

October 24, 2017

VIA ELECTRONIC MAIL

Alan Hirsch, Chief Deputy Director
Michelle Ramirez, Proposition 65 Implementation
Office of Environmental Health Hazard Assessment
1001 I Street
Sacramento, California 95812

Re: *Comments in Opposition to Listing Chlorpyrifos as “Known the State to Cause Reproductive Toxicity” Under Proposition 65*

Dear Mr. Hirsch and Ms. Ramirez:

As you know from previous correspondence, Dow AgroSciences LLC (“DAS”) is the principal producer in the United States of agricultural crop protection products containing chlorpyrifos. This chemical is scheduled to be considered for designation (or “listing”) at a meeting of the Developmental and Reproductive Toxicant Identification Committee (“DART IC”) at a public meeting to be held on November 29, 2017.

I am transmitting for the DART IC’s consideration the attached paper prepared by DAS, entitled “Evaluation of the Data for Chlorpyrifos Pursuant to the DART Criteria: Why the Weight of Evidence Does Not Support Listing Chlorpyrifos as a Developmental Toxicant Under Proposition 65.” The paper demonstrates that chlorpyrifos, an important pest management tool that supports California’s agricultural industry, is not “clearly shown through scientifically valid testing according to generally accepted principles to cause . . . reproductive toxicity” within the meaning of Proposition 65.

The Notice announcing the meeting of the DART IC indicates that interested persons may appear in person to comment, and that persons who desire to speak for more than five minutes may submit such requests by October 30, 2017. Our client, as the principal producer of chlorpyrifos products and the primary registrant and primary developer of toxicological data to support such products pursuant to the Federal Insecticide, Fungicide and Rodenticide Act and the California Food & Agricultural Code and other laws around the world hereby requests additional speaking time. We will contact you to make those arrangements within the coming week, as we learn what other parties are planning to make presentations.

We look forward to seeing you at the DART IC meeting on November 29.

Sincerely,

/s/ Stanley W. Landfair

Stanley W. Landfair
Counsel for Dow AgroSciences LLC

cc: Carol Monahan-Cummings, Chief Counsel

**EVALUATION OF THE DATA FOR CHLORPYRIFOS
PURSUANT TO THE DART CRITERIA:**

**WHY THE WEIGHT OF EVIDENCE DOES NOT SUPPORT LISTING
CHLORPYRIFOS AS A DEVELOPMENTAL TOXICANT UNDER
PROPOSITION 65**

OCTOBER 24, 2017

Dow AgroSciences LLC

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I. INTRODUCTION AND EXECUTIVE SUMMARY

Dow AgroSciences LLC appreciates the opportunity to demonstrate that the agricultural pesticide known as chlorpyrifos should not be listed as a developmental toxicant for purposes of Proposition 65.¹ Specifically, the Developmental and Reproductive Toxicity Identification Committee (DART IC) is to deliberate as to “whether or not chlorpyrifos has been ‘*clearly shown*’ by scientifically valid testing according to generally accepted principles to *cause* developmental toxicity.”² For the reasons discussed below, the data do not support listing.

The toxicological database for chlorpyrifos is one of the more extensive for any regulated chemical. Simply put, the substance has been studied extensively by many regulatory agencies around the world, and none have concluded that chlorpyrifos causes developmental toxicity within the meaning of Proposition 65.

The starting point in evaluating the data is the Hazard Identification Document developed by OEHHA for the DART IC’s consideration in 2008. The DART IC considered chlorpyrifos for listing for female and male reproductive toxicity as well as developmental toxicity, and voted nearly unanimously not to list for any of those endpoints. *See infra*, Section IV. At that time, the DART IC assessed both epidemiological and experimental animal research and concluded that the weight of the evidence did not demonstrate that chlorpyrifos had been “clearly shown through scientifically valid testing according to generally accepted principles to cause” developmental or reproductive toxicity. *See infra*, Section IV and V.

Presently, chlorpyrifos is being considered for listing for developmental toxicity, on the premise that “[s]ubstantial new, relevant data” on developmental toxicity have become available. Importantly, the August 2017 announcement of availability of Hazard Identification Materials for chlorpyrifos (see below) and the September 1, 2017 Announcement of the DART IC meeting Scheduled for November 29, 2017, indicate that chlorpyrifos is to be considered for listing *only* as causing developmental toxicity.³ Accordingly, the comments herein are limited to developmental toxicity.

In addition to the 2008 Hazard Identification Document, the DART IC has been provided with other “Hazard Identification Materials” that include two reviews by the United States Environmental Protection Agency (which evaluates pesticidal chemicals like chlorpyrifos for reproductive and developmental toxicity in the course of regulating those substances under standards mandated by the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), the first in 2014 and the other completed in 2016. The Committee further has been provided seventy-two

¹ Proposition 65 is the popular name for California’s Safe Drinking Water & Toxic Enforcement Act of 1986, Cal. Health & Safety Code Section 25249.5 *et seq.*

² Hazard Identification Materials for Consideration of the Developmental Toxicity of Chlorpyrifos, (Aug. 2017), available at <https://oehha.ca.gov/media/downloads/crn/himchlorpyrifos2017.pdf>.

³ *Ibid.*

“additional studies” apparently *not* reviewed in the 2008 HID or in the 2014 or 2016 US EPA reports.⁴

As set forth in Sections IV and V below, there is nothing in these “new data” that should change the conclusion reached by the DART IC in 2008. Many of the epidemiology studies continue to be not valid or reliable, and thus do not provide a sufficient dataset for purposes of regulatory decision-making. On the other hand, there is a robust and reliable animal toxicology dataset which continues to demonstrate that chlorpyrifos is not a developmental toxicant. Neither the human data nor the animal data clearly show, using a weight of the evidence approach, that chlorpyrifos causes developmental toxicity. *See id.* Thus, *none* of the three criteria are met for the DART IC to recommend listing chlorpyrifos as known to the State to cause developmental toxicity. More specifically, as set forth below:

- the epidemiology studies do not provide “sufficient evidence in humans” (DART Criteria 3.A.) that chlorpyrifos causes developmental toxicity;
- the animal studies do not provide “sufficient evidence in experimental animals (mammals)” (DART Criteria 3.C.) that chlorpyrifos causes developmental toxicity; and
- neither the epidemiology studies nor the animal studies provide “limited evidence or suggestive evidence in humans” (DART Criteria 3.B.) that chlorpyrifos causes developmental toxicity.

A. Epidemiology Studies Do Not Clearly Show Developmental Toxicity

As discussed in Section IV, *infra*, the weight of the epidemiology evidence does not “clearly show” an association, much less a causal relationship, between exposure to chlorpyrifos and developmental effects in humans; indeed, interpretation using newly published quality assessment tools shows that not only do the three prospective cohort studies of chlorpyrifos have conflicting and contradictory results but two studies demonstrate high risk of bias. None of the associations was confirmed in the newly published epidemiological studies with low risk of bias. As explained more fully herein, the available epidemiological studies do not provide “sufficient evidence in humans,” as that term is explained in Section 3.A.(1) of the DART IC criteria.

To summarize the key points:

- An important quality element for all epidemiology studies is the use of valid and reliable methods to assign exposure. Since 2008, numerous methodological studies of exposure have been published. The introduction of several quality assessment instruments has changed the interpretation of environmental epidemiology. Assumptions regarding personal pesticide exposure and proximity to an application require validation. Single biological samples of short-lived chemicals, such as chlorpyrifos, are not reliable.

⁴ *Ibid.*

- The study conducted by researchers at Columbia University and initiated in 1998 (“the Columbia study”) relies upon a single serum sample of chlorpyrifos with an unvalidated analytical method, and no adjustment for lipids. The resulting chlorpyrifos concentrations are both unreliable and invalid, making the exposure-health effects correlations unreliable and invalid. Moreover, evidence for random error is exhibited by internally inconsistent results. The chlorpyrifos-related observations in this study thus do not meet the threshold for consideration as “scientifically valid testing.” *See id.*
- Many of the published studies since 2008 also rely upon poor exposure assessment methods. *See, id.* Notably, the fundamental limitation of the exposure assessment remains and exposure-outcome correlations are unreliable and not valid. The most robust studies found no adverse association of chlorpyrifos and adverse development, behavior or intelligence. *See id.*

Taken as a whole, and given these key points, the relevant studies do not meet the standard of “scientifically valid according to generally accepted principles,” and thus do not provide “convincing evidence to support a causal relationship between exposure to the chemical, and the developmental . . . effect in question.”

B. Animal Studies Do Not Clearly Show Developmental Toxicity

Chlorpyrifos has been evaluated for developmental toxicity in four well-conducted studies of conventional experimental design by oral administration in three species: rats, mice and rabbits.⁵ *See* Section V, *infra*. These “guideline” studies are designed to follow US EPA’s “Guidelines for Developmental Toxicity Risk Assessment,” which outline principles and methods for study conduct and subsequent evaluation of data to characterize risk to human development, growth, survival and function because of exposure prior to conception, prenatally, or to infants and children (USEPA, 1991).

In every one of these studies, no developmental toxicity was observed in the absence of significant maternal toxicity. *See id.* Indeed, what is remarkable is the absence of developmental toxicity at doses that cause severe maternal toxicity. Other studies that associate exposure with developmental outcomes have serious limitations and do not represent “scientifically valid testing according to generally accepted principles.” *See id.* As explained more fully below, the available animal studies do not provide “sufficient evidence in experimental animals (mammals)” and do not meet the criteria for the DART IC to list chlorpyrifos as a developmental toxicant.

⁵ Deacon *et al.*, 1980; Ouellette *et al.*, 1983; Rubin *et al.*, 1987a; Rubin *et al.*, 1987b.

Summarizing the key points:

- Chlorpyrifos has a complete toxicological database as required by global regulatory agencies and has never been associated with, or designated as a developmental toxicant. This conclusion is supported by four guideline developmental toxicity studies and a developmental neurotoxicity study. *See Section II, infra.*
- Chlorpyrifos was evaluated for listing as a developmental and reproductive toxicant in 2008, and the data were deemed insufficient for listing under either category, based on both human and animal evidence. *See Sections IV and V, infra.*
- Despite reference to “growing evidence” surrounding neurodevelopmental toxicity in humans and animals and a multitude of investigative studies (similar to the evidence and study type that were evaluated in 2008 by the DART IC), there are no new evidence or data that satisfy the standard of “clearly shown through scientifically valid testing according to generally accepted principles” that support even a tenuous association between chlorpyrifos and developmental toxicity. *See Section V, infra.*
- Critical review of the studies provided to the DART IC in 2017 shows multiple experimental design variables, confounders, and omissions that fail to meet the standard of “scientifically valid testing.” Both the US EPA and its Scientific Advisory Panel (“SAP”) have similarly concluded that the vast majority of this new literature contains experimental challenges and is devoid of any identified or proven mode of action as related to neurodevelopmental outcomes. *See id.*

Taken as a whole, and given these key points, there is not “sufficient evidence in experimental animals” to support a conclusion that chlorpyrifos causes developmental toxicity. Based upon qualified guideline developmental toxicity studies, the strict criteria required under Proposition 65 for listing, the consistent failings of the “new” studies to incorporate rigorous study designs to critically explore developmental toxicity, and the confirmed absence of biological plausibility associated with any of the reported outcomes, there is no basis to designate chlorpyrifos as a developmental toxicant.

II. BACKGROUND

A. Use of Chlorpyrifos in California

Chlorpyrifos is an important pest management tool for the control of insect pests in California’s \$30 billion agricultural industry. It is a non-systemic insecticide used on a wide variety of crops to control insect pests such as aphids, scales and various species of lepidoptera larvae. In 2006,

the use on five major crops—oranges, almonds, cotton, alfalfa, and walnuts—accounted for almost 75 percent of the chlorpyrifos used in the state. Chlorpyrifos is often used for the most severe pest outbreaks that threaten California’s agricultural production.

B. History of Developmental Toxicity Reviews by Regulatory Authorities and Independent Scientists

Chlorpyrifos has been evaluated for developmental toxicity by global regulatory bodies and authorities (US EPA, 2000, 2002; EU, 2005; ANRA, 2000a; CalEPA DPR, 2001) as well as by independent scientists and review boards (Eaton *et al.*⁶, 2008; Schardein and Scialli, 1999; Jackson *et al.*, 1999). Eaton *et al.* evaluated the animal and human data for evidence of teratogenicity and/or reproductive effects and concluded that:

“Taken together, the studies [experimental animal] found *no consistent evidence for teratogenicity or abnormal reproduction* with daily oral dose of chlorpyrifos up to 5 mg/kg-day. Indications of *prenatal growth retardation and increased pre-perinatal death* were seen in some studies at 5 mg/kg, *associated with signs of maternal toxicity.*”⁷

“Results of human surveys on malformation rates after chlorpyrifos exposure are few, and *existing data are too limited to allow firm conclusions.*”⁸

Collectively, these agencies, independent scientists and review boards have consistently concluded that chlorpyrifos is not associated with developmental toxicity.

In its review of existing studies submitted to support the registration of chlorpyrifos, as well as many other studies that have been included in the HID, the California Department of Pesticide Regulation (2001) concluded that “[t]here is insufficient evidence that human infants are more susceptible to the toxicity of chlorpyrifos than adults and small children and there is no compelling evidence that chlorpyrifos causes any developmental neurotoxicity under physiologically relevant conditions.” As part of the European Commission reevaluation of chlorpyrifos toxicology and human health (European Food Safety Authority (EFSA, 2014)), the EFSA peer review made the following conclusion regarding the body of epidemiology studies: “*The epidemiology data are not sufficiently robust to support the hypothesis that CPF is a causal factor for neurodevelopmental effects.*” EFSA (2014). Final addendum to the Art. 21 Review on chlorpyrifos—public version—Initial risk assessment provided by the Rapporteur Member State Spain for the exiting substance CHLORPYRIFOS as referred to in Article 21 of regulation (EC) No. 1107/2009. February, 2014. Chapter: Add. III to Vol. 3, Ch. 6 to DAR. Pg. 53-54 (emphasis added).

⁶ Authors include Eaton, D.L., Daroff, R.B., Autrup, H., Bridges, J., Buffler, P., Costa, L.G., Coyle, J., McKhann, G., Mobley, W.C., Nadel, L., Neubert, D., Schulte-Hermann, R., and Spencer, P.S.

⁷ *Ibid.* at 39 (emphasis added).

⁸ *Ibid.*

III. CRITERIA FOR REVIEWING DATA FOR LISTING UNDER PROPOSITION 65

A. The Statutory Standard and the Regulatory Mandate

The text of Proposition 65 recites that:

“[a] chemical is known . . . to cause . . . reproductive toxicity . . . if in the opinion of the state’s qualified experts it has been *clearly shown* through *scientifically valid testing according to generally accepted principles* to cause . . . reproductive toxicity.”⁹

The Proposition 65 implementing regulations list among the duties of the DART IC the responsibility to “[r]ender an opinion as to whether specific chemicals have been *clearly shown* through scientifically valid testing according to generally accepted principles, to *cause* reproductive toxicity.”¹⁰ Hence, the standard that the DART IC is to apply is referred to as the “clearly shown” standard.

This standard is intended to be restrictive, to be implemented by the DART IC to limit listing to chemicals that are “known” to cause cancer or reproductive toxicity and to prevent the listing of those that are not. As background, Proposition 65 was enacted through the initiative process, rather than by the Legislature. There was considerable electioneering debate whether Proposition 65, if enacted, would result in the listing of too many chemicals based on incomplete or unconvincing evidence. The “Ballot Argument” in favor of Proposition 65 thus clarified that

“Proposition 65 [would] focus only on chemicals that are ‘*known*’ to the state to cause cancer or reproductive disorders. *Chemicals that are only suspect are not included*. The Governor must list these chemicals, after full consultation with the state’s qualified experts.”¹¹

B. The DART Criteria for Determining Whether a Chemical Should Be Listed

Notwithstanding this concern for “over-listing,” the term “reproductive toxicity” was not defined in the statute and/or in the implementing regulations (even now). Rather, in 1989, to clarify the use of this term and others, and to identify the kinds of scientific data that would be required to support the designation of chemicals as reproductive toxicants, the DART IC developed, adopted and published written “Criteria for Recommending Chemicals for Listing as ‘Known to the State to Cause Reproductive Toxicity.’” As short-hand, these criteria for listing generally are referred to as the DART Criteria.

⁹ Cal. Health & Safety Code § 254249.8(b) (emphasis added).

¹⁰ Cal. Code Regs., tit. 27, § 25305(b)(1).

¹¹ Ballot Argument in Favor of Proposition 65, available at <https://oehha.ca.gov/media/downloads/proposition-65/general-info/prop65ballot1986.pdf>

The DART Criteria are available on the OEHHA website, where the agency explains:

“Apr 23, 2004

The Criteria for Recommending Chemicals for Listing as “Known to the State to Cause Reproductive Toxicity” was accepted and approved by the Developmental and Reproductive Toxicant (DART) Identification Committee at its meeting on October 25, 1993. The criteria were approved before the Office of Environmental Health Hazard Assessment (OEHHA) web site was established. OEHHA has received requests for the DART criteria; therefore, OEHHA is making the document available on the Internet at this time. The DART criteria are unchanged from its adoption in 1993.”¹²

The first paragraph of the DART Criteria provides that the

“criteria included [in the DART Criteria] *shall be used* by the Office of Environmental Health Hazard Assessment Science Advisory Board Developmental and Reproductive Toxicant (DART) Identification Committee to identify chemicals to be recommended as known to the State to cause reproductive toxicity, for purposes of . . . Proposition 65.”¹³

According to the DART Criteria, “reproductive toxicity” includes “developmental toxicity.”¹⁴ “Developmental toxicity” is defined to include adverse effects on the products of conception (*i.e.*, the conceptus), including but not limited to:

- (1) Embryo/fetal mortality (including resorption, miscarriage/spontaneous abortion, or stillbirth), malformations, structural abnormalities and variations, altered fetal growth, and change in gestational age at delivery.
- (2) Postnatal parameters including growth and development, physiological deficits and delay, neurological, neurobehavioral and psychological deficits, altered sex ratio, abnormal sexual development or function and morbidity or mortality.
- (3) Transplacental carcinogenesis.
- (4) Somatic or genetic (germ cell) mutations in the conceptus.¹⁵

¹² <https://oehha.ca.gov/proposition-65/general-info-background/criteria-listing-chemicals-listing-known-state-cause>

¹³ DART Criteria at 1.A.

