

**Dow AgroSciences Comments on OEHHA Proposed Amendments to Section 25805(b), Specific Regulatory Levels: Chemicals Causing Reproductive Toxicity. Maximum Allowable Dose Levels for Chlorpyrifos (Oral, Inhalation, and Dermal Exposures)**

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## I. Executive Summary

Dow AgroSciences (DAS) submits the following comments on the OEHHA proposal *Proposed Amendments to Section 25805(b), Specific Regulatory Levels: Chemicals Causing Reproductive Toxicity. Maximum Allow Dose Levels for Chlorpyrifos (Oral, Inhalation and Dermal Exposure)* released May 24, 2019. OEHHA proposes establishing MADLs (Maximum Allowable Dose Levels) of 0.58 ug/day for both oral and inhalation routes of exposure and 7.2 ug/day for dermal exposure. DAS demonstrates that a comprehensive and robust examination of the available science does not support the validity of the proposed MADLs.

On December 15, 2017 chlorpyrifos was added to the Proposition 65 list as a reproductive toxicant (for an unidentified development endpoint) based on findings of the Developmental and Reproductive Toxicant Identification Committee (DARTIC). As presented in comments submitted to the DARTIC (Dow AgroSciences, 2017), DAS respectfully disagrees with the Committee's recommendation to list chlorpyrifos under Prop 65. DAS continues to maintain that a full evaluation of the relevant science did not support the listing.

Proposition 65 Regulations identify specific criteria for the types and quality of studies to be considered.

Critical in the consideration of OEHHA's proposed MADLs is that, within the Proposition 65 Regulations under Section 25803 (Assessment), it is stated that "[o]nly studies producing the reproductive effect which provides the basis for the determination that a chemical is known to the state to cause reproductive toxicity shall be utilized for the determination of the NOEL." Since chlorpyrifos was listed as a developmental toxicant, this means that the MADL must be based on an endpoint of developmental toxicity. Any adverse developmental effect does not come within the purview of Proposition 65 for a developmental toxicant unless it is attributable to pre-natal exposure. Thus, studies utilized must be able to show that the developmental effects were the result of pre-natal and not post-natal exposures.

Consideration of study quality is also specifically spelled out in the Regulation “The results obtained for the most sensitive study deemed to be of sufficient quality shall be applicable to all routes of exposure for which the results are relevant”.

Of the four studies identified by OEHHA as the purported bases for its MADL proposal, three do not meet the required standard of sufficient quality and, for the fourth, OEHHA failed to consider the full data package.

Overall OEHHA’s selection of one study and inclusion of three supportive studies is not reflective of the full toxicological database for chlorpyrifos and potential neurodevelopmental toxicity. A more complete evaluation of all relevant studies which meet the standards of the Regulation shows that chlorpyrifos does not cause neurodevelopmental effects at exposure levels below the current regulatory endpoint of Red Blood Cell Cholinesterase Inhibition (RBC ChEI).

As discussed in more detail in these comments, three of the four studies, including the primary study cited by OEHHA, do not meet the regulatory definition “of sufficient quality” as required under the Statute (Cal. Health & Safety Code 25249.5 et seq.) and therefore should not serve as the bases for derivation of a MADL, while the fourth study produced adverse effects in offspring only at maternally toxic doses.

OEHHA relies on the study by Silva et al. (2017) as the primary foundation for development of the proposed MADL. However, as discussed in these comments, this study does not meet the standard of “sufficient quality” and therefore cannot be used as the basis for a MADL for chlorpyrifos. The fundamental scientific limitations of this study include questions concerning the biological significance of the effect cited, the failure to measure red blood cell cholinesterase inhibition (which is the regulatory endpoint), and the absence of a well-defined dose response, which is an accepted scientific principle for confirming a cause-and-effect relationship.

Further, the significance OEHHA has placed on this study as the principal basis for the proposed MADL, is unclear. Based on a review of the transcript of the DARTIC hearing, none of the

Committee members identified or cited this study as a basis for listing even though it was available in the public, published literature well before the hearing.

OEHHA also lists two studies by Gomez-Gimenez (2017a, 2017b) as supportive of the proposed MADL, but these studies involved both pre-and post-natal exposure. Due to the investigators' inability to confirm that the reported effects resulted from only pre-natal exposure, along with other limitations of these studies discussed below, these studies do not meet the standard of "sufficient quality" required in the Regulation and therefore are unsuitable for determining a MADL for chlorpyrifos.

OEHHA cites the Developmental Neurotoxicity (DNT) study by Hoberman (1998) as the third study supportive of Silva et al. (2017). While this study does meet the standard of "sufficient quality," DAS disagrees with OEHHA's contention that the LOEL is 1 mg/kg/day based on parietal cortex morphometry in PND 66 rats. The study authors concluded the developmental NOEL was 1 mg/kg/day. OEHHA failed to consider supplemental information and the full data record for this study. No historical DNT morphometric control data were available at the time this chlorpyrifos DNT study was conducted, but the lead researchers for the study conducted five DNT studies soon after the chlorpyrifos study, at the same laboratory and using the same methods, and issued Supplement 3, Historical Control Morphometric Data (Hoberman, 2000). The importance and relevance of the additional available control morphometric data help to place the results of the chlorpyrifos DNT study results into proper context and demonstrate that the changes observed in the Hoberman study cannot be attributed to chlorpyrifos.

OEHHA's derivation of a dermal absorption value from one study is scientifically inappropriate and results in a dermal MADL that is incorrect and significantly over-estimates absorption

The dermal absorption value selected is critical since it is the most sensitive variable in evaluating potential bystander exposure. As discussed in more detail later in these comments, OEHHA relied upon a single dermal absorption value (the highest) from