues at comparable CPF blood levels (e.g., approximately 27% RBC and plasma ChE inhibition and 2% brain ChE inhibition with CPF blood levels of 0.60 ng/g in pups compared with 71% RBC and plasma ChE inhibition and 9% brain ChE inhibition with 0.54 ng/g blood CPF in adults). The later sampling time in adults (8 h vs. 6 h in pups), coupled with a somewhat higher metabolic rate in adults, may have resulted in comparable blood levels. At 1 mg/kg/day, it appeared that RBC and plasma were less affected in pups, whereas brain was more affected; however, the magnitude of the brain ChE inhibition in pups was consistent with other brain ChE results for these blood CPF levels (i.e., mean pup blood CPF was 1.69 ng/g with 76% of control brain ChE activity; these values were between the blood CPF levels (0.54 and 2.21 ng/g, respectively) and brain ChE activity (91.1 and 31% of control, respectively) in adults given 1 or 3.5 mg/kg/day CPF, respectively). At 0.5 mg/kg/day, there was no significant brain ChE inhibition in either adults or

Table 7
ChE Inhibition in PND 21 pups and adult (~PND 80) females following repeated doses of CPF in corn oil.

Dose (mg/kg/day)	RBC ChE (U/L)	RBC ChE (% Control)	Brain ChE (U/L)	Brain ChE (% Control)	Plasma ChE (U/L)	Plasma ChE (% Control)
Adults – CPF in corn oil						
0	4903 ± 276	100.0	51,978 ± 1803	100.0	2254 ± 744	100.0
0.05	4655 ± 713[a]	94.9	51,929 ± 1031[a]	99.9	2892 ± 1052[a]	128.3
0.1	4120 ± 489*	84.0	51,990 ± 3291	100.0	2617 ± 770	116.1
0.5	**3946 ± 722***	80.5	51,694 ± 1862	99.5	**1220 ± 562***	54.1
1.0	**1335 ± 395***	27.2	**47,357 ± 1938***	91.1	**691 ± 316***	30.7
3.5	**135 ± 185***	2.7	**16,093 ± 2362***	31.0	**262 ± 73***	11.6
PND 21 male pups – CPF in corn oil						
0	6410 ± 1605	100.0	43,265 ± 1500	100.0	903 ± 111	100.0
0.05	5517 ± 1180	86.1	43,558 ± 1981	100.7	949 ± 148	104.9
0.1	5450 ± 1294	85.0	42,657 ± 1576	98.6	902 ± 136	99.6
0.5	**4054 ± 1112***	63.2	40,780 ± 1865	94.3	**645 ± 58***	71.3
1.0	**2478 ± 533***	38.7	**31,006 ± 4154***	71.7	**508 ± 70***	56.5
3.5	**540 ± 332***	8.4	**13,898 ± 988***	32.1	**187 ± 22***	20.8
PND 21 female pups – CPF in corn oil						
0	5951 ± 2082	100.0	42,289 ± 1616	100.0	928 ± 122	100.0
0.05	6388 ± 1021	107.3	44,726 ± 2598	105.8	921 ± 45	99.2
0.1	5882 ± 1282	98.8	43,499 ± 1409	102.9	864 ± 95	93.1
0.5	**4870 ± 829**	81.8	42,810 ± 1258	101.2	**713 ± 173***	76.8
1.0	**3333 ± 821***	56.0	**34,266 ± 2009***	81.0	**519 ± 64***	55.9
3.5	**723 ± 264***	12.1	**17,344 ± 2414***	41.0	**266 ± 51***	28.7

$n = 8$ pups/sex/dose level or 8 adult females/dose level unless otherwise indicated.
U/L, international units/L.
[a] $n = 7$ adult females in the 0.05 mg/kg/day dose group.
* Significantly different from controls at alpha = 0.05 using Dunnett's test when raw ChE data were analyzed (treatment-related effects in bold text).