¹⁴ DART Criteria at 2.B.

¹⁵ DART Criteria at 2.C.

Equally clearly, however, developmental toxicity is to be considered only with regard to “prenatal exposures.”¹⁶ That is the reason for the use of the word “conceptus” above. It is the long-standing position of OEHHA and the Office of the Attorney General that “chemicals that have been clearly shown to cause birth defects as a result of prenatal exposure, whether the effect is manifested prior to birth, at birth, or during the postnatal period, clearly should be listed under Proposition 65. . . . However, they adhered to the position that a chemical known to cause developmental toxicity only as a result of exposure during the postnatal period could not be listed under Proposition 65.”¹⁷ To simplify, “postnatal exposures were not encompassed by Proposition 65.”¹⁸

The DART Criteria provide that “[i]n evaluating the sufficiency of data, a *weight of evidence approach shall be used* to evaluate the body of information available for a given chemical,” and outline certain weight of evidence considerations that apply for certain types of evidence. In relevant part, the chemical’s developmental effects “shall” meet at least one of three criteria for the DART IC to recommend listing as known to the State to cause developmental toxicity:

- (1) **Sufficient evidence in humans**, defined to include “any of a variety of epidemiological studies, so long as the study or studies are scientifically valid according to generally accepted principles and provide convincing evidence to support a causal relationship between exposure to the chemical, and the developmental . . . effect in question.
- (2) **Limited evidence or suggestive evidence in humans**, supported by sufficient experimental animal (mammalian) data, as described in (3) below.
- (3) **Sufficient evidence in experimental animals (mammals)**, such that extrapolation to humans is appropriate, in most cases based on the adequacy of several factors, including the experimental design, the exposure, the number of dose levels, consideration of maternal and systemic toxicity, the number of tests or experimental animal species and other considerations.¹⁹

Finally, the Criteria note the importance of both statistical considerations and biological plausibility:

- (1) Statistical analyses are important in determining the effect of a particular agent; however, the biological significance of the data should not be overlooked. Given the number of endpoints that can be quantified in developmental and reproduction studies, a few statistically significant differences may occur by chance alone. Conversely, apparent dose-related trends may be biologically relevant even though statistical analyses do not indicate a significant effect.

¹⁶ Transcript of Public Meeting of Developmental and Reproductive Toxicant Identification Committee, Testimony of OEHHA Chief Counsel William Soo Hoo, December 4, 1996, at 12-15.

¹⁷ *Id.*, at 14, lines 16-35.

¹⁸ *Id.*, at 13, lines 17-21.

¹⁹ DART Criteria at 3.A.-C.

(2) In determining whether a chemical is to be recommended to be listed as known to the State to cause reproductive toxicity, the biological plausibility of the association between the adverse reproductive effects observed and the chemical in question should be considered. Confidence is increased when, based on known principles of developmental and reproductive biology, physiology, and toxicology, a sound scientific basis exists for the observed adverse effects and the known characteristics of the particular chemical. Conversely, confidence is decreased if the observed adverse effects are contradictory to the known characteristics of the particular chemical.²⁰

IV. THE WEIGHT OF EVIDENCE FROM EPIDEMIOLOGY STUDIES DOES NOT PROVIDE “SUFFICIENT EVIDENCE IN HUMANS”

A. Epidemiology Overview

As noted above, the DART IC decided against listing chlorpyrifos as a developmental toxicant, female reproductive toxicant, or a male reproductive toxicant in 2008, following a public meeting. At that time, the epidemiology literature featured three prospective cohort studies of mother and infant pairs, or birth cohort studies, by researchers at Columbia University, Mount Sinai Hospital and the University of California at Berkeley. The many relationships investigated were conflicting and contradictory. These studies relied largely upon biological samples of blood and/urine collected during the period for 1998 – 2002, to estimate exposure. In the ensuing 9 years, there have been additional observations from these and other epidemiology studies. In addition, the shortcomings of using biological samples of short-lived chemicals, such as chlorpyrifos, have become more clear.

The studies published since the 2008 HID—which are limited by unreliable exposure assessment, lack of demonstrated exposure to chlorpyrifos, and inconsistent results across specific health endpoints—should not change the 2008 DART IC conclusion (Burns *et al.*, 2013; Eaton *et al.*, 2008; Goodman *et al.*, 2013; Li *et al.*, 2012; Mink *et al.*, 2012; Needham, 2005; Weselak *et al.*, 2007; Zhao *et al.*, 2005). In the context of all of the epidemiology data, these limited and ambiguous results clearly are not “sufficient evidence in humans” that chlorpyrifos causes any DART effect, including “delayed neurodevelopment.”

With respect to epidemiological studies, the term “sufficient evidence in humans” is defined to include:

“[A]ny of a variety of epidemiological studies, so long as the study or studies are scientifically valid according to generally accepted principles and provide convincing evidence to support a causal relationship between exposure to the chemical, and the developmental . . . effect in question. This requires accurate

²⁰ DART Criteria at 4.

exposure and toxicity endpoint classification and proper control of confounding factors, bias, and effect modifiers. . . .”²¹

In evaluating the sufficiency of human data, reviewers should apply the following “[w]eight of evidence considerations:”

- (a) “Data from multiple studies increase the confidence for classification of an agent as a developmental . . . toxicant, and unless there is an exceptionally strong study (see below), effects should occur in more than one human study for a chemical to be recommended for listing on the basis of epidemiologic evidence alone.”
- (b) “Data from a single well conducted epidemiologic developmental . . . toxicity study showing a clear relationship between exposure and effect may be sufficient to classify an agent as a developmental . . . toxicant, provided there are not equally well conducted studies which do not show an effect and which have sufficient power to call into question the repeatability of the observation in the positive study.”²²

The weight of the epidemiology evidence does not “clearly show” an association, much less a causal relationship between chlorpyrifos exposure and developmental effects; indeed, the studies have conflicting and contradictory results. Taken as a whole, there is not “sufficient evidence [of developmental toxicity] in humans” for the DART IC to list chlorpyrifos for developmental toxicity.

B. Certain Data Should Be Excluded from the DART IC’s Consideration

Certain data are not relevant to the DART IC’s weight of evidence analysis. These data include the following:

(1) Effects from Postnatal Exposure

Proposition 65 limits developmental toxicity to developmental effects that occur from prenatal exposure, and exclude effects from post-natal exposure.²³ Several publications identified in the Hazard Identification Document and/or by the US EPA are not useful for hazard identification because the studies identified and evaluated exposure in children and adults that do not meet Proposition 65 criteria for developmental toxicity, “*defined to include adverse effects on the products of conception (i.e., the conceptus).*” Note that some publications evaluated exposures in both pregnant women and their children (*e.g.*, Cartier *et al.*, 2016; Eskenazi *et al.*, 2007). The results for *in utero* exposures are discussed below.

²¹ DART Criteria at 3.A.(1).

²² DART Criteria at 3.A.(3).

²³ *See* Testimony of Chief Counsel Soo Hoo, at notes 17-19, *supra*, and accompanying text.

(2) Health Outcomes Not Evaluated

The DART IC also should not consider case reports, reviews, and studies that did not evaluate health outcomes, but were conducted instead merely to monitor for the presence of chemical residues in environmental media. Studies that did not evaluate chlorpyrifos (*i.e.*, relied upon assumptions derived from general agricultural exposure, cholinesterase or organophosphates) should be excluded. For example, recent studies that relied on categorization of persons based upon their parental occupation in floriculture (Moreno-Banda *et al.*, 2009) or residence in an organic or traditional farm (Lu *et al.*, 2009) as the basis for categorizing subjects as exposed or not exposed should be excluded. Without further information on use of specific insecticides, these studies provide no information on the putative role of chlorpyrifos in influencing health outcomes.

(3) General, Non-Specific Analytes

Urinary metabolites dialkylphosphates (DAPs) are associated with the entire class of organophosphates. Hence, the total DAPs cannot be used to specifically estimate exposure to any single OP source, including chlorpyrifos. Within the DAPs, the diethylphosphate (DEP) metabolites are known fragments of chlorpyrifos, but are also associated with nine other organophosphate metabolites. In contrast the dimethylphosphate (DMP) metabolites are fragments of up to a dozen other organophosphates, but not chlorpyrifos. Notably, the California Department of Pesticide Regulation recently recommended:

“[b]ecause each urinary metabolite has multiple sources, the presence of any DAP metabolite in urine (*e.g.*, DMP, DEP, DMTP, DETP, *etc.*) may result from exposure to the parent compound (such as an OP pesticide) or an environmental degradate. DEP and DETP are common metabolites for many O,O-diethyl substituted pesticides such as diazinon, and therefore they cannot be considered specific markers of chlorpyrifos exposure.”²⁴

Because they cannot be linked directly to chlorpyrifos, for the purposes of this Proposition 65 review, analytical results that rely upon urinary DAP cannot be considered relevant for chlorpyrifos. However, those results for urinary DEP will be discussed in the context of reliability, validity and causal interpretation. These publications are listed in Appendix Tables 1 and 2.

C. Epidemiology Studies Are Not Valid and Reliable for Proposition 65 Purposes Unless They Meet Certain Best Practices

The DART Criteria describe “sufficient evidence in humans” to

²⁴ California Department of Pesticide Regulation, 2017 Draft Evaluation of Chlorpyrifos as a Toxic Air Contaminant, at 65.

“[i]nclude[] any of a variety of epidemiological studies, so long as the study or studies are scientifically valid according to generally accepted principles and provide convincing evidence to support a causal relationship between exposure to the chemical, and the developmental or reproductive effect in question. This requires accurate exposure and toxicity endpoint classification and proper control of confounding factors, bias and effect modifiers.”²⁵

The principles for causal assessment for epidemiology studies have been guided by post hoc evaluations using parameters suggested by Bradford Hill (Hill, 1965). These guidelines for comparing consistency and strength of associations across studies, for example, incorporate the fundamental scientific tenet of reproducibility. However, elements of bias, control of confounding and accurate exposure assessment are not included in the Hill parameters. In the last decade, additional principles have been introduced to evaluate the reporting of evidence (*e.g.*, Little *et al.*, 2009; Sanderson *et al.*, 2007; Vandembroucke *et al.*, 2007; von Elm *et al.*, 2014) and the quality of the underlying data (*e.g.*, LaKind *et al.*, 2014; LaKind *et al.*, 2015; Muñoz-Quezada *et al.*, 2013; Youngstrom *et al.*, 2011). Regulatory agencies in the US (including the Office of Pesticide Programs and National Toxicology Program), EU, and Japan, have begun to use these guidelines and to recommend omitting studies of poor quality before evaluating the causal evidence.

An important quality element for all epidemiology studies is the use of valid and reliable methods to assign exposure. Self-reported exposure information via questionnaires is one of several tools to obtain historical data. Differential recall and lack of knowledge of specific pesticides are recognized limitations of questionnaires. Other approaches have been introduced to reduce these limitations and to improve the specificity of exposure. These include correlation of pesticide application records and proximity to an application, and biological samples of short-lived chemicals. While introduced to ameliorate exposure identification, without evaluation of error or repeated sampling, the reliance upon these approaches is not scientifically valid.

(1) Assumptions Relating Exposure From Proximity to a Pesticide Application Require Validation

The fallacy of using residence and pesticide application as a proxy for valid exposure is discussed by Chang *et al.*, (2014) and addressed in a letter to the editor (Burns *et al.*, 2015). In short, these assignments do not take into consideration the precautions and restrictions of the application methods, formulation and properties of the pesticide moiety or weather patterns. A body of literature has compared levels of multiple pesticides in indoor dust with farm vs. non-farm homes but the associations were not consistently seen for individual pesticides or when paired with urinary data (Arbuckle *et al.*, 2002; Deziel *et al.*, 2015; Fenske *et al.*, 2002). Efforts to validate assumption of bystander exposure have indicated that physical proximity to the application is not a predictor of exposure and that personal behaviors are better determinants (Alexander *et al.*, 2006; Galea *et al.*, 2015). An evaluation of carpet dust and data from the California Pesticide Use Report noted that while the overall concentration was correlated with

²⁵

DART Criteria at 3.A.(1).

nearby applications, these associations were inexplicably weaker for distances within 500 meters and applications within the last 6 months (Gunier *et al.*, 2011).

(2) Single Biological Samples of Short-Lived Chemicals Do Not Provide Reliable Information

Single biological samples of short-lived chemicals are not sufficiently robust to infer past exposure. Specifically, urine or blood collected late in pregnancy or at delivery will not alone reflect temporally relevant exposure to the developing fetus. The overall range may be informative of peak exposures to population but not for an individual. Investigators that collected repeated samples of urine have reported high within and between individual variability (Bradman *et al.*, 2013; Fortenberry *et al.*, 2014; Kissel *et al.*, 2005), indicating that the point estimate based upon a single urinary concentration may be invalid. Others have cautioned against relying upon a single sample to estimate long-term exposure (Aylward *et al.*, 2014; LaKind and Naiman, 2015; Morgan *et al.*, 2016; Spaan *et al.*, 2015). Exposure assessments based upon a “single sample without considering error” are considered to be of low utility (LaKind *et al.*, 2014) as adopted by the National Toxicology Program Office of Health Assessment and Translation and European Food Safety Authority. Due to the short half-life of chlorpyrifos in the body ($t_{1/2} = 27$ hrs), the concentration of chlorpyrifos or its metabolite(s) are not a valid or reliable estimate of the exposure levels throughout the prenatal period. According to Spaan *et al.*, (2015), spot samples should not be used to establish prenatal exposure for epidemiology studies:

“The weak correlation between the samples from the same mother emphasizes the need to use multiple urine samples in order to reduce misclassification of exposure and increase power, if the exposure of interest is an average of gestational exposure.”

D. The Columbia Study Does Not Meet the Standard for Scientific Validity

The Columbia study investigators have correlated chlorpyrifos levels to adverse infant health (birth weight and birth length), (Whyatt *et al.*, 2004), poor performance on the Bayley Physical Development Index (PDI) at age 36 months (Rauh *et al.*, 2006) and lower scores on the Working Memory domain of IQ (Rauh *et al.*, 2011). The investigators analyzed chlorpyrifos in maternal and cord blood, rather than a less specific urinary metabolite, such as 3,5,6-trichloro-2-pyridinol (TCPy) or the sum of diethylphosphates (DEP).

However, the Columbia study 1) relies upon a single concentration of chlorpyrifos, 2) uses an unvalidated analytical method, and 3) made no adjustment for lipids. Evidence for random error is exhibited by 4) internally inconsistent results for the Columbia study. As a result, the chlorpyrifos related observations of this study are no longer considered reliable or valid and do not meet the Proposition 65 criteria to be “clearly shown through scientifically valid testing according to generally accepted principles.” Each point is discussed further below.

(1) Single Blood Sample is an Unreliable Estimate of *In Utero* Exposure

The Columbia study investigators collected maternal and cord blood at or near delivery. These single samples at only one point in time were collected for convenience (at birth) and with little information regarding the chlorpyrifos home application. Given the rapid metabolism of chlorpyrifos in humans and subsequent short residence time in the body, one sample obtained at the time of delivery or shortly after would have little relationship or meaning to exposure levels that may have been present during most of the pregnancy (or thereafter), or even if there was a presence in the body at any time in pregnancy other than at the time of the blood sampling.

Notably, the maternal blood chlorpyrifos concentrations were not correlated with the personal air samples when collected more than a month from delivery (Spearman rank = 0.09) (Whyatt *et al.*, 2003). The maternal blood chlorpyrifos levels were also not associated with past exterminator applications or self-reported use (Whyatt *et al.*, 2003). Exposure in the Columbia cohort is marked by heterogeneity among the children born in 1998 – 1999 with little exposure detected in children born thereafter. Validation efforts published in later years found “no association between chlorpyrifos levels in maternal and cord blood and TCPy levels in maternal urine samples during pregnancy or after delivery” (Whyatt *et al.*, 2009).

The Scientific Advisory Panel (SAP) convened by the US Environmental Protection Agency under FIFRA in 2016 (2016 SAP) cautioned that basing conclusions on once-in-time cord blood measurements is not scientifically justified because a once-in-time measurement is not representative of long-term exposure. 2016 SAP Minutes at 42. (“[R]eliance on single cord blood measurements from only one study (*i.e.*, the [Columbia] study) as a primary basis for a highly impactful regulatory decision goes against standard practices of science in the fields of toxicology and pharmacology.”)

(2) Lack of Validation of Analytical Method at the Low Concentrations Reported

It is a basic foundation of the scientific process that researchers must show that a quantitative exposure measurement is accurate, precise and reproducible across the range of values determined within a study. For example, the US EPA method validation guidelines call for replicate determinations of analyte recovery from a given matrix (substrate) down to the stated limit of detection (LOD) (US EPA 1998). However, this did not occur within the Columbia study. There were no data generated during validation of the plasma/serum analysis method (Barr *et al.*, 2002) or during the subsequent analysis of the Columbia cohort samples to show that chlorpyrifos levels could be accurately measured in plasma/serum matrix down to the stated LOD of 0.5-1 pg/g (The Columbia study authors use the term LOD when discussing limit of quantitation.). The lowest concentration for which analyte recovery in plasma/serum was determined using this method was 15 pg/g. This is a critical point, as more than 80% of the Columbia subjects had levels below this validation level. Further, there was no evaluation of possible sample contamination during blood collection in the hospital, processing to plasma, or during shipment to the Centers for Disease Control and Prevention (CDC). Analysis of sample integrity is a critical parameter of all biomonitoring studies, especially those at the trace levels

reported for this cohort. Barr *et al.* (2002) also reported background chlorpyrifos levels of 9 pg/g in control serum samples, 50% higher than the “high” exposure Columbia cohort criteria, the source of which was never determined. Since the blood test results for the Columbia cohort are not valid measures of true exposure, the study’s classification of the blood test results into high (above 6.17 pg/g) and low (below 6.17 pg/g) is inherently suspect. This, in turn, raises serious doubt about any claimed correlation between exposure groups and effects.

The 2016 SAP expressed significant concerns regarding the validity and reliability of the blood test results upon which the Columbia study’s published conclusions were based:

“A major source of uncertainty for the Panel was the lack of verification and replication of the analytical chemistry results that reported very low levels of chlorpyrifos (pg/g). Imputing quantitative values when the concentration of analyte falls below the level of detection (LOD) was a particular concern, especially given that a large fraction of cord blood samples included in the analyses presented with levels below LOD.” 2016 SAP Minutes at 18; *see also id.* at 41 (“[T]he use of means with large standard deviations that extend below the level of detection that are included in the analysis . . . further decreases the value and increases uncertainties associated with the raw data that cannot and has not been independently reviewed or verified.”).

(3) Failure of Authors to Make Adjustments for Lipid Levels

The Columbia study analyses made no lipid adjustments to the plasma chlorpyrifos concentrations. Chlorpyrifos is a lipophilic compound ($\log K_{ow}$ 4.96), which is known to partition into lipids (Lowe *et al.*, 2009). Studies have shown that the blood: tissue partition coefficients for chlorpyrifos are altered during pregnancy, consistent with documented changes in blood lipid chemistry during gestation (Lowe *et al.*, 2009; McMullin *et al.*, 2008). Estimates of internal exposure are best made by adjusting plasma concentrations to lipid levels (Haddad *et al.*, 2000; Lin *et al.*, 2002). For example, if two women were exposed to the same dose of chlorpyrifos, and one woman had higher levels of plasma lipids, her plasma chlorpyrifos concentration would be higher, even though total body burdens are equivalent, due to a higher blood: adipose partition coefficient.

(4) Internal Inconsistencies and Indications of Random Exposure Determinations

The results of the Columbia study are internally inconsistent. In other words, the results are contradictory with other analyses from the same study population. For example, when evaluating performance on the Bayley Scales of Infant Development, statistically significant associations were observed for chlorpyrifos for the Columbia study children at 36 months but not at ages 12 or 24 months. Additional inconsistencies were observed when the Bayley tests were evaluated as continuous scores and as dichotomous classifications. The outcomes were not evaluated using longitudinal analytic approaches (as recommended by a FIFRA 2010 SAP). This is poor evidence that the association at 36 months is a “true positive.”

The Columbia publications of IQ reported a significantly inverse association with chlorpyrifos and IQ (Horton *et al.*, 2012; Rauh *et al.*, 2011), while other Columbia study analyses that focused on polycyclic aromatic hydrocarbons (PAH) and phthalates (Factor-Litvak *et al.*, 2014; Perera *et al.*, 2009) excluded chlorpyrifos from the multivariate models because chlorpyrifos was a not significant predictor of IQ. This suggests that chlorpyrifos is not causally related to the outcome of interest and may be due to random error or bias.

E. Inconsistent Results in Epidemiology Studies for Infant Health Outcomes

The Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) is another prospective cohort study of women and infant pairs. This California study collected 2 urine samples during pregnancy with well described (and compliant) quality assurance/quality control procedures (Eskenazi *et al.*, 2004). The investigators evaluated concentrations of TCPy as well as the broad class of dialkyl phosphates (DAP), and ethyl groups that include chlorpyrifos, diethyl phosphates (DEP). It is well-described that urinary TCPy is the more specific metabolite of chlorpyrifos (Barr and Angerer, 2006; Sudakin and Stone, 2011), but also cannot be distinguished from TCPy in residues in the environment or diet. Due to the large intra-individual variability in concentrations, a mean of the 2 urine samples for TCPy and DEP was reported by the CHAMACOS investigators as an estimate of *in utero* exposure to chlorpyrifos.

As reviewed by OEHHA in 2008, Eskenazi *et al.*, (2004), reported no adverse association of urinary TCPy (or DEP) and any birth outcome. As shown in Table 1, Summary of Infant Health Epidemiology Study Results, three additional studies estimated exposure from occupational use or more than one biological sample (Sathyanarayana *et al.*, 2010; Rauch *et al.*, 2012; Naksen *et al.*, 2015). Overall, none reported an adverse association of chlorpyrifos (or a metabolite) and birth weight, birth length, head circumference and/or gestational age. Only Rauch *et al.*, (2012) observed a statistically significant inverse association between DEP metabolite levels and birth weight, limited to the black women. A nonsignificant positive association was observed among the white women. Naksen *et al.*, (2015) did not report the detailed results for combined urinary metabolites over pregnancy, except to note that none was associated with birth outcomes. Taken together, the four studies do not support a cause and effect relationship for infant health and chlorpyrifos exposure.

A number of other studies relied upon a single sample of blood, urine, or amniotic fluid to estimate *in utero* exposure (Barr *et al.*, 2010; Berkowitz *et al.*, 2004; Koutroulakis *et al.*, 2014; Wang *et al.*, 2012; Whyatt *et al.*, 2004; Wickerham *et al.*, 2012; Wolff *et al.*, 2007). As discussed previously, this does not meet the standard of “scientifically valid testing” to determine exposure to chlorpyrifos. The results are summarized in Table 1, below. The Columbia study is the only study to report a statistically significant inverse association with birth weight and birth length (Rauh *et al.*, 2004).

Table 1: Summary of Infant Health Epidemiology Study Results

Author, year	Exposure indicator	Study area	Birth weight	Birth Length	Head Circumference	Gestation Age
Eskenazi, 2004	TCPy, in urine (mean of 2 samples)	CHAMACOS, CA	+, No	+, No	+, No	-, No
	DEP, in urine (mean of 2 samples)		+, No	+, No	+, No	-, No
Sathyanarayana <i>et al.</i> (2010)	Ever used chlorpyrifos in farming	AHS, IA and NC	-, No	NR	NR	NR
Rauch <i>et al.</i> (2012)	DEP, in urine (mean of 2 samples)	HOME, OH	-, No (All) -, Yes (B) +, No (W)	NR	NR	-, No (All) -, No (B) -, No (W)
Naksen <i>et al.</i> (2015)	DEP, in urine (~ 8 samples)	SAWASDEE Thailand	No, direction NR	No, direction NR	No, direction NR	No, direction NR

Evidence from studies with 1 sample, do not meet criteria for scientifically valid exposure

Whyatt <i>et al.</i> (2004)	Chlorpyrifos in maternal/ cord blood	Columbia, NYC	-, Yes	-, Yes	-, No	NR
	Personal air samples (48 hours)		-, No	-, No	-, No	NR
Berkowitz <i>et al.</i> (2004)	TCPy in urine	Mt Sinai, NYC	+, No	+, No	=, No	=, No
Wolff <i>et al.</i> (2007)	DEP in urine	Mt Sinai, NYC	-, No	+, No	-, No	-, No
Barr <i>et al.</i> (2010)	Chlorpyrifos in maternal/ cord blood	NJ	-, No (MS) +, No (CS)	-, No (MS) -, No (CS)	-, No (MS) -, No (CS)	
Wang <i>et al.</i> (2012)	DEP in urine	China	+, No	+, No	NR	+, No
Wickerham <i>et al.</i> (2012)	Chlorpyrifos in cord blood	China	No Direction NR	NR	NR	NR
Koutroulakis <i>et al.</i> (2012)	DEP in amniotic fluid	Greece	+, No	NR	+, No	NR

Yes indicates statistically significant at $p < 0.05$; No indicates no statistical significance.

+ positive association, - adverse association, = the groups were equal.

NR: Not reported; B: Black women, W: White women; MS: Maternal sera, CS: Cord sera

F. Inconsistent Results in Epidemiology Studies of Neurodevelopment in the Growing Child

(1) Newborns

Limitations in exposure assessment were similarly found in studies of outcomes in newborns that relied upon a single urine or blood sample (Engel *et al.*, 2007; Silver *et al.*, 2017; Y. Zhang *et al.*, 2014). Only the prospective studies in California (CHAMACOS) and Ohio (HOME) collected more than one urinary sample ($n = 2$) and reported results relevant to chlorpyrifos (DEP).

Using the Brazelton Neonatal Behavioral Assessment Scale (BNBAS) assessment at ≤ 2 months, CHAMACOS investigators Young *et al.* (2005) observed a statistically inverse association with urinary DEP levels for reflexes, but no adverse association for other BNBAS parameters of habituation, orientation, motor performance, range of state, regulation of state and autonomic

stability. Despite having data for urinary TCPy, the CHAMACOS investigators limited the analyses to the less specific metabolite DEP (Young *et al.*, 2005). In contrast, the HOME study investigators used the NICU Network Neurobehavioral Scale (NNNS) at 5 weeks and reported significantly *improved scores* with DEP levels (Yolton *et al.*, 2013). In summary, findings from two studies are both negative and positive and do not do not support a cause and effect relationship chlorpyrifos-induced neurodevelopmental effects.

(2) Bayley Scale of Infant Development

The prospective studies in California (CHAMACOS) and Ohio (HOME) each administered the Bayley Scale of Infant Development (BSID) at routine intervals. Neither reported any statistically significant association with either the Mental Developmental Index (MDI) or the Psychomotor Developmental Index (PDI) and urinary chlorpyrifos metabolite levels (Donauer *et al.*, 2016; Eskenazi *et al.*, 2007). Another Ohio study similarly reported no adverse association with the BSID and maternal urinary TCPy at the second or third trimester (Fluegge *et al.*, 2016). The most robust studies found no adverse association of chlorpyrifos and infant development.

The findings from other studies (Columbia, Mt. Sinai, and Thailand) that relied upon single biological samples are provided in Table 2, Summary of Bayley Scales of Infant Development Epidemiology Study Results. The Columbia study reported a statistically significant inverse association with chlorpyrifos and as mentioned above, only when the child attained 36 months (*i.e.*, not at 12 or 24 months). In contrast, at 5 months of age, the Thailand study reported significantly lower (inverse) MDI and PDI scores with higher DEP levels (Kongtip *et al.*, 2017). If the association were truly causative, the relationships should be observed at similar ages. The Columbia study analyses for MDI and PDI also reported an exceedingly low R^2 for the regression models (10 – 25%), which indicates that the variability of the scores is poorly explained. Collectively, this reinforces the problem of weak exposure assessment and that the inconsistent observations are likely due to random error or bias.

Table 2: Summary of Bayley Scales of Infant Development Epidemiology Study Results

Author, year	Exposure indicator	Study area	Age	MDI (Mental)	PDI (Psychomotor)
Eskenazi <i>et al.</i> (2007)	TCPy, in urine (mean of 2 samples)	CHAMACOS, CA	6 m	+, No	-, No
			12 m	-, No	-, No
			24 m	-, No	-, No
	DEP, in urine (mean of 2 samples)		6 m	-, No	+, No
			12 m	-, No	+, No
			24 m	-, No	-, No
Donauer <i>et al.</i> (2016)	DEP, in urine (mean of 2 samples)	HOME, OH	12 m	=, No	=, No
			24 m	+, No	=, No
			36 m	=, No	=, No
Fluegge <i>et al.</i> (2016)	TCPy, in urine (2 samples, reported separately)	OH	3 m	-, No	-, No
				-, No	=, No

Table 2: Summary of Bayley Scales of Infant Development Epidemiology Study Results

Author, year	Exposure indicator	Study area	Age	MDI (Mental)	PDI (Psychomotor)
Evidence from studies with 1 sample, do not meet criteria for scientifically valid exposure					
Rauh <i>et al.</i> (2006)	Chlorpyrifos in cord blood	Columbia study, NYC	12 m - linear	-, No	-, No
Lovasi <i>et al.</i> (2011)			24 m - linear	-, No	+, No
			36 m - linear	-, No	-, Yes
			12 m - delay	-, No	-, No
			24 m - delay	-, No	=, No
			36 m - delay	-, Yes	-, Yes
Engel <i>et al.</i> , (2011)	DEP, in urine	Mt. Sinai, NYC	12 m	+, No	-, No
Kongtip <i>et al.</i> (2017)	DEP, in urine	Thailand	5 m	-, Yes	-, Yes

Yes indicates statistically significant at $p < 0.05$;
+ indicates positive association, - indicates adverse association, = the groups were equal ($\beta \leq 0.003$).
Note: the Columbia study evaluated the BSID using linear regression for the scores and categorically, as defined as delay ≤ 85 .

(3) Autism spectrum disorders, developmental delay, attention/ADHD outcomes, etc.

The Columbia and Mt. Sinai studies and others have evaluated other neurodevelopmental outcomes such as attention problems and ADHD in the growing children. Notably, the fundamental limitation of the exposure assessment remains, and exposure-outcome correlations are unreliable (Engel *et al.*, 2011; Fortenberry *et al.*, 2014; Furlong *et al.*, 2014; Rauh *et al.*, 2012; Rauh *et al.*, 2015; Shelton *et al.*, 2014). Specifically, the results of a case control study of autism spectrum disorders relied upon the California Pesticide Use Report to assign exposure based upon residential address (Shelton *et al.*, 2014). As previously discussed, this method is unlikely to validly assign exposure and the reported associations with applications of chlorpyrifos (and other pesticides) and cases of autism spectrum disorder are likely due to chance and/or confounding.

Using the Child Behavior Checklist (CBCL) at age 24, the CHAMACOS investigators reported no significant association between maternal TCPy or DEP and any CBCL outcome (Eskenazi *et al.*, 2007). These outcomes were re-assessed at ages 3.5 and 5 years for the dialkylphosphate metabolites only (Marks *et al.*, 2010). The authors reported many analyses, most of which were statistically nonsignificant. At 5 years, the categorical ADHD Confidence Index (> 70th percentile) and composite ADHD indicator were statistically associated with log DEP, but no other behavioral assessments were associated with DEP in the CHAMACOS study.

(4) Intelligence (IQ)

Recent publications have highlighted results related to childhood IQ and specific testing domains at ages 6 to 11 years (*see* Table 3, Summary of Epidemiology Study Results for Intelligence Testing). The CHAMACOS investigators observed a statistically significant association with maternal urinary DEP levels and Processing Speed, but not Full-Scale IQ or other domains such

as Working Memory (Bouchard *et al.*, 2011). Since the CHAMACOS investigators previously reported analyses using urinary TCPy, it can only be assumed that no association was present for this metabolite that is more specific to chlorpyrifos. The HOME study reported no inverse association with IQ and DEP (Donauer *et al.*, 2016).

The findings from publications from the Columbia, Mt. Sinai, and PELAGIE studies that relied upon a single sample are also provided in Table 3. The Columbia study investigators reported statistically significant decrement of log transformed Working Memory scores with increasing chlorpyrifos blood levels. Significant decrements were not observed for other IQ indices of Verbal Comprehension, Perceptual Reasoning and Processing Speed (Rauh *et al.*, 2011). The Mt. Sinai and the PELAGIE investigators reported no statistically significant association with any IQ function and DEP (Cartier *et al.*, 2016; Engel *et al.*, 2011). Taken together, the epidemiology data do not support a cause and effect relationship for impaired intelligence and prenatal chlorpyrifos exposure.

Table 3: Summary of Epidemiology Study Results for Intelligence Testing

Author, year	Exposure indicator	Study area	Test	Full-Scale IQ	Working Memory	Verbal Comprehension	Perceptual Reasoning	Processing Speed
Bouchard <i>et al.</i> (2011)	DEP, in urine (mean of 2 samples)	CHAMACOS, CA	WISC-IV	-, No	-, No	-, No	-, No	-, Yes
Donauer <i>et al.</i> (2016)	DEP, in urine (mean of 2 samples)	HOME, OH	WPPSI-III	+, No	NR	Verbal IQ -, No	Performance IQ -, No	NR
Evidence from studies with 1 sample, do not meet criteria for scientifically valid exposure								
Rauh <i>et al.</i> (2011)	Chlorpyrifos	Columbia, NYC	WISC-IV	-, Yes	-, Yes	-, No	-, No	-, No
Engel <i>et al.</i> (2011)	DEP, in urine	Mt. Sinai, NYC	WPPSI-III	-, No	NR	-, No	-, No	-, No
Cartier <i>et al.</i> (2016)	DEP, in urine	PELAGIE, France	WISC-IV	NR	+, No	+, No	NR	NR

Yes indicates statistically significant at $p < 0.05$, No indicates not statistically significant
+ indicates positive association, - indicates adverse association, NR Not reported.
WISC-IV: Wechsler Intelligence Scale for Children—4th Edition, administered at age 7 – 11 years.
WPPSI-III: Wechsler Preschool and Primary Scale of Intelligence—3rd Edition, administered at age 6 years

G. Epidemiology Studies Subsequent to the DART IC’s 2008 Decision Not to List Do Not Provide a Basis to List Now

As noted above and further in this section, additional studies published since the OEHHA review in 2008 have important limitations and do not demonstrate evidence of chlorpyrifos-induced neurodevelopmental. These limitations include reliance upon a single biological sample to infer past exposure and lack of specificity of exposure to chlorpyrifos *per se*.

A number of recent publications reported on birth outcome (*e.g.*, birth weight) (Acosta-Maldonado *et al.*, 2009; Barr *et al.*, 2010; Koutroulakis *et al.*, 2014; Moreno-Banda *et al.*, 2009; Naksen *et al.*, 2015; Rauch *et al.*, 2012; Sathyanarayana *et al.*, 2010; Wang *et al.*, 2012; Wickerham *et al.*, 2012). However, only three studies collected occupational information specific to chlorpyrifos or used at least 2 urinary samples. The results were mixed, reporting no association with parental use of chlorpyrifos and birth weight (Sathyanarayana *et al.*, 2010), an inverse association of urinary DEP (average of 2 samples) and lower birth weight among blacks (but not whites) (Rauch *et al.*, 2012); and no association with any birth outcome and urinary DEP (average of 8 samples) (Naksen *et al.*, 2015). In summary, there is no consistent evidence of an adverse association of birth outcome and chlorpyrifos exposure.

The study limitations are similarly found in publications of newborns (Samarawickrema *et al.*, 2008; Silver *et al.*, 2017; Zhang *et al.*, 2014) and developing children (Cartier *et al.*, 2016; Engel *et al.*, 2011; Fortenberry *et al.*, 2014; Furlong *et al.*, 2014; Kongtip *et al.*, 2017; Rauh *et al.*, 2011; Rauh *et al.*, 2012; Rauh *et al.*, 2015; Shelton *et al.*, 2014). Only the prospective studies in California (CHAMACOS) and Ohio (HOME) collected more than one urinary sample ($n = 2$) and reported results relevant to chlorpyrifos (urinary DEP). Their publications reported “no detrimental effects” with urinary DEP at 5 weeks (Yolton *et al.*, 2013), 12 months (Donauer *et al.*, 2016), or at school age (Bouchard *et al.*, 2011; Donauer *et al.*, 2016). As a result, findings from newly published studies, many of which are methodologically weak, are also inconsistent and do not contribute sufficiently to a claim of chlorpyrifos-induced neurodevelopmental effects. Many of the epidemiology studies, most prominently the Columbia study, do not use a reliable exposure classification. The body of information is largely inconsistent for specific endpoints, particularly for the most methodologically robust studies. As a result, the data do not “provide convincing evidence to support a causal relationship between exposure to the chemical, and the developmental or reproductive effect in question.”

H. Conclusions from Epidemiology Studies

Based on the foregoing discussion, it is clear that:

- The epidemiology data do not meet the criteria for sufficient evidence in humans.
- Data from multiple studies do not consistently show similar adverse association with chlorpyrifos.
- The CHAMACOS study is one of the better epidemiology studies for developmental toxicity because of the strong QA/QC procedures, collection of two biological samples during pregnancy, and analyzing for urinary TCPy (in addition to the less specific DEP). The results of this study for chlorpyrifos (urinary TCPy) do not show a clear relationship between exposure and effect.

In sum, the weight of the epidemiology evidence does not clearly show an association, much less a causal relationship between chlorpyrifos exposure and developmental effects. The epidemiology studies do not constitute “limited evidence or suggestive evidence in humans” that chlorpyrifos causes developmental toxicity (DART Criteria at 3.B.) and certainly do not represent “sufficient evidence in humans” of causation (DART Criteria at 3.A.).

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V. THE WEIGHT OF EVIDENCE FROM ANIMAL STUDIES DOES NOT PROVIDE “SUFFICIENT EVIDENCE IN EXPERIMENTAL ANIMALS”

A. Animal Study Overview

Chlorpyrifos is being considered for listing as a developmental toxicant due to “substantial new, relevant data on developmental toxicity.” Per the OEHHA announcement:

Chlorpyrifos was previously considered by the DART IC in 2008, but was not added to the Proposition 65 list at that time. Substantial new, relevant data on developmental toxicity have become available since the chemical was previously considered for listing.²⁶

Chlorpyrifos was evaluated for listing under Proposition 65 in 2008 and the DART IC at that time determined that the scientific evidence for chlorpyrifos did not support listing as either a developmental toxicant or male/female reproductive toxicant. As set forth herein, the available “substantial new, relevant data,” which are the sole basis for the current reevaluation, do not provide any compelling or consistent evidence that would merit reconsideration or provide the basis for listing as a developmental toxicant as it is presently being considered for under Proposition 65.

DAS summarizes below: (a) the evidence that was provided in 2008 related to guideline-compliant, Good Laboratory Practice studies that meet the DART Criteria for evaluation of developmental and neurodevelopmental toxicity; (b) the USEPA perspective and SAP response related to the 2012 SAP meeting on Chlorpyrifos Health Effects in which much of the available animal literature related to reported neurodevelopmental effects was assessed; (c) the US EPA’s perspective on the available literature relative to neurodevelopmental/behavioral studies as reviewed in the 2014 Revised Human Health Risk Assessment (RHHRA) and 2016 updated RHHRA; and (d) an analysis of the studies identified by US EPA in its 2014 RHHRA and additional evidence (studies) provided by OEHHA to the DART IC and dated August (Rev. Sept. 1 and Sept. 8), 2017.

The DART Criteria for evaluation of animal studies are as follows:

Sufficient evidence in experimental animals (mammals), such that extrapolation to humans is appropriate, in most cases based on the adequacy of the following:

- (1) The experimental design, including overall protocol and numbers of animals, and presence of appropriate controls.

²⁶ OEHHA, Announcement of the Developmental and Reproductive Toxicant Identification Committee Meeting Scheduled for November 29, 2007, September 1, 2017.

- (2) The exposure, in terms of route of administration, is relevant to expected human exposures, and in terms of timing, with regard to critical periods of development for developmental toxicity, sexual maturation, stage of pregnancy, or other critical periods for female reproductive toxicity, and sexual maturation, spermatogenesis, or other critical periods for male reproductive toxicity.
- (3) Number of dose levels, so that the presence of a dose-response relationship can be evaluated. It is desirable that the high dose level should elicit maternal toxicity in developmental studies, and systemic toxicity in female and male reproductive studies, and that the low dose should elicit no observable adverse effect for adult and offspring.
- (4) Consideration of maternal and systemic toxicity. The Criteria Document notes that “Differentiating between (a) the effects of a toxic agent on the conceptus or reproduction and (b) the effects on the conceptus or reproduction that are secondary to the maternal or systemic toxic effects is sometimes difficult and may require special attention, on a case by case basis.”
- (5) Number of tests or experimental animal species.
 - a. In general, effects should occur in multiple studies or multiple species for a substance to be recommended for listing.
 - b. Weight of evidence “considerations” are identified for this criterion:
 1. Data on a single species from a well conducted developmental or reproduction study may be sufficient to classify an agent as a reproductive toxicant provided there are not equally well conducted studies which do not show an effect and which have sufficient power to call into questions the repeatability of the observation in the positive study.
 2. Data on more than one species or from more than a single study increase the confidence for classification of an agent as a reproductive toxicant.
- (6) Other considerations, including, but not limited to those listed below, which can increase or decrease the confidence for classification of an agent as a reproductive toxicant.
 - a. Severity or consistency of findings.

- b. Metabolic and pharmacokinetic data.
- c. Time course of events.

B. Animal Studies Demonstrate That Chlorpyrifos Is Not a Developmental Toxicant

Chlorpyrifos has been extensively evaluated in four standard guideline studies covering three different species and in a developmental neurotoxicity study in rats. These study designs are developed from multi-stakeholder expert input over the course of many years (and continual review) to explicitly include those parameters required for a thorough and robust design aimed at identification of DART effects. DAS believes this is an important and relevant point to be included in the HID for DART IC consideration. Based on the collective results of the four studies discussed in this submission and in comparison to DART IC Criteria, there was no evidence of treatment-related or dose-related effects on embryo/fetal mortality, malformations, structural abnormalities and variations, altered fetal growth or change in gestational age at delivery.

Nor was there any evidence of chlorpyrifos-induced postnatal developmental effects, including physiological deficits and neurological or neurobehavioral deficits. Chlorpyrifos did not induce transplacental carcinogenesis or somatic or genetic mutations in the conceptus. Findings in one of the developmental studies (Deacon *et al.*, 1979.) were limited to embryo/fetal mortality at the highest dose, accompanied by pronounced maternal toxicity. This was not observed in the other three developmental toxicity studies. In all cases, developmental toxicity NOAELs were at or above those associated with maternal toxicity. Finally, there was no evidence of developmental neurotoxicity following exposure to chlorpyrifos in the DNT study (Maurissen *et al.*, 2000).

The results of these studies are summarized below as “scientifically valid testing according to generally accepted principles,” which demonstrate that chlorpyrifos is not a developmental toxicant. In addition, we include a summary of the developmental neurotoxicity study for chlorpyrifos, and comments from global regulatory authorities on chlorpyrifos and developmental toxicity.

(1) Developmental toxicity studies show that chlorpyrifos is not a developmental toxicant

Four developmental toxicity studies (one in mice, two in rats and one in rabbits) have been conducted that are compliant with existing test guidelines for the evaluation of reproductive and developmental toxicity. These studies show that chlorpyrifos is not a developmental toxicant. The results of these studies are summarized in Table 4, Summary of Guideline Developmental Studies with Chlorpyrifos.

TABLE 4: SUMMARY OF GUIDELINE DEVELOPMENTAL STUDIES WITH CHLORPYRIFOS

Species	Route	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Reference	Conclusions
Rat	Oral gavage	Dam, 3 Litter, 15	Dam, 15 Litter, none	Ouelette <i>et al.</i> , 1983	Not a developmental toxicant
Rat	Oral gavage	Dam, 2.5 Litter, 15	Dam, 15 Litter, none	Rubin <i>et al.</i> , 1987a	Not a developmental toxicant
Rabbit	Oral gavage	Dam, 81 Litter, 81	Dam, 140 Litter, 140	Rubin <i>et al.</i> , 1987b	Not a developmental toxicant
Mouse	Oral gavage	Dam, 1 Litter, 10	Dam, 10 Litter, 25	Deacon <i>et al.</i> , 1979	Not a developmental toxicant

The data from each of these studies are discussed in detail below. The studies demonstrate that chlorpyrifos is not a developmental toxicant as evaluated in the rat, the rabbit or the mouse.

(a) *Oral studies in rats*

Ouelette *et al.*, 1983. A prenatal rat developmental toxicity study was conducted in 1983 according to GLP standards (Ouelette *et al.*, 1983). Groups of 31 to 33 bred female Fischer 344 rats received oral doses of chlorpyrifos (Dursban® F, 96.6% chlorpyrifos) by gavage on days 6 through 15 of gestation at dose levels of 0 (corn oil vehicle), 0.1, 3 or 15 mg/kg bw/day. On day 21 of gestation, dams were euthanized and examined post mortem. A minimum of 24 litters/dose group were obtained. Ovarian, uterine and fetal observations were recorded. All fetuses were weighed, measured for crown-rump length and examined externally. One-half of each litter was examined immediately for visceral alterations by the Staples technique. The heads of the rat fetuses selected for visceral examination were preserved in Bouin's and examined by the serial sectioning technique of Wilson. All fetuses were then preserved, processed, stained with alizarin red-s and examined for skeletal alterations. Additional subgroups of 10 bred rats/dose group were dosed on days 6 through 15 of gestation and euthanized on gestation day 15, four hours after dosing, for plasma and erythrocyte cholinesterase determinations.

A summary of selected observations/parameters is presented in Table 5, Data from Oral Teratology Study with Chlorpyrifos. Dams given 15 mg/kg bw/day showed signs of severe maternal toxicity that included clinical signs of cholinergic effects (excessive salivation and tremors) and decreased body weight and body weight gain. Dams given 3 or 15 mg/kg bw/day had dose-related decreases in plasma and erythrocyte cholinesterase levels. In isolation, the depressions in plasma and erythrocyte cholinesterase levels were not considered adverse or toxicologically significant. ***No adverse developmental effects (embryo toxicity, fetal toxicity or teratogenicity) were observed at any dose level*** (see Table 6, Oral Teratology Study of Chlorpyrifos-Fetal Alterations, below).

Table 5. Data From Oral Teratology Study With Chlorpyrifos

Chlorpyrifos: Oral Teratology Study In Fischer 344 Rats				
Observations Made at the Time of Caesarean Section of Bred Rats				
	Chlorpyrifos mg/kg/day			
	0	0.1	3.0	15
Number of females bred	31	32	33	31
Number of maternal deaths	0	0	0	0
Pregnancies detected by stain only	0/1	0/4	0/5	1/3
% Pregnant, (total) ^a	97(30/31)	88(28/32)	84(26/31) ^b	94(29/31)
Number of litters	29	26	24	26
Corpora lutea/dam ^c	11±2	11±2	11±2	12±1
Implantation sites/dam ^c	11±2	10±2	10±2	10±3
% Preimplantation loss ^d	8±12	12±16	11±21	15±20
Fetuses/litter ^c	10±3	9±2	10±3	9±3
Resorptions/litter ^c	0.9±1.3	0.8±0.8	0.6±0.8	0.6±0.8
% Implantations resorbed	9(26/304)	9(22/259)	6(15/245)	6(15/259)
% Litters with resorptions	45(13/29)	62(16/26)	46(11/24)	42(11/26)
Litters totally resorbed	0	0	0	0
Resorptions/litters with resorptions	2.0(26/13)	1.4(22/16)	1.4(15/11)	1.4(15/11)
Dead fetuses	0	0	0	0
Sex ratio, M:F, %	48:52	53:47	52:48	54:46
Fetal body weight (grams)	4.28±0.15	4.37±0.32	4.52±0.14*	4.46±0.40*
Fetal crown-rump length (mm)	43.91±2.71	43.93±2.64	43.96±2.06	43.64±2.33

a Number of females detected as being pregnant by visual inspection of the uterus or by sodium stain/total bred.

b Two animals were removed from study during dosing (one exhibited glaucoma, one was inadvertently deprived of water overnight).

c Mean ± S.D.

d Percent per litter, mean ± S.D.

e Mean of litter means ± S.D.

* Indicates statistical difference from control, $\alpha = .05$.

Table 6: Oral Teratology Study of Chlorpyrifos-Fetal Alterations

		Chlorpyrifos, mg/kg/day			
		0	0.1	3.0	15
		Number Fetuses (Number Litters) Examined			
External Examination		278 (29)	237 (26)	230 (24)	244 (26)
Soft Tissue Examination		147 (29)	126 (26)	124 (24)	130 (26)
Skeletal Examination		278 (29)	237 (26)	230 (22)	244 (26)
Bones of the Skull		129 (29)	110 (25)	106 (22)	115 (25)
		Percent Affected (Number Affected)			
External Observations					
Microphthalmia [†]	F ^a	0.4 (1)	0	0.4 (1)	0.8 (2)
	L	3 (1)	0	4 (1)	8 (2)
Anophthalmia [†]	F ^a	0.4 (1)	0	0.4 (1)	0.8 (2)
	L	3 (1)	0	4 (1)	8 (2)
Soft Tissue Observations					
Cleft soft palate [†]	F	0	0	0	0.8 (1)
	L	0	0	0	4 (1)
Patent ductus arteriosus	F	0.7 (1)	0	0	0
	L	3 (1)	0	0	0
Hemorrhage of the liver	F	0	0.8(1)	0	0
	L	0	4 (1)	0	0
Convoluted ureter	F	0	0.8 (1)	0	2 (2)
	L	0	4 (1)	0	8 (2)
Severely dilated renal pelvis	F	0	0	0	2 (3)
	L	0	0	0	4 (1)
Skeletal Observations					
Vertebrae - Delayed ossification of centrum	F	6 (16)	6 (13)	1* (2)	5 (12)
	L	41 (12)	42 (11)	8 (2)	42 (11)
Ribs					
-Fused [†]	F	0	0.4 (1)	0	0
	L	0	4 (1)	0	0
-Spur	F	0.7 (2)	2 (4)	0	1 (2)
	L	3 (1)	15 (4)	0	8 (2)
Sternebrae					
-Delayed ossification	F	41 (115)	44 (103)	37 (85)	34(84)
	L	97 (28)	89 (23)	96 (23)	92 (24)
-Extra site of ossification	F	0.4 (1)	0	0	0
	L	3 (1)	0	0	0

a F = fetuses, L = litters.

* Different from the control value by Wilcoxon (modified by Haseman and Hoel) test, = 0.05

† Considered to be a malformation.

The NOAEL for developmental toxicity was 15 mg/kg bw/day, the highest dose level tested; the maternal NOAEL was 3 mg/kg bw/day, based on clinical signs of cholinergic effects and decreased body weight gain at 15 mg/kg/day. A maternal NOEL of 0.1 mg/kg bw/day was established based on plasma and erythrocyte cholinesterase depression.

Significantly, the California Department of Pesticide Regulation and US EPA separately concluded as follows:

- DPR Conclusion: Maternal NOEL (excluding cholinesterase (ChE inhibition) = 3.0 mg/kg/day (cholinergic effects). Maternal ChE inhibition NOEL = 0.1 mg/kg/day (inhibition of plasma and RBC ChE). Developmental toxicity NOEL = 15 mg/kg/day (HDT). ACCEPTABLE due to submission of supplementary information.
- US EPA 1-liner [Teratology – rat; Toxicology. Research Lab; 7/5/83] Teratogenic and fetotoxic NOEL > 15 mg/kg/day (HDT); Maternal NOEL = 0.1 mg/kg; Maternal LEL = 3.0 (ChE inhibition).

The results from this study demonstrate that chlorpyrifos is not a developmental toxicant as evaluated in rats.

Rubin *et al.*, 1987a. A second prenatal rat developmental toxicity study was conducted in 1987 according to GLP standards (Rubin *et al.*, 1987a). Groups of 32 bred female CD rats received oral doses of chlorpyrifos (Pyrinex, 96.1% chlorpyrifos), by intragastric gavage, on days 6 through 15 of gestation at dose levels of 0 (corn oil vehicle), 0.5, 2.5 or 15 mg/kg bw/day. On day 15 of gestation, 10 females/dose group were euthanized for determination of plasma cholinesterase levels. The remaining 22 females/dose group were euthanized and examined post mortem on day 20 of gestation. A minimum of 21 litters/dose group was obtained. Ovarian, uterine and fetal observations were recorded. All fetuses were weighed, measured for crown-rump length and examined externally. Approximately one-half of each litter was examined immediately for thoracic and abdominal visceral alterations. These fetuses were then preserved, processed, stained with alizarin red and evaluated for skeletal alterations. The remaining fetuses were preserved in Bouin's fixative and examined by the serial sectioning technique of Wilson.

Adverse maternal effects were limited to dams given 15 mg/kg bw/day and included an increased incidence of tremors (3 of 21 animals), a transient decrease in feed consumption, and decreased body weight. Although plasma cholinesterase was decreased in a dose-related manner at all exposure levels, this effect was not considered adverse or toxicologically significant.

No adverse developmental effects were seen at doses below maternal toxicity. The authors concluded that adverse fetal effects, consisting of a very slight but statistically significant increase in post-implantation loss, accompanied the maternal toxicity observed at 15 mg/kg bw/day (*see* Table 7, Group Mean Litter Data on Day 20 of Gestation). This very slight increase in post-implantation loss was not considered by the notifiers to be treatment-related as the magnitude of the apparent increase was very small and within the range of normal variability, the increase was found to be significant in only one of the two statistical methods used to analyze this parameter, and an effect on this parameter was not observed in the previous rat teratology study. No other adverse treatment-related developmental effects (embryo toxicity, fetal toxicity or teratogenicity) were noted at any exposure level.

The NOAEL for developmental toxicity was 15 mg/kg bw/day, the highest dose level tested; the maternal NOAEL was 2.5 mg/kg bw/day, based on clinical signs of tremors, transient decreases in feed consumption and decreased body weight at 15 mg/kg bw/day. A maternal NOEL, based on depressions in plasma cholinesterase, was not established.

Table 7: Group Mean Litter Data on Day 20 of Gestation

Group: 1 2 3 4
 Test material: PYRINEX
 Dosage (mg/lg/day): 0 0.5 2.5 15

Group	N	Corporalutea	Weight of gravid uterus (g)	Live fetuses			Resorptions			Implantation loss		Mean fetal wt. and SD (g)	Mean CRL and SD (mm)	Mean placental wt. and SD (g)
				M	F	Total	Early	Late	Total	(%) *	(%) *			
1 i	22	17.1	75.3	7.6	6.8	14.4	1.0	0.0	1.0	8.6	7.0	3.33	36.0	0.48
ii		2.2	9.2	1.8	2.2	1.8	1.4	0.0	1.4	2.6	2.1	0.27	1.0	0.04
iii														
2 i	21	18.2	80.3	8.0	7.0	15.0	0.9	0.0	0.9	12.20	6.0	3.40	36.2	0.50
ii		2.4	9.1	2.1	1.8	1.7	1.1	0.2	1.1	2.1	1.8	0.18	0.8	0.04
Iii												0.26	1.4	0.06
3 i	22	16.9	76.1	6.8	7.3	14.1	0.8	0.0	0.8	11.50	5.8	3.44	36.3	0.49
ii		2.1	8.0	1.9	1.9	1.6	1.1	0.0	1.1	1.8	1.8	0.17	0.6	0.04
												0.25	1.3	0.06
4 i	21	17.0	73.4	6.0 ^a	7.5	13.4	1.3	0.0	1.3	11.40	9.0 ^b	3.51 ^b	36.7 ^b	0.48
ii		2.6	10.1	2.2	1.9	1.7	1.5	0.0	1.5	2.7	2.4	0.25	0.9	0.05
												0.22	1.2	0.06

* Freeman - Tukey arcsino transformed data.

i Group mean

ii Standard deviation

iii Pooled weighted within - litter standard deviation

M Male

F Female

CRL crown - rump length

a: Significantly different from control. P < 0.05. Student's t-test

b: Significantly different from control. P < 0.01. Student's t-test

c: Significantly different from control. P < 0.001. Student's t-test

California DPR concluded that:

- No maternal ChE NOEL was identified (dose-related plasma ChE inhibition at all dose levels at day 15 p.c., with restoration of normal ChE activity in all but high dose dams by p.c. day 20).
- Maternal functional NOEL = 2.5 mg/kg/day (tremors in 3/21 dams, transient food consumption reduction, modest but consistent body weight decrement).
- Developmental NOEL = 2.5 mg/kg/day (slight increase in early resorptions). No adverse reproductive effect at dose levels sufficient to elicit cholinergic responses. Acceptable.

Overall, for both rat developmental toxicity studies (Ouelette and Rubin), the NOAEL for developmental toxicity was 15 mg/kg bw/day, the highest dose level tested; the maternal NOAEL was 3 mg/kg bw/day, based on clinical signs of cholinergic effects, decreased feed consumption and decreased body weight. A maternal NOEL of 0.1 mg/kg bw/day was established based on plasma and erythrocyte cholinesterase depression.

The results from this study demonstrate that chlorpyrifos is not a developmental toxicant as evaluated in rats.

(b) Oral study in rabbits

Rubin *et al.*, 1987b. A prenatal rabbit developmental toxicity study was conducted in 1987 according to GLP standards (Rubin *et al.*, 1987b). Groups of 14 to 21 mated female HY/CR rabbits received oral doses of chlorpyrifos (Pyrinex, 96.1% chlorpyrifos), by intragastric gavage, on days 7 through 19 of gestation at dose levels of 0 (corn oil vehicle), 1, 9, 81 or 140 mg/kg bw/day. On day 29 of gestation, females were euthanized and examined post mortem. A minimum of 11 litters/dose group was obtained. Ovarian, uterine and fetal observations were recorded. All fetuses were weighed, measured for crown-rump length and examined for external, visceral and skeletal alterations. Plasma cholinesterase activity was measured once before mating and again after at least 10 days of dosing.

Adverse maternal effects were limited to dams given 140 mg/kg bw/day and consisted of decreased body weight gain. Although plasma cholinesterase was decreased in a dose-related manner at all exposure levels, this effect was not considered adverse or toxicologically significant.

No adverse developmental effects were seen at doses below maternal toxicity. Adverse developmental effects were limited to the high dose and included a slight reduction in fetal growth and a possible increase in post-implantation loss (*see* Table 8, Group Mean Litter Development on Day 29 of Gestation, below). No other adverse treatment-related

developmental effects (embryo toxicity, fetal toxicity or teratogenicity) were noted at any exposure level.

The NOAEL for developmental toxicity in the rabbit was 81 mg/kg bw/day, based on fetal growth and possibly post-implantation loss at 140 mg/kg/day; the maternal NOAEL was 81 mg/kg bw/day, based on decreased body weight gain at 140 mg/kg/day. A maternal NOEL, based on depression in plasma cholinesterase, was not established.

California DPR drew the following conclusions:

- Maternal NOEL = 81 mg/kg day (body weight gain decrement during treatment period).
- Developmental NOEL = 81 mg/kg/day [reduced crown/rump length, reduced fetal weight, ossification delays (indicated by non-ossification of fifth sternebra and/or xiphisternum)].
- No adverse effects are indicated. Acceptable.

The results from this study demonstrate that chlorpyrifos is not a developmental toxicant as evaluated in rabbits.

Table 8: Group Mean Litter Data on Day 29 of Gestation

Group	Corporalute a	Weight of gravid uterus (g)	Live fetuses			Resorptions			Implantation loss		Mean fetal wt. and SD (g)	Mean CRL wt. and SD (mm)	Mean placental wt. and SD (g)
			M	F	Total	Early	Late	Total	Pre – (%) *	Post – (%) *			
1 i	10.4	515.5	3.8	4.5	8.3	0.1	0.4	0.5	15.2	6.5	45.4	97.4	6.0
ii	1.4	99.0	1.9	1.5	2.1	0.3	0.8	0.8	4.2	2.2	5.7	4.2	1.4
iii											5.7	5.5	1.0
N	13.	13.	13.	13.	13.	13.	13.	13.	13.	13.	13.	13.	13.
2 i	11.1	556.4	4.1	4.9	9.0	0.3	0.5	0.8	13.1	8.6	44.3	95.9	6.0
ii	2.1	109.3	1.7	1.7	2.4	0.5	0.7	0.9	3.4	2.5	4.7	4.3	0.7
iii											5.6	4.7	0.9
N	13.	13.	13.	13.	13.	13.	13.	13.	13.	13.	13.	13.	13.
3 i	12.8 ^b	582.8	4.9	4.8	9.7	1.2	0.7	1.9 ^a	8.9 ^c	13.8 ^c	43.3	95.0	5.8
ii	2.0	131.8	2.6	1.6	3.1	3.3	0.9	3.2	3.1	7.5	6.0	4.6	1.1
iii											5.8	5.3	0.9
N	13.	13.	13.	13.	13.	13.		13	13.	13.	13.	13.	13.
4 i	11.9	555.6	4.7	4.7	9.5	0.4	0.4	0.8	11.9 ^a	8.7	41.8	94.2	5.5
ii	2.8	117.3	1.8	1.8	1.7	0.7	0.6	0.9	3.4	2.4	6.8	5.9	1.1
iii											4.6	4.2	0.7
N	15.	15.	15.	15.	15.	15.	15.	15.	15.	15.	15.	15.	15.
5 i	12.1	518.5	4.9	4.1	9.0	0.7	1.0	1.7	11.5 ^a	13.9 ^c	40.7	93.2 ^a	5.4
ii	1.8	82.2	1.4	1.6	2.0	1.2	1.5	1.8	3.6	4.8	6.0	4.9	0.7
iii											4.8	5.2	1.0
N	11.	11.	11.	11.	11.	11.	11.	11.	11.	11.	11.	11.	11.

* Freeman - Tukey arcsino transformed data.

i Group mean

ii Standard deviation

iii Pooled weighted within - litter standard deviation

M Male

F Female

CRL crown - rump length

a: Significantly different from control. P < 0.05. Student's t-test

b: Significantly different from control. P < 0.01. Student's t-test

(c) *Oral study in mice*

Deacon *et al.*, 1979. A prenatal mouse developmental toxicity study was conducted in 1983. Groups of 35 to 47 bred female CF-1 mice received oral doses of chlorpyrifos (Dursban® F, 96.8% chlorpyrifos) by gavage on days 6 through 15 of gestation at dose levels of 0 (cottonseed oil vehicle), 0.1, 1, 10 or 25 mg/kg bw/day. On day 18 of gestation, dams were euthanized and examined post mortem. A minimum of 24 litters/dose group was obtained. Ovarian, uterine and fetal observations were recorded. All fetuses were weighed, measured for crown-rump length and examined externally. One-half of each litter was examined immediately for visceral alterations by the Staples technique. The heads of the fetuses selected for visceral examination were preserved in Bouin's and examined by the serial sectioning technique of Wilson. All fetuses were then preserved, processed, stained with alizarin red-s and examined for skeletal alterations. Additional groups of bred mice were administered chlorpyrifos during gestation and euthanized on gestation day 15 for maternal plasma and erythrocyte and total fetal cholinesterase determinations.

Table 9, Observations at Time of Cesarean Section of Bred Mice Given Chlorpyrifos by Gavage, demonstrates that severe maternal toxicity was present at 25 mg/kg bw/day and included clinical signs of cholinergic effects, decreased feed and water consumption, decreased body weight and death. Maternal effects attributed to treatment were also observed at 10 mg/kg bw/day and included signs of cholinergic effects and a single maternal death. Plasma and erythrocyte cholinesterase levels were decreased at 1 mg/kg bw/day and higher but, in isolation, these effects were not considered adverse or toxicologically significant.

No adverse developmental effects were seen at doses below maternal toxicity. Adverse developmental effects were limited to the high dose and consisted of decreased fetal growth and skeletal maturation. Fetuses were less sensitive to cholinesterase inhibition than the dams, showing depressions in total cholinesterase at maternal exposure levels of 10 mg/kg bw/day and higher.

The NOAEL for developmental toxicity was 10 mg/kg bw/day, based on decreased fetal growth and skeletal maturation at 25 mg/kg bw/day. The maternal NOAEL was 1 mg/kg bw/day, based on clinical signs of cholinergic effects and a single maternal death at 10 mg/kg bw/day. Developmental and maternal NOELs were 1.0 and 0.1 mg/kg bw/day, respectively.

California DPR and US EPA concluded as follows:

- California DPR:
 - NOEL for maternal functional toxicity = 1 mg/kg/day[cholinesterase (ChE) effects as salivation, tremors, *etc.*].
 - ChE enzyme NOEL = 0.1 mg/kg/day (significant inhibition of maternal plasma ChE at 1 mg/kg/day).

- Developmental toxicity NOEL = 10 mg/kg/day (decreased fetal length and weight, delayed ossification in skull, sternebrae). ACCEPTABLE
- US EPA 1-liner:
 - Teratology – mice; Toxicology. Research Lab; 7/24/74 [sic:presumed this is the 7/24/79 study]; Teratogenic NOEL > 25 mg/kg/day (HDT)
 - fetotoxic NOEL = 10 mg/kg fetotoxic LEL = 25 mg/kg (decreased fetal length, increased skeletal variants)
 - Plasma and RBC ChE NOEL = 0.1 mg/kg/day.

The results from this study demonstrate that chlorpyrifos is not a developmental toxicant as evaluated in mice.

Table 9: Observations at Time of Cesarean Section of Bred Mice Given Chlorpyrifos by Gavage

	Dose Levels of Chlorpyrifos, mg/kg			
	<u>0</u>	<u>1</u>	<u>10</u>	<u>25</u>
No. of Bred Females	51	40	44	47
No. of Maternal Deaths	0/51	1/40	1/44	4/47
% Pregnant ^c	70 (36/51)	75 (30/40)	70(31/44)	70(33/47)
% pregnant, Total ^d	78 (40/51)	78 (31/40)	73(32/44)	72(34/47)
Pregnancies Detected by Stain ^e	<u>4</u>	<u>1</u>	<u>1</u>	<u>1</u>
No. of Litters	<u>36</u>	<u>29</u>	<u>30</u>	<u>29</u>
Implantation Sites/dam ^f	13±3	13±2	13±1	12±3
Fetuses/Litter ^f	11±3	12±2	12±2	11±3
Resorptions/Litter ^{f, g}	1±2	1±1	1±2	1±2
% Implantations resorbed ^g	11(50/458)	9(35/382)	8(30/389)	11(39/365)
%Litters with Resorptions ^g	67(24/36)	55(16/29)	53(16/30)	66(19/29)
Litters Totally Resorbed ^g	0	0	0	0
Resorptions/Litters with Resorptions ^g	2.1(50/24)	2.2(35/16)	1.9(30/16)	2.0(39/19)
% Dead Fetuses	0(0/408)	0(0/347)	1(2/359)	0(0/326)
Sex Ration, M : F	51:49	46:54	44:56	50:50
Fetal Body Weight, g ^h	1.14±0.10	1.13±0.07	1.15±0.08	1.03±0.17 ^j
Fetal Crown-rump length, mm ^h	25.0±1.2	25.2±0.9	25.3±0.9	24.4±1.6 ⁱ

- a Bred mice were given 0, 1, 10 or 25 mg/kg/day chlorpyrifos by gavage on days 6 through 15 of gestation.
- b Significantly different from control values by Fisher's Exact Probability Test p < 0.05.
- c Number of females with visible implantation sites at the time of cesarean section or necropsy/total number of bred females.
- d Number of females with implantation sites as observed either visually at cesarean section or necropsy, or after staining with sodium sulfide/total number of bred females.
- e Number of females with implantation sites detected only after staining the uterus with sodium sulfide.
- f Mean ± S.D.
- g Resorptions which were detected only by sodium sulfide staining were not included in these calculations.
- h Mean of litter means ± S.D.
- i Significantly different from the control values by Dunnett's test, p<0.05.

(2) The Only Developmental Neurotoxicity Study Shows that Chlorpyrifos Is Not a Developmental Toxicant

The only chlorpyrifos developmental neurotoxicity (DNT) study available today that best meets the study-design requirements of regulatory agencies world-wide is the guideline compliant, Good-Laboratory-Practices compliant, chlorpyrifos DNT study conducted by Drs. Alan Hoberman (study director) and Robert Garman (pathologist) at Argus Laboratories in 1998 (Hoberman, 1998). This study meets global standards and requirements for study design to evaluate neurotoxicity, sensitivity, and non-cholinergic effects in young animals, and also supersedes all *in vitro* and other laboratory animal studies that use inappropriate doses and routes of administration, key factors when considering relevance to humans.

Because of the newness of guideline-based developmental neurotoxicity (DNT) studies in 1997, the chlorpyrifos DNT study was conducted under a protocol developed by Dr. Jacques Maurissen and other toxicologists at The Dow Chemical Company (Dow), in consultation with US EPA toxicologists. Although the study was conducted according to the 1991 DNT guidelines, the 1998 DNT guidelines were under preparation and the purpose of the consultation with US EPA was to design a study that would meet all current expectations for a state-of-the-art DNT study. Although the draft protocol recommended dietary exposure to chlorpyrifos, the US EPA strongly recommended oral gavage. The maternal doses were 0, 0.3, 1 or 5 mg/kg/day. The route of exposure was oral gavage in vegetable oil of dams from gestation day 6 to lactation day 10 (birth = lactation day 0). A publication by Marty et al. (2007) demonstrates that oral gavage of chlorpyrifos in vegetable oil to pregnant rats causes a blood chlorpyrifos C_{max} approximately 13X higher than chlorpyrifos administered in the diet.

The US EPA analyzed samples from the chlorpyrifos DNT study for maternal plasma, RBC and brain ChE activity. Because of his experience with chlorpyrifos and cognitive testing, Dr. Mark Stanton of the US EPA was consulted on the design of the cognitive test that was conducted just after weaning and again when the pups were about two months old (a T-maze spatial-delayed alternation task to evaluate learning and memory).

The chlorpyrifos DNT study was published in the open literature (Maurissen *et al.*, 2000). No historical DNT morphometric control data were available at the time the chlorpyrifos DNT study was conducted, but Drs. Hoberman and Garman conducted five DNT studies soon after the chlorpyrifos study, at the same laboratory and using the same methods, and issued a Supplement 3, Historical control morphometric data (Hoberman, 2000). Notably, the morphometric historical control data (Supplement 3) were submitted five months after the US EPA June 8, 2000 risk assessment was released.

High-dose dams had clinically-evident toxic signs just before and for four days subsequent to giving birth (*e.g.*, muscle fasciculations, hyperpnea, hyperactivity, diminished weight and weight gain). Several pups of high-dose dams died at this time, some in entire litters and some without milk in their stomachs. When maternal clinical signs abated, no more pup deaths occurred. Pups from high-dose dams gained weight more slowly than controls, and several of the developmental measures showed effects consistent with slightly delayed maturation. Although there were many

signs of delayed maturation, pups of high-dose dams performed as well as controls in post-weaning tests of learning and memory (T-maze spatial delayed-alternation task). There was no evidence of maternal toxicity at 1 mg/kg/day, and pups of these dams had no differences from control that were attributed to treatment. Small but statistically significant differences in the thickness of the parietal cortex of high- and mid-dose female pups at two months of age were considered to be random effects and not treatment related for several reasons (discussed below).

The DNT study concluded the maternal and developmental NOAEL was 1 mg/kg/day. Cognitive function such as learning, memory and habituation were not impaired in the pups at any of the dose levels. All adverse effects in offspring of high-dose dams in this study were interpreted by Drs. Hoberman and Garman as secondary to pup undernutrition due to excessive maternal toxicity in high-dose dams (*i.e.*, secondary to maternal toxicity). There was no evidence of developmental neurotoxicity following exposure to chlorpyrifos in the DNT study (Maurissen *et al.*, 2000).

Notably, the US EPA did not consider this DNT study to be of concern relative to developmental neurotoxicity during the revised organophosphate cumulative risk assessment (US EPA, 2002) or in their final cumulative risk assessment (US EPA, 2006). Both of these subsequent EPA reviews considered the published literature on chlorpyrifos developmental toxicity, including Supplement 3, and the FQPA factor for chlorpyrifos (repeated exposures) was determined to be 1X.

(3) Numerous Regulatory Bodies Have Evaluated Chlorpyrifos for Developmental Toxicity

Various regulatory bodies have evaluated chlorpyrifos for developmental toxicity, based in part on the DNT study. Statements from various agencies relevant to the DART IC's consideration follow.

WHO 1999 Toxicology Assessment. “The NOAEL for toxic effects in the pups was 1 mg/kg bw per day on the basis of the decreased viability index, relative brain weight, and delayed sexual maturity, possibly associated with maternal toxicity and subsequent diminished maternal care at the high dose. Cognitive function (learning, memory, and habituation) in the pups were not affected by treatment (Hoberman, 1998).” (WHO, 1999.)

2001 California Department of Pesticide Regulation. Concluding comments about Supplement 3: “In the context of the demonstrated high maternal and neonatal toxicity of this dose, the supplemental data reinforce the lack of demonstrated special toxicity of the test article toward the developing nervous system. Supplemental to a previously acceptable study with no adverse effects.” (CalEPA, 2001).

United Kingdom Advisory Committee on Pesticides (ACP 6(299/03)). “By contrast, the OECD Guideline-compliant developmental neurotoxicity study performed with chlorpyrifos covered similar endpoints and established a clear NOAEL (1 mg/kg bw/day) for effects on pups following oral exposure (see Appendix 2, Hoberman, 1998 at section 5.1.7.1(q), and the evaluation of a supplement to this study at Appendix 3),” at 3. (UK, ACP, 2003.)

APPENDIX 2 - Taken from ACP 264 (277/00) considered by ACP 6 July 2000: “The NOAEL for effects on pups was 1 mg/kg bw/day, based on decreased viability, lower pup bodyweights and brain weights and delayed sexual maturity at 5 mg/kg bw/day. These effects were consistent with being secondary to maternal toxicity. Cognitive functions in the pups (learning, memory and habituation) were not affected by treatment at any dosage. There were no neuropathology findings in pups at 12 or 66 days of age.” (UK, ACP, 2003.)

Australia 2000a Chlorpyrifos Toxicology Assessment (Supplement 3 not included): “The morphometric measurements reveal minor variations (ca. 5%) which might be expected for such a small sample (6 animals). The neuropathological microscopical examinations (generally 48 sites/tissues reported) were restricted to the control and high dose animals and no effects of treatment were evident. While data comprising the morphometric measurements were provided for mid-dose DPP 66 females (1 mg/kg/d), no neuropathological examinations were reported for this group. These results suggest that the animals had generally recovered from the delayed development that was evident at DPP 12.” (Australia, 2000a.)

Australia 2000b NRA Chlorpyrifos Summary. “There was no evidence that significant developmental or neurological effects were caused by chlorpyrifos in young animals at doses below those that inhibited plasma cholinesterase activity”. . . “The data on effects of chlorpyrifos in young or developing animals have been reviewed and infants and children are not considered to be at an increased risk from chlorpyrifos products that are used according to label instructions.” (Australia, 2000b.)

(4) Regulatory Authorities and Independent Experts Have Concluded That Chlorpyrifos Is Not a Developmental Toxicant

Regulatory Bodies. Several expert regulatory bodies, including the US EPA, have recently examined the potential health effects of chlorpyrifos. These thorough evaluations were performed after 1999, using not only the comprehensive developmental and reproductive toxicity studies required by the regulatory agencies, but also some of the published academic literature on potential DART effects that has become available in recent years. The regulatory agencies used criteria generally recognized by the scientific community and analogous to the DART Criteria used by the DART IC.

US EPA 2000 Human Health Risk Assessment of Chlorpyrifos. The US EPA's June 2000 Human Health Risk Assessment of chlorpyrifos is one of several key documents that the EPA developed to contribute to its toxicological conclusions within the IRED (US EPA 2000). The Human Health Risk Assessment's evaluation of the developmental toxicity data concludes that "*in both mice and rabbits, the developmental effects occurred at maternally toxic doses as indicated by reduced weight gain, and food consumption in both species, and increased mortality in mouse dams.*" *Id.* at 16 (emphasis added). As to the rat developmental studies, EPA similarly concludes that "*In one rat study, developmental effects (increased post-implantation loss) were noted at 15 mg/kg/day (highest dose tested, HDT), that were also associated with maternal toxicity, while another rat study failed to observe developmental effects at 15 mg/kg/day.*" *Id.* at 15 (emphasis added).

European Commission – Classification and Labeling (2005). The European Commission recently completed its risk classification process for chlorpyrifos (European Commission, 2005). This process is coordinated by the Commission Working Group on the Classification and Labeling of Dangerous Substances. This Working Group comprises representatives from several member states with expertise in toxicology and other disciplines involved in the EC Risk Phrases. At its February 2002 meeting, the Working Group considered potential changes to the Risk Phrases for chlorpyrifos. In particular, its debate focused on the possible classification of R64, the Risk Phrase "May cause harm to breastfed babies." The Working Group concluded that this Risk Phrase would be inappropriate for chlorpyrifos. The Working Group also concluded that none of the other potential Risk Phrases involving developmental and reproductive effects (R47, R60, R61, R62, and R63) were appropriate for chlorpyrifos classification.

Australian National Registration Authority (2000). The Australian National Registration Authority ("ANRA") completed its comprehensive evaluation of chlorpyrifos in 2000 (Australia, 2000b). ANRA's over-600-page toxicology evaluation summarized its conclusions regarding animal studies of developmental and reproductive effects by stating that "*exposure to chlorpyrifos had no adverse effects on reproduction. The data on effects of chlorpyrifos in young or developing animals have been reviewed and infants and children are not considered to be at an increased risk from chlorpyrifos products that are used according to label instructions.*" (ANRA 2000b) (emphasis added).

California Department of Pesticide Regulation (2001). DPR updated its Summary of Toxicology Data on chlorpyrifos (CalEPA/DPR 2001) and completed a comprehensive draft Risk Characterization Document (RCD) on chlorpyrifos. The conclusions of DPR's evaluation of the toxicology data on chlorpyrifos are summarized in Cochran (2002). DPR's evaluations included developmental toxicity and reproductive toxicology studies required by DPR and EPA, along with several other documents relating to DART. Furthermore, the studies cited in the OEHHA survey of chlorpyrifos as indicative of DART were

assessed by DPR (Cochran 2002), which concluded that *“There is insufficient evidence that human infants are more susceptible to the toxicity of chlorpyrifos than adults and small children and there is no compelling evidence that chlorpyrifos causes any developmental neurotoxicity under physiologically relevant conditions.”* In their evaluation of the comprehensive studies required under FIFRA by the EPA and DPR, for the developmental and reproductive toxicity categories, the DPR Summary notes that there is *“no data gap, no adverse effect”*. DPR’s more detailed conclusions are similar to those of the other agencies discussed previously. For example, in its evaluation of the most recent and comprehensive two-generation dietary reproductive toxicity study, DPR concludes that *“the reproductive findings at 5 mg/kg/day do not warrant a possible adverse effects designation, since brain ChE levels were very markedly depressed at that dose level, and all observed reproductive effects appeared to be due to failure of dams to nurture pups which were otherwise normal”* (CalEPA/DPR 2001).

Independent Review. Experts in reproductive and developmental toxicology also reviewed the chlorpyrifos reproductive and developmental literature in 1999, and concluded:

“As can be seen, the young in all studies conducted evidenced toxicity at the same or higher dose levels than the adult parent. Chlorpyrifos did not adversely affect reproduction and was not developmentally neurotoxic or teratogenic, and no selective toxicity or sensitivity of the fetus or young animals was apparent in any guideline studies that were scientifically acceptable.”

(Schardein and Scialli, 1999). The CDC similarly reviewed the developmental toxicity data and concluded that chlorpyrifos was not a teratogen (Jackson *et al.*, 1999).

(5) The US EPA and its Scientific Advisory Panel Have Significantly Discounted the Weight of Evidence Value of Studies That Attempt to Associate Chlorpyrifos With Developmental Effects

Because there is a substantial body of literature/studies that exists pertaining to investigative research on chlorpyrifos and various endpoints related to neurodevelopmental and neurobehavioral outcomes, it is relevant to review this science in light of both the DART Criteria and a 2012 US EPA Scientific Advisory Panel meeting in which much of this literature was reviewed and evaluated.

i. 2012 United States Environmental Protection Agency Scientific Advisory Panel

The following are relevant perspectives from the US EPA on studies that have associated chlorpyrifos with neurodevelopmental and neurobehavioral outcomes. These perspectives are

taken from the **EPA Meeting of the FIFRA Scientific Advisory Panel, Draft Issue Paper: Scientific Issues Concerning Health Effects of Chlorpyrifos (US EPA, 2012a)**

- **Summary**

“[T]here are several lines of evidence for actions of chlorpyrifos distinct from the classical mode of action of cholinesterase inhibition . . . however, most of these studies have not been designed with the specific goal of construction or testing an adverse outcome pathway. Thus, there are not sufficient data available to test rigorously the causal relationship between effects of chlorpyrifos at the different levels of biological organization in the nervous system.”

EPA Issues Paper at 35.

- **Developmental Impacts on Neurological Domains**

“Because many of these papers report a number of positive as well as negative findings, *the Agency had previously taken the approach of comparing responses that were observed following various exposures to a common dose, 1 mg/kg/d* (FIFRA Scientific Advisory Panel (SAP), 2008a; U.S. Environmental Protection Agency, 2011). A more robust approach is taken here, to include important factors such as dose-response and differences in exposure scenarios. . . . *unfortunately, many of the chlorpyrifos studies have evaluated only one dose.*”

Id. at 39 (emphasis added).

- **Conclusions**

“All testing reported herein was conducted after weaning, and there is a presumption that the effects are permanent; however, no study has directly addressed this issue, and there is a range in test ages. *Dose-response is not always evident, since many studies only use one dose*, and of those using two or more doses, there is not always a monotonic response. Furthermore, the summary presented herein combines studies of different dosing regimens. . . .”

Id. at 52 (emphasis added).

“Overall, these data do not clearly show specific critical periods of exposure, or definitive sensitive behavioral outcomes. *Unfortunately, no laboratory has provided systematic comparisons across exposure period, dosing regimen, and age of testing*; such studies would improve understanding of the impact of these critical factors.”

Id. (emphasis added).

“These studies have almost exclusively focused on doses that could produce some degree, however minimal, of AChE inhibition. Thus *it is not possible to know whether effects would be present at lower doses, since they have not been adequately studied; thus far, only one study (Braquenier, et al., 2010) has tested a dose lower than the point of departure.* The broad profile of neurological effects that have been reported do not aid in the development of a specific AOP, and as described in section 3.2.1., existing experimental studies have not been designed to examine and track possible mechanisms from early initiating events to the final neurological outcome. Such studies represent longer term research efforts by the different laboratories.”

Id. at 52-53 (emphasis added).

The following represent statements and conclusions from the subsequent SAP meeting (US EPA, 2012b) in response to EPA charge questions:

[From EPA to SAP] Question 2.1

“As discussed in Section 3.2.1, although there are numerous mechanistic studies in the scientific literature, the research on different hypotheses does not provide sufficient data to establish causal linkages among different levels of biological organization to show how effects lead to adversity. As such, a mode of action or adverse outcome pathway leading to effects on the developing brain cannot be established at this time. Moreover, although multiple biologically plausible hypotheses are being pursued by researchers, based on the current state of the science, no one pathway has sufficient data to be considered more credible than the others. Please comment on the Agency’s preliminary conclusion that although there are multiple biologically plausible hypotheses being evaluated by research scientists, the mechanistic experimental toxicology data do not yet support a coherent set of key events in a mode of action/adverse outcome pathway.”

EPA, Transmittal of Meeting Minutes of the FIFRA Scientific Advisory Panel held April 10-12, 2012 on “Chlorpyrifos Health Effects” (US EPA, 2012b) at 13 (July 11, 2012).

“The Panel agrees with the Agency’s conclusion that based on the current state of the science, no one pathway has sufficient data to be considered more credible than the others with respect to a causal link between chlorpyrifos exposure and neurodevelopmental outcome.”

2012 SAP Minutes at 13.

“The Panel additionally notes that studies evaluating neurodevelopmental effects entailed experimental designs that do not permit an efficient means of determining a point of departure for chlorpyrifos. Thus, just as . . . in the 2008 SAP, this Panel advises that the Agency continue to use AChE data at the most sensitive lifestages for dose-response analysis and deriving points of departure. *Also in keeping with the 2008 SAP, this Panel expresses concern about the use of Dimethyl Sulfoxide (DMSO) as a vehicle because of its intrinsic toxicity, its potential influence on absorption and interaction with chlorpyrifos, and the impact of this interaction on the developing organism.*”

Id. at 12 (emphasis added).

“Of the neurobehavioral effects reported in the reviewed experiments that assessed AChE inhibition, no studies were identified that showed effects on behavior at low levels of AChE inhibition, including at 1.0 mg/kg of chlorpyrifos. *Doses below 1.0 mg/kg/day chlorpyrifos did not show convincing evidence of neurobehavioral effect; hence, no extrapolation to lower doses in terms of AChE inhibition is possible* from the data reviewed herein.”

Id. at 39 (emphasis added).

ii. US EPA 2014 Revised Human Health Risk Assessment

The following provides some Agency perspectives on the body of literature evaluating possible neurodevelopmental and/or neurobehavioral effects in laboratory animals and is taken from the 2014 EPA RHHRA. (USEPA, 2014).

• Neurodevelopmental Outcomes in Laboratory Animals

“In the 2008 and 2012 SAP reviews, the Agency evaluated the neurobehavioral studies available at that time; the literature review has been updated for the revised risk assessment (Appendix 1). Papers considered by EPA as addressing long-term outcomes from developmental exposure include only those where chlorpyrifos is administered during the pre-weaning period (gestational and/or postnatal) and the offspring are examined at some time after weaning. That is, papers reporting evaluations shortly after birth or during the pre-weaning period do not reflect long-term consequences and may also be confounded by AChE/ChE inhibition during concurrent or recent exposure. In addition, the Agency focused its efforts on studies using relatively low doses (*e.g.*, 1 mg/kg/day), that is, doses that would not be expected to produce a considerable degree of brain AChE inhibition and resultant cholinergic toxicity. These constraints aid in the unencumbered evaluation of longer-term effects compared to acute impacts of AChE inhibition. In total, *the Agency has reviewed 31 papers generated from 14 different laboratories on areas such as cognitive function,*

anxiety/emotion, social behaviors/interaction and motor activity. Twenty five papers were reviewed for the 2012 SAP, and another six have been published and reviewed by ORD since then.”

EPA, Chlorpyrifos: Revised Human Health Risk Assessment for Registration Review (Dec. 29, 2014) at 25 (emphasis added).

“Overall, these data do not clearly show specific critical periods of exposure, or definitive sensitive behavioral outcomes. Unfortunately, no laboratory has provided systematic comparisons across exposure period, dosing regimen, and age of testing; such studies would improve understanding of the impact of these critical factors.”

Id. at 27 (emphasis added).

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

Id. (emphasis added).

The following statements are taken from **Appendix 1** from the 2014 EPA RHHRA.

- **Adverse Outcome Pathway: Neurodevelopmental Outcomes**

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

Id. App. 1, p. 144 (emphasis added).

“In summary, in the late 2000s, a number of papers were published on the *in vitro* modification of various proteins by chlorpyrifos or chlorpyrifos oxon (Grigoryan, Li, *et al.*, 2009; Li *et al.*, 2009), including tubulin (Grigoryan, Li, *et al.*, 2009; Grigoryan & Lockridge, 2009; Grigoryan, Schopfer, *et al.*, 2009; Grigoryan, *et al.*, 2008). ***Although interesting and provocative, these studies were usually conducted with exceedingly high concentrations (high micromolar to millimolar) of the OP compound, making the connection to a “real world” human exposure tenuous.***”

Id. at 158 (emphasis added).

iii. US EPA 2016 Revised Human Health Risk Assessment

US EPA updated its 2014 RHHRA and provided the following perspective on the body of scientific literature pertaining to chlorpyrifos and possible neurodevelopmental and/or neurobehavioral effects. (US EPA, 2016).

“A review of the scientific literature on potential MOAs/AOPs⁵ leading to effects on the developing brain was conducted for the 2012 FIFRA SAP meeting (US EPA, 2012) and updated for the December 2014 chlorpyrifos HHRA (D. Drew *et al.*, D424485, 12/29/2014). ***In short, multiple biologically plausible hypotheses and pathways are being pursued*** by researchers that include targets other than AChE inhibition, including cholinergic and non-cholinergic systems, signaling pathways, proteins, and others. ***However, no one pathway has sufficient data to be considered more credible than the others.*** Published and submitted guideline developmental neurotoxicity (DNT) laboratory animal studies have been reviewed for OPs (D. Drew *et al.*, D424485, 12/29/2014 and US EPA, D331251, 09/15/2015). Neurobehavioral alterations in laboratory animals were often reported; however, at AChE inhibiting doses. ***Moreover, there was generally a lack of consistency in pattern, timing, and dose-response for these effects; and a number of studies were of low quality.***” (EPA, Chlorpyrifos Revised Human Health Risk Assessment for Registration Review (“2016 EPA RHHRA”) (Nov. 3, 2016) at 11 (emphasis added).)

C. “Additional” Animal Studies Among the Hazard Identification Materials Do Not “Clearly Show” That Chlorpyrifos Is a Developmental Toxicant

As noted above, OEHA included among the Hazard Identification Materials a 2016 report from the US EPA, and two lists of studies, one on September 1, 2017, and another on September 8, 2017. Dow AgroSciences has reviewed these studies, reports and data, and presents them in tabular form below. Excluded from this review are *in vitro* studies, studies in zebrafish, studies

involving postnatal exposure and studies involving test systems/designs that do not conform with DART Criteria.

Additional DART Criteria for consideration of experimental studies include the following:

- The experimental design, including overall protocol and numbers of animals, and presence of appropriate controls.
- The exposure, in terms of route of administration, is relevant to expected human exposures, and in terms of timing, with regard to critical periods of development for developmental toxicity.
- Number of dose levels, so that the presence of a dose-response relationship can be evaluated. It is desirable that the high dose level should elicit maternal toxicity in developmental studies . . . and that the low dose should elicit no observable adverse effects for adults and offspring.
- Consideration of maternal and systemic toxicity.
- Number of tests or experimental animal species.²⁷

For each set of studies/citations (*i.e.*, six new EPA studies, OEHHA Sept. 1 studies, OEHHA Sept. 8 additional studies) Dow AgroSciences lists below the only studies that involve gestational exposure and which could be evaluated for developmental toxicity.

Table 10: New Studies Cited by US EPA Since 2012 SAP and Included in 2014 RHHRA

1 st Author	Test system	Route of admin	Vehicle	Dose	Exposure Period	Eval. period	D-R	ChE Activity	Comments
Mullen, 2013	Mouse	Implanted osmotic pump	DMSO/PBS	6 mg/mL CPO oxon	GD13.5-20	PND 7, 14, 30	No	Yes	Decreased reelin expression following oxon treatment
Chen, 2012	Mouse	SC	DMSO	1 or 5 mkd	GD 13-17	PND 45-60	No	No	Cognitive impairment in hippocampus and prefrontal cortex

Assessment: Only two studies involve gestational exposure, but these would be excluded based on DART Criteria (*i.e.*, use of an inappropriate route of exposure (subcutaneous, SC) and the confounding use of DMSO as vehicle). Moreover, Mullen *et al.* (2013) used only one dose and used CPF-oxon (not even chlorpyrifos) while Chen *et al.* (2012) only assessed two doses which

²⁷ DART Criteria at 3.C.(1)-(5).

do not permit evaluation of dose-response. In summary, these studies do not offer new additional insight or a defensible basis for associating chlorpyrifos with developmental toxicity based on DART Criteria specifying “scientifically valid testing according to generally accepted principles.”

(1) The References Provided on September 1, 2017 Do Not Clearly Show Chlorpyrifos To Be a Developmental Toxicant

Of the 308 cited references included in the August 2017 (OEHHA September 1 Rev.), 26 were deemed suitable for evaluation of potential developmental toxicity and are included in the Table below. One hundred ten studies were studies not involving chlorpyrifos, 50 were human or human-related studies or topics (covered within the epidemiology section), 60 involved or included postnatal exposure/treatment, 32 were not related to developmental toxicity as an outcome/endpoint, 28 involved cellular or *in vitro* (non-mammal) systems and two used zebrafish as the test system. Zebrafish have been used for screening purposes for developmental toxicity, but are not surrogates for standard developmental toxicity investigations in rats or rabbits. *In vitro* studies were not assessed because OEHHA criteria specify studies for consideration for listing under Proposition 65 be conducted in mammals and more fundamentally, it is not possible for a cellular or *in vitro* system to represent a gestational exposure as is the case for *in vivo* test systems. Furthermore, *in vitro* experimental conditions are often far removed from relevant human exposure scenarios, do not report findings/outcomes that have been demonstrated *in vivo*, and do not supersede guideline compliant developmental toxicity studies that do exist for chlorpyrifos.

Table 11: Selected Cited References on the Developmental Toxicity of Chlorpyrifos Provided to the DART IC Rev. September 1, 2017

1 st Author	Test System	Route of Admin	Vehicle	Dose	Exposure Period	Evaluation Period	D-R	ChE Activity	Focus of Study
Akhtar 2006	Rat	Gavage	Corn oil	9.6, 12, 15 mkd	GD 0-20	Pups at Day 21 of gestation following sacrifice	Yes	No	Evaluation of teratogenicity
Tian 2005	Mouse	IP	Olive oil	40 or 80 mkd	GD10	Pups evaluated following sac on GD17	No	No	Teratogenicity and developmental toxicity
Farag, 2003	Rat	Gavage	Corn oil	5, 15, or 25 mkd	GD 6-15	Examinations following sac on GD 21	Yes	Yes	Developmental toxicity evaluation
Pope, 1991	Rat	SC	Peanut oil	MTD of 45 mkd	Neonates	1-8 days posttreatment	No	Yes	Comparison of <i>in vivo</i> cholinesterase inhibition following treatment with MTD doses
				MTD of 279 mkd	Adults				

**Table 11: Selected Cited References on the Developmental Toxicity of Chlorpyrifos
Provided to the DART IC Rev. September 1, 2017**

1 st Author	Test System	Route of Admin	Vehicle	Dose	Exposure Period	Evaluation Period	D-R	ChE Activity	Focus of Study
Slotkin, 2007	Rat	SC	DMSO	1 or 5 mkd	GD 9-12 or GD 17-20	PND 30	No	No	Evaluation of serotonergic and dopaminergic hyperactivity at adolescence
Chanda, 1995	Rat	SC	Peanut oil	200 mkd	GD12	GD 16, GD 20, or PND 3	No	Yes	Comparative maternal/developmental neurotoxicity
Turgeman, 2011	Mouse	SC	DMSO	3 mkd	GD 9-18	Transplanted cells to animals on PND 35; Behavioral exam at PND80	No	No	Reversal of CPF neurobehavioral teratogenicity by transplantation of adult neural stem cells
Venerosi, 2009	Mouse	Gavage	Peanut oil	6 mkd	GD 15-18	Various evaluations from PND 3-15	No	No	Motor behavior and ultrasonic vocalization
Chen, 2010	Rat	SC	DMSO	5 mkd	GD 7.5 to 11.5 or GD 13-17	GD 17, PND, 14 and PND 60	No	No	Assessment of dopamine content
Qiao, 2002	Rat	SC	DMSO	1, 2, 5 mkd 1, 2, 5, 10, 20 or 40 mkd	GD 9-12 GD 17-20	GD 17 and GD 21 24 hrs after last dose on GD 21	Yes	No	Developmental neurotoxicity evaluations
Chanda, 1996	Rat	SC	Peanut oil	25 mkd 6.25 or 12.5 mkd	GD12-19 GD12-19	GD20 or PND3 GD20	No	Yes	Neurochemical and neurobehavioral Effects
Hunter, 1999	Rat	Gavage	Corn oil	7 mkd 3 or 7 mkd	GD14-18 GD 14-18	GD 18-20 5 hrs after last dose	No	Yes	Comparative distribution of TCPy
Tian, 2003	Mouse	IP	Olive oil	40 or 80 mkd	GD0	Day 3 blastocysts collected	No	No	Micronucleus formation in 3-day mouse embryos
Lassiter, 1998	Rat	Gavage	Corn oil	7 mkd	GD14-18	GD18-20	No	Yes	Gestational exposure may be protective to Fetus
Venerosi, 2010	Mouse	Gavage	Peanut oil	6 mkd	GD15-18	PND90	No	No	Social-emotional behavior

Table 11: Selected Cited References on the Developmental Toxicity of Chlorpyrifos Provided to the DART IC Rev. September 1, 2017

1 st Author	Test System	Route of Admin	Vehicle	Dose	Exposure Period	Evaluation Period	D-R	ChE Activity	Focus of Study
Deacon, 1980	Mouse	Gavage	Cotton-seed oil	1, 10, 25 mkd; also 0.1, 1, 10 mkd	GD6-15	GD 16	Yes	Yes	Guideline developmental toxicity study
Ouellette, 1983	Rat	Gavage	Corn oil	0.1, 3.0, or 15 mkd	GD6-15	GD 21	Yes	Yes	Guideline developmental toxicity study
Thompson, 1971	Rat	Gavage	Corn oil	0.1, 0.3, 1.0 mkd	GD6-15	GD21	Yes	Yes	Guideline developmental toxicity study
Breslin, 1996	Rat	Gavage	Corn oil	0.1, 3, or 15 mkd	GD6-15	GD21	Yes	Yes	Guideline developmental toxicity study
Icenogle 2004	Rat	SC	DMSO	1 or 5 mkd	GD 9-12	Weeks 4-8; Weeks 8-13	No	No	Behavioral alterations
Qiao 2004	Rat	SC	DMSO	1 or 5 mkd	GD 9-12	PND30 and PND 60	No	Yes	Cholinergic synaptic dysfunction and cellular alterations
Haviland, 2009	Mouse	SC	DMSO	1 or 5 mkd	GD 17-20	60 days of age	Yes	No	Behavioral/hormonal alterations (thyroid)
Qiao, 2003	Rat	SC	DMSO	1 or 5 mkd	GD17-20	PND30 and PND 60	No	No	Evaluation of brain development and cholinergic biomarkers
Billauer-Haimovitch	Mouse	SC	DMSO	1, 3, 5, 10, 20 mkd	GD 9-18	PND 3, 7, and 75	Yes	No	Reversal of chlorpyrifos neurobehavioral effects by nicotine administration and neural stem cell transplantation
Levin, 2002	Rat	SC	DMSO	1 or 5 mkd	GD 17-20	Weeks 4-6, weeks 8-13 and weeks 14-17	No	No	Behavioral alterations
Abou-Donia, 2006	Rat	Dermal	70% ethanol	1 mkd	GD 4-20	PND 90	No	Yes	Sensorimotor deficits and neuron loss in cerebellum of offspring rats

Assessment: From this listing of “new” studies, virtually none would meet the DART Criteria of “scientifically valid testing according to generally accepted principles.” All studies have an experimental design deficiency such as inappropriate route of exposure, single dose level, high

dose exposures or use of DMSO as a vehicle. In fact, the few studies on this list that would meet the DART Criteria for “scientifically valid testing according to generally accepted principles” are guideline developmental toxicity studies employing gestational exposure, multiple dose levels for dose-response and appropriate/relevant route of exposure. In summary, the vast majority of the studies from the September 1 list do not offer new additional insight or a defensible basis for associating chlorpyrifos with developmental toxicity based on DART Criteria specifying “scientifically valid testing according to generally accepted principles.”

(2) Additional References Not Cited in OEHHA (2008), US EPA (2014) or US EPA (2016) Do Not “Clearly Show” Chlorpyrifos To Be a Developmental Toxicant

Of the 72 citations included in the OEHHA August 2017 (Rev. September 8) communication, 14 were deemed suitable for evaluation of potential developmental toxicity and are included in the Table below. Twelve publications involved experimentation with zebrafish (see comment above), 4 were human studies (considered within the epidemiology section), 16 involved cellular or non-mammal test systems, 12 involved or included postnatal exposure and 14 were unrelated to developmental toxicity as an outcome/endpoint.

Table 12. Selected Additional References on Developmental Toxicity of Chlorpyrifos Provided to DART IC and Not Cited in OEHHA (2008), US EPA (2014) or US EPA (2016)

1 st Author	Test System	Route of Admin	Vehicle	Exposure/Dose	Exposure Window	Evaluation period	Dose-Response	ChEI Measured	Focus of Study
DeFelice 2015	Mouse	Gavage	Peanut oil	6 mkd	GD14-17	PND 4, 6, 8, 12	No	No	Delayed motor development
DeFelice 2016	Mouse	Gavage	Peanut oil	6 mkd	GD14-17	PND 1, 21, 70	No	No	Effects on brain oxidative stress and prostaglandin synthesis
Chen et al 2014	Mouse	SC	DMSO	2 mkd	E7.5 to E11.5?	Embryo brains isolated on E16	No	No	Cleavage plane orientation alteration in neocortex
Buratti, 2011	Mouse	Gavage	Peanut oil	3 or 6 mkd	GD15 to GD18	PND 0, 9, 15, and 150	No	No	Biochemical and metabolic alterations
De Felice 2014	Mouse	Gavage	Peanut oil	6 mkd	GD 14-17	PND 70	No	No	Sex-dimorphic effects on social investigation
Lan, 2017	Mouse	Gavage	Corn oil	2.5 or 5 mkd	GD 12 to GD 15	PND 6-12 and PND 90	No	No	Autism-like deficits
Rubin, 1987	Rabbit	Gavage	Maize oil	1, 9, 81, and 140 mkd	GD 7 to 19	Dams sacrificed on GD 29	Yes	Yes	Guideline developmental study used for registration
Grabovsk,	Rat	?	Sunflow-	5, 10, 15	Adult rats	Pups	Yes	No	ADHA-like

Table 12. Selected Additional References on Developmental Toxicity of Chlorpyrifos Provided to DART IC and Not Cited in OEHHA (2008), US EPA (2014) or US EPA (2016)

1 st Author	Test System	Route of Admin	Vehicle	Exposure/Dose	Exposure Window	Evaluation period	Dose-Response	ChEI Measured	Focus of Study
2015			er oil	mkd	received exposure for 30 days, then no exposure for 4 months before pregnancy; other group received 30 mkd on GD6	evaluated at 2 months of age			behavior
Mamczarz, 2016	Guinea Pig	SC	Peanut oil	25 mkd	10 days starting around GD 53-55	PND 30	No	Yes	Spatial learning impairment
Mullins, 2015	Guinea pig	SC	Peanut oil	25 mkd	10 days starting around GD50	PND 40-45	No	No	Structural and functional brain disruption
Richardson, 2004	Rat	Via vanilla wafer	NA	3 or 7 mkd	GD 6-20	PND 1, 3, 6, 9, 12, and 30	No	Yes	Neurochemical effects
Chen, 2016	Mouse	SC	DMSO	5 mkd	GD 13-17	PND 60	No	No	Neuron and glial loss
Cole, 2005	Mouse	Dermal	Acetone	Not described	8-12 week old mice	15 min, 1, 3, 4, 6, 12, and 24 h	No	No	Mouse model of human PON1 Q192R polymorphism
Silva 2017	Rat	Gavage	9% saline + Tween 20	0.01, 0.1, 1, 10 mkd	GD14-20	PND 21 and 70	Yes	No	Induction of anxiety-like behavior

Assessment: From this additional listing of “new” studies, virtually none would meet the DART Criteria specifying “scientifically valid testing according to generally accepted principles.” Similar to the September 1 list of studies, the remaining studies included above have experimental design deficiencies including the use of DMSO as a vehicle solvent, use of singular or high dose levels, or inappropriate route of exposure. In summary, these studies do not offer new additional insight or a defensible basis for associating chlorpyrifos with developmental toxicity based on DART Criteria specifying “scientifically valid testing according to generally accepted principles.”

As discussed below, many of the studies that were included in the previous three tables did not measure cholinesterase inhibition, which is an important experimental variable when evaluating this broad literature as it is recognized that protection against cholinesterase inhibition is protective against all other toxicities, including developmental toxicity (US EPA, 2012a, 2012b; Li *et al.*, 2012).

(3) None of the “New Evidence” Clearly Shows Chlorpyrifos To Be a Developmental Toxicant

Despite the number of studies/citations included in the preceding three tables which investigate numerous developmental/behavioral observations/outcomes, collectively there is no new evidence that satisfies the DART Criteria that would clearly show chlorpyrifos to be a developmental toxicant by “scientifically valid testing according to generally accepted principles.” Moreover, the existing guideline-compliant, GLP developmental toxicity studies (four studies in three different species) supporting the registration of chlorpyrifos globally clearly demonstrate that it is not a developmental toxicant, a fact which is supported by regulatory agencies globally.

A critical review of those studies referenced by OEHHA in the two lists (September 1 and September 8) demonstrate that the number of “new” studies available for assessment of developmental toxicity is very few and a review of these few studies reveals that they are limited by other factors including lack of suitable number of doses for dose-response, the inclusion of an inappropriate (non-relevant for humans) route of exposure, use of a neurotoxic vehicle which the 2012 EPA SAP specifically cautioned against when evaluating this same literature for possible developmental/behavioral toxicity, and the failure of many investigators to concurrently measure cholinesterase activity which is a key variable for consideration when reporting on neurodevelopmental effects below a putative threshold for cholinesterase inhibition. Considering these limitations, coupled with the DART IC criteria for “scientifically valid testing” and the endpoints/outcomes that would be considered indicative of developmental toxicity, there is no new evidence presented that would constitute a scientific basis for listing chlorpyrifos as a developmental toxicant under Proposition 65. The following examples showcase how experimental design and variables are important and why DART IC dictates that studies conform to “scientifically valid testing according to generally accepted principles.”

(a) *Inappropriate route of exposure*

Many of the studies cited above utilize subcutaneous administration with a vehicle (DMSO) that is known to exert neurobehavioral effects of its own (Fossum *et al.*, 1985; Cavaletti *et al.*, 2000; Cavas *et al.*, 2005). This type of study design would never be identified as appropriate or relevant when considering human exposures, and yet it has been used consistently by some investigators for many years. It is this experimental study design and reported experimental results from studies on laboratory animals, in large part, that have served as the basis for the allegations of developmental effects associated with chlorpyrifos (*e.g.*, Rauh *et al.*, 2006).

(b) *Delays in Absorption Due to Presence of DMSO*

It is frequently stated in many of the studies cited above in which DMSO is used as a vehicle that injection of DMSO subcutaneously is conducted to “ensure rapid and complete absorption.” Data from five-day old rat pups provide evidence that subcutaneous administration in DMSO actually delayed absorption of most of the chlorpyrifos for at least two hours (Marty *et al.*, 2007). Chlorpyrifos remained near or at the site of injection instead of being absorbed along with the

DMSO. This finding raises questions since it is not possible to characterize chlorpyrifos' behavior or effects in this experimental system in a manner relevant to human exposures or potential health effects.

(c) ***Confounding of CNS responses by cell signaling from local irritation***

The lag in absorption of chlorpyrifos, and the localization of the radiolabel at the site of injection or in the carcass indicate a depot effect. Chlorpyrifos is recognized as a mild dermal irritant by regulatory authorities (WHO 1999). The *in vivo* studies involving subcutaneous administration of chlorpyrifos with DMSO have not evaluated local, systemic, or central nervous system (CNS) consequences of the irritant properties of chlorpyrifos. There is a growing body of literature on the CNS effects of peripheral pain and inflammation (Swarm *et al.*, 2001; Ruda *et al.*, 2000; Chatterjee *et al.*, 2006).

(d) ***CNS Effects from DMSO***

DMSO has been reported to have adverse effects on PNS and CNS function (Fossum *et al.*, 1985; Cavaletti *et al.*, 2000; Cavas *et al.*, 2005; Authier *et al.*, 2002), as noted above, and in effect represents a confounding variable when evaluating neurodevelopmental/behavioral/toxicological effects. Numerous regulatory authorities globally have cautioned against the use of DMSO, including recent US EPA SAPs. To this point, the 2012 EPA SAP that specifically reviewed much of this literature noted the following:

“The Panel concurred with the 2008 Panel in express caution on the use of DMSO as a vehicle because of its intrinsic toxicity and potential influence on absorption. Again, uncertainty was expressed about potential interactions between DMSO and low doses of chlorpyrifos and the effect of this interaction on the developing organism. In addition to the three papers cited by the 2008 SAP (FIFRA Scientific Advisory Panel, 2008b), more recent evidence is available to support the potential toxicity of DMSO. Hanslick *et al.* (2009) reported that following acute intraperitoneal injection of DMSO into 7 day-old mice, there was a significant increase in the number of apoptotic neurons at dosages as low as 0.3 ml/kg. ***An increased number of apoptotic neurons was also observed at 1 ml/kg which is the most frequent volume of DMSO administered in the cited studies using DMSO as a vehicle.*** Recent reports from the zebrafish literature suggest that DMSO has the capacity to directly induce neurobehavioral effects. Exposure to 0.05% DMSO induces anxiolytic behavior in adult zebrafish (Sackerman *et al.*, 2010) and exposure to 0.01% DMSO alters locomotor activity in larval zebrafish exposed embryonically (Chen *et al.*, 2011). Also, based on earlier studies observing that DMSO induces a stress protein response in zebrafish embryos (Hallare *et al.*, 2004; 2006), Turner *et al.* (2012) reported that levels of DMSO as low as 25 µl/L (0.0025%) were sufficient to induce gene expression changes in embryonic zebrafish. While altered gene expression does not indicate a toxic response, it suggests disruption of homeostasis by low levels of this

solvent. *While the experimental studies reviewed in the White Paper all had controls with DMSO only, there is no way to rule out the potential for an interaction between DMSO and the OP. . . .* It should be noted that the concentration of 100% DMSO is approximately 14 M. Because of the potential biological/ cellular changes noted above, the lack of evaluation of potential interactions between DMSO and chlorpyrifos, and the well-known effects of DMSO on membrane permeability (Gurtovenko and Anwar, 2007), caution should be exercised in the use of data for quantitative risk assessment from *in vivo* (or *in vitro*) studies using DMSO as a solvent.”

2012 SAP Minutes at 28-29 (emphasis added).

Beyond these important experimental design deficiencies which preclude many of the studies from consideration according to DART IC criteria, the vast majority of the studies do not use dose levels below 1 mg/kg/day and are further hampered by not incorporating sufficient dose levels (*i.e.*, 3) for use in assessing dose-response. Of growing and critical relevance is the additional central need to measure cholinesterase activity concurrently or concomitantly in any and all experimental investigations assessing possible toxicities related to chlorpyrifos exposure as this remains the conservative and sensitive point of departure used globally for risk assessment.

As stated by the US EPA in the 2012 SAP:

“It is well established that AChE inhibition is the primary mode of action/ adverse outcome pathway for OPs, like chlorpyrifos. Because AChE inhibition is the initiating event for this mode of action/adverse outcome pathway, using AChE inhibition as a regulatory endpoint is protective of downstream cholinergic effects. *Moreover, historically, given the sensitivity of AChE inhibition data for OPs, these data have been considered to be protective of other potential toxicities and/or modes of action for OPs.* In 2008, the Agency performed a comprehensive review of the available AChE data from multiple lifestages. This review has been supplemented with the newest studies. Consistent with the recommendations from the 2008 SAP, the Agency believes that AChE data remain the most robust dose-response data for deriving points of departure in *in vivo* experimental toxicology studies with laboratory animals. Please comment on the Agency’s preliminary conclusion that AChE data remain the most robust source of data for deriving points of departure for chlorpyrifos.

Id. at 12 (emphasis added).

The SAP Panel responded as follows:

“The Panel concurs with the Agency’s position that AChE data continue to be the strongest resource of data for deriving points of departure for chlorpyrifos. *The*

Panel's conclusion is based on the premise that all studies reporting neurobehavioral changes following in vivo prenatal or postnatal exposures to chlorpyrifos have been accompanied by AChE inhibition when measured at an appropriate time following administration of chlorpyrifos."

Id. (emphasis added).

It is significant that US EPA took careful note when reviewing much of this literature whether cholinesterase activity was measured and in many instances, it was not. Li *et al.* (2012) and others have conducted extensive reviews and reported that "...the most sensitive endpoint for CPF is RBC AChE inhibition. Taking into consideration consistency of outcomes across studies, and strength of experimental design and methods for risk assessment purposes, the NOAEL for behavioral effects is 1 mg/kg-d. There is strong evidence from the animal literature that AChE inhibition (RBC or brain from adult or offspring) is a sensitive endpoint that is protective of neurobehavioral, neuropharmacologic, and morphologic alterations that were measured following gestational, lactational and/or early postnatal exposures to 1 to 6 mg/kg-d."

Finally, it should be noted that in the guideline developmental studies reported on earlier in this assessment, 3 of the 4 studies used dose levels below 1 mg/kg/day, notably 0.1, 0.5, and 0.1 mg/kg/day, respectively in the two rat and one mouse developmental toxicity studies (Ouelette *et al.*, 1983; Rubin *et al.*, 1987a; Deacon *et al.*, 1979). This is specifically illustrated as the NOAELs for all developmental endpoints/outcomes were well above these low dose levels and cholinesterase activity was measured in all studies. Very few of the current studies reported in the tables above have assessed neurodevelopmental/behavioral outcomes below 1 mg/kg/day and moreover have often not included cholinesterase activity measurements in the experimental design.

(4) Lack of Biological Plausibility and Validated Mode of Action

In addition to the numerous challenges relative to study design, which would preclude the vast majority of these studies for consideration based on DART IC criteria, there is little consistency in the postnatal parameters reported as evidenced by myriad and wide-ranging reported outcomes across the studies (as noted in the final right column in each table above). This observation has been noted by several US EPA SAP panels and demonstrates the overall lack of biological plausibility and absence of a defined and validated mode of action for reported non-cholinergic neurodevelopmental outcomes. For example, the 2016 SAP said that

“[W]ithout any evidence in the animal literature or elsewhere of a mechanism of action that could explain how pg/g levels in blood could impair IQ and/or working memory, there does not appear to be biological plausibility.”

2016 SAP Minutes at 40-41.

In summary, despite the reporting of numerous animal studies on developmental toxicity following chlorpyrifos exposure, in fact, there is no new evidence that would qualify as “clearly shown through scientifically valid testing according to generally accepted principles to cause . . . developmental toxicity.” Furthermore, there is no biological plausibility or defined and validated mode of action to explain the outcomes in these studies despite the vast and myriad number of endpoints reported. This collective literature fails to provide a defensible, biologically plausible or supportive weight of evidence that would associate chlorpyrifos with developmental toxicity. The animal studies do not constitute “sufficient evidence in experimental animals (mammals)” (DART Criteria 3.C.) and are not “sufficient experimental animal (mammalian) data” to support “limited evidence or suggestive evidence in humans” (DART Criteria 3.B.). There is simply no evidence based on qualified studies that have been conducted “through scientifically valid testing according to generally accepted principles” to list chlorpyrifos under Proposition 65 as a developmental toxicant.”

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VI. CONCLUSION

After due consideration of epidemiology and animal toxicology studies, the DART IC in 2008 determined that chlorpyrifos should not be listed as a reproductive or developmental toxicant under Proposition 65. The DART IC now is to consider chlorpyrifos for listing as a developmental toxicant again on the basis of the studies presented to it in 2008 as well as additional “new” studies. However, based on a weight of evidence review encompassing *all* of the relevant human epidemiology and experimental animal studies, chlorpyrifos has not been “clearly shown . . . to cause” developmental toxicity. The epidemiological evidence presented to the DART IC related to chlorpyrifos is weak, inconsistent, and fails to meet the criteria required to establish a causal relationship between exposure and effect. The experimental animal studies that have been conducted using “scientifically valid testing according to generally accepted principles” consistently and across species demonstrate no evidence of developmental toxicity in the absence of maternal toxicity.

For these reasons, chlorpyrifos does not meet the listing criteria of Proposition 65. Specifically, the epidemiology studies do not provide “sufficient evidence in humans” (DART Criteria at 3.A.) that chlorpyrifos causes developmental toxicity; the animal studies do not provide “sufficient evidence in experimental animals (mammals)” (DART Criteria at 3.C.) that chlorpyrifos causes developmental toxicity; and neither the epidemiology studies nor the animal studies provide “limited evidence or suggestive evidence in humans” (DART Criteria at 3.B.) that chlorpyrifos causes developmental toxicity.

Appendices for Epidemiology Data

Appendix 1: Epidemiology Studies Not Relevant Because in Children or Adults

Appendix 1 lists the epidemiology studies and analyses of exposure in children or adults, which are not relevant for consideration under Proposition 65 because they studied the effects of post-natal exposures.

Author, Year of Publication	Study Description/Reason Not Relevant
Bouchard <i>et al.</i> (2010)	NHANES, Age 8 – 15 years
Bouchard <i>et al.</i> (2011)	CHAMACOS, Age 7 years
Cartier <i>et al.</i> (2016)	PELAGIE, Age 6 years
Crane <i>et al.</i> (2013)	Egypt, Applicators Ages 12 -21 years
Ellison, Crane, <i>et al.</i> (2012b)	Egypt applicators Adults
Eskenazi <i>et al.</i> (2007)	
Marks <i>et al.</i> , (2010)	CHAMACOS, Age 6, 12, 24 months
Fenske <i>et al.</i> (2012)	Egypt, Applicators, Adults
Fiedler <i>et al.</i> (2015)	Thailand, Age 6 - 8 years
González-Alzaga (2015)	Spain, Age 6 – 11 years
Guodong <i>et al.</i> (2012)	Shanghai, China, Age 23-25 months
Harari <i>et al.</i> (2010)	Ecuador, Age 6- 8 years
Hoppin <i>et al.</i> (2012)	AHS, Applicators Adults
Karunanayake <i>et al.</i> (2012)	Adults, Hodgkin lymphoma
Khan <i>et al.</i> (2014)	Egypt, Applicators, Adolescent
Lein <i>et al.</i> (2012)	Egypt, Adults
Lerro <i>et al.</i> (2015)	AHS, Adults
Lizardi <i>et al.</i> (2008)	Arizona, Age 7 years
Lu <i>et al.</i> (2009)	Costa Rica, Age 4 – 10 years
Lurati (2013)	Adults, Case report (n = 1)
Niu <i>et al.</i> (2014)	China, Adult workers
Oulhote and Bouchard (2013)	Canadian National Population Age 6 – 11 y
Phung <i>et al.</i> (2013)	Vietnam, Farmers
Raanan <i>et al.</i> (2015)	CHAMACOS, Age 5 – 7 years
Rohlman <i>et al.</i> (2005)	Oregon and NC, Adults
Zhang <i>et al.</i> (2014)	Chinese factory workers, Adults

Appendix 2: Epidemiological Reports and Reviews Not Relevant Under DART Criteria

Appendix 2 lists epidemiology studies that are not relevant because these case reports and reviews do not evaluate effects from exposure to chlorpyrifos, or are not health studies.

Author, Year of Publication	Comments / Reason Not Relevant
Acosta-Maldonado <i>et al.</i> (2009)	No information on any class or pesticide
Cavari <i>et al.</i> (2013)	Not specific to chlorpyrifos. Anticholinesterase intoxication in children
Cecchi <i>et al.</i> (2012)	Not specific to chlorpyrifos Evaluated hormones and liver enzymes
Costa <i>et al.</i> (2013)	Review
Dabrowski <i>et al.</i> (2003)	Organophosphates
Dalvie <i>et al.</i> (2014)	Environmental exposure in air, dust, grass
De Cock <i>et al.</i> (2012)	Review
Ellison, Abou El-Ella, <i>et al.</i> (2012a)	Frequencies of CYP2B6 and CYP2C19 polymorphisms
Fenske <i>et al.</i> (2013)	Exposure only
Freire and Koifman (2012)	Review of Parkinson's
Freire and Koifman (2013)	Review of depression and suicide
Goodman <i>et al.</i> (2013)	Review
Grandjean <i>et al.</i> (2006)	Analyses limited to "exposed" to pesticides
Hengel and Lee (2014)	Method paper for air monitoring
Huen <i>et al.</i> (2012)	Exposure only
Kim <i>et al.</i> (2013)	Exposure study only
Kofman <i>et al.</i> (2006)	Organophosphates
Larsen <i>et al.</i> , (2017)	No information on any class or pesticide
Lee <i>et al.</i> (2013)	Incident cases of idiopathic PD and controls
Llop <i>et al.</i> (2013)	No information about chlorpyrifos, Spray likely to be pyrethroid
Moreno-Banda <i>et al.</i> (2009)	No data on chlorpyrifos
Ostrea <i>et al.</i> (2012)	Did not detect or report chlorpyrifos
Peter <i>et al.</i> (2013)	Clinical scoring systems in acute organophosphate poisoning.
Quiros-Alcala <i>et al.</i> (2012)	Exposure study only
Samarawickrema <i>et al.</i> (2008)	Cholinesterase
Savitz <i>et al.</i> (1997)	Organophosphates
Thivakaran <i>et al.</i> (2012)	A case report
Wang, Yi <i>et al.</i> (2012)	Food residue study
Whyatt <i>et al.</i> (2002)	Exposure data only
Wu, Hao, and Yu (2012)	Report of four cases. No exposure to chlorpyrifos