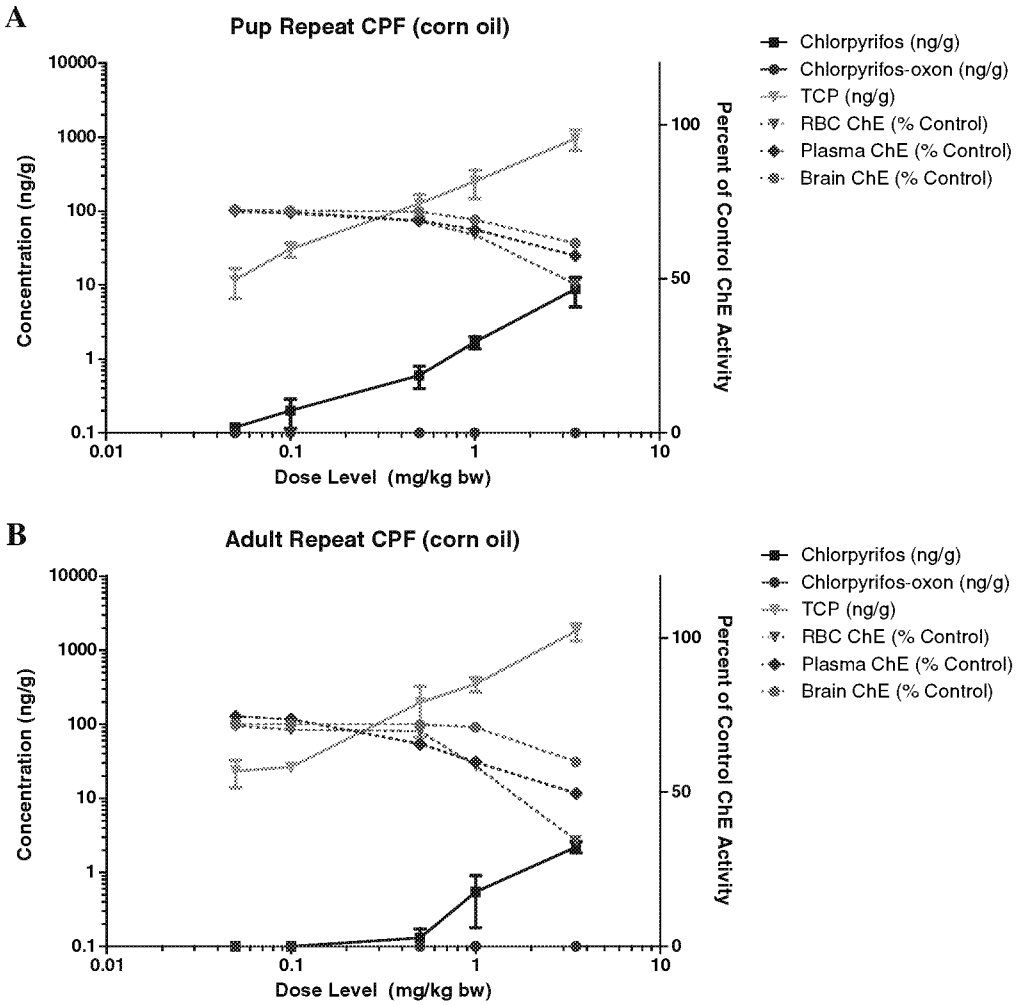


Fig. 3. Mean RBC, brain and plasma ChE inhibition relative to mean blood CPF, CPFO and TCP levels in PND 21 pups (A; sampled at 6 h after the last dose) or adult female rats (B; sampled at 8 h after the last dose) following daily gavage dosing with CPF in corn oil vehicle at multiple dose levels for 11 days. Data show mean values of combined male and female pup data as there were no gender-related differences in either ChE inhibition or blood CPF/CPFO levels. (n = 8 pups/sex/dose level or 7–8 adult females/dose level for ChE measurements; n = 4 pups or 4 adults in each dose group for determination of blood CPF, CPFO and TCP levels except for 3 pups in the control group and 3 adults in the 0.05 mg/kg/day group).

pups. Both age groups had significant decreases in RBC and plasma ChE activity with no apparent difference in sensitivity, despite a 4.6× higher blood CPF level in pups. Interestingly, the pup blood CPF level at 0.5 mg/kg/day was similar to blood levels in adults given 1 mg/kg/day CPF, a dose at which significant ChE inhibition was seen in all tissues in the adults. Thus, for a given blood CPF level, pups were equally or less sensitive to ChE inhibition as adults. As chlorpyrifos doses decrease below 1 mg/kg/day, there was no evidence of increased sensitivity in pups to CPF-induced ChE inhibition. At 0.1 mg/kg/day, CPF and CPFO were below the LLQ and TCP values were similar in adults and pups at 0.1 mg/kg/day, supporting the concept that pups would not be more sensitive to CPF at lower dose levels.

(b) CPFO in corn oil: in the repeat dose study, there were no treatment-related clinical observations in male or female rat pups (8/sex/dose; dosed PND 11–21) or adult female rats (8/dose; dosed PND 70–80) following exposure to eleven daily gavage doses of 0 (corn oil vehicle), 0.01 or 0.5 mg/kg/day CPFO (data not shown). There were no treatment-related effects on body weight and neither pups nor adult females exhibited effects on neurobehavioral endpoints (i.e., FOB and motor activity; Supplemental data 2) evaluated at the time-of-peak ChE inhibition after the 10th CPFO dose (i.e., 4 h post-dosing; Supplemental data 1).

Data for ChE activity measured in PND 21 male and female rat pups and 80-day-old female rats exposed to various doses of CPFO for eleven days are shown in Table 8. Once again, the high dose was selected to provide consistent, measurable RBC and plasma ChE inhibition. Samples for ChE inhibition were collected at the time-of-peak ChE inhibition determined in the acute studies. In response to repeated CPFO treatment, there was no discernable gender or age-related difference in ChE activity in male or female PND 21 pups or PND 80 adult females. There was no significant inhibition of brain ChE at any doses of CPFO (⩽0.5 mg/kg/day) in either pups or adults. The magnitude of RBC and plasma ChE in pups and adults was similar at 0.5 mg/kg/day CPFO, although adults had 2× the levels of TCP as pups. ChE was not inhibited in either of these tissues at 0.01 mg/kg/day in either adults or pups.

Fig. 4 shows blood levels of CPFO relative to ChE inhibition across tissues with samples collected at 4 h post-dosing. In both pups and adults, TCP was detectable in blood at all doses of CPFO (including two PND 21 control samples, which reflected some variability in detection around the LLQ; data not shown). TCP levels were increased in a dose-related fashion, although the increase was lower than dose proportional in pups (i.e., with a 50× increase in CPFO dose (0.01 to 0.5 mg/kg/day), TCP levels increased only 20×) vs. approximately dose proportional in adults (i.e., with a

Table 8
ChE inhibition in PND 21 pups and adult (~PND 80) females following repeated doses of CPFO in corn oil.

Dose (mg/kg/day)	RBC ChE (U/L)	RBC ChE (% control)	Brain ChE (U/L)	Brain ChE (% control)	Plasma ChE (U/L)	Plasma ChE (% control)
Adults – CPFO in corn oil						
0	4903 ± 276	100.0	51,978 ± 1803	100.0	2254 ± 744	100.0
0.01	5227 ± 1065	106.6	51,480 ± 1954	99.0	2814 ± 1122	124.8
0.5	**619 ± 228**[*]	12.6	51,161 ± 2204	98.4	**541 ± 160**[*]	24.0
PND 21 male pups – CPFO in corn oil						
0	6410 ± 1605	100.0	43,265 ± 1500	100.0	905 ± 111	100.0
0.01	5849 ± 1153	91.2	43,429 ± 1395	100.4	933 ± 136	103.9
0.5	**1013 ± 537**[*,a]	15.8	43,003 ± 1747[a]	99.4	**344 ± 51**[*,a]	38.3
PND 21 female pups – CPFO in corn oil						
0	5951 ± 2082	100.0	42,289 ± 1616	100.0	928 ± 122	100.0
0.01	5163 ± 1199	86.8	44,273 ± 1639	104.7	903 ± 95	97.2
0.5	**813 ± 304**[*]	13.7	38,762 ± 12,286	91.7	**362 ± 34**[*]	39.0

$n = 8$ pups/sex/dose level or 8 adult females/dose level unless otherwise indicated.
U/L, international units/L.
[a] $n = 7$ male pups in the 0.5 mg/kg/day dose group
[*] Significantly different from controls at alpha = 0.05 using Dunnett's test when raw ChE data were analyzed (treatment-related effects in bold text).

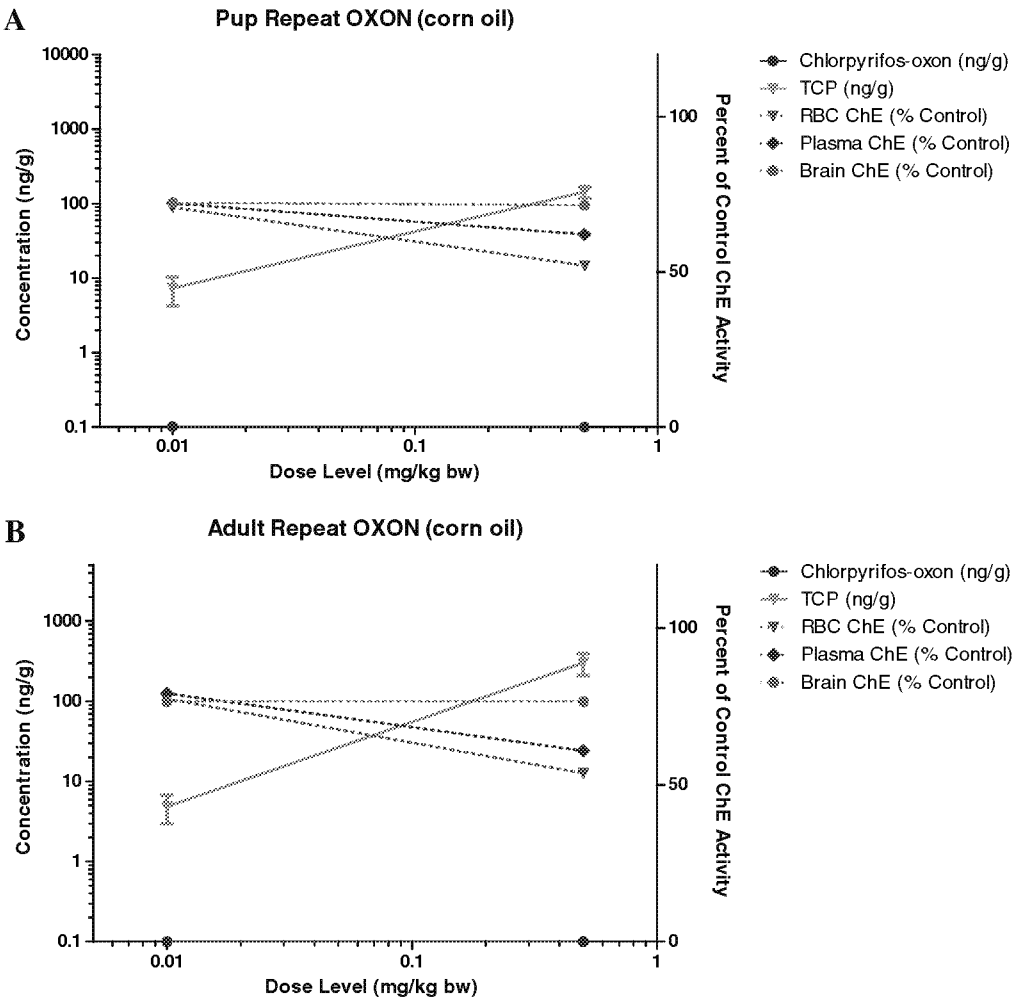


Fig. 4. Mean RBC, brain and plasma ChE inhibition relative to mean blood CPFO and TCP levels in PND 21 pups (A; sampled 4 h after the last dose) or adult female rats (B; sampled 4 h after the last dose) following daily gavage dosing with CPFO in corn oil vehicle at two dose levels for 11 days. Data show mean values of combined male and female pup data as there were no gender-related differences in either ChE inhibition or blood CPFO levels. ($n = 7$–8 pups/sex/dose level or 8 adult females/dose level for ChE measurements; $n = 4$ pups or 4 adults in each dose group for determination of blood CPFO and TCP levels except for 3 pups in the control group).

50× increase in CPFO dose, TCP levels increased 62×). Thus, adults had higher levels of TCP than pups at 0.5 mg/kg/day CPFO, whereas pups had 1.5× TCP blood levels compared with adults at the lower dose level of CPFO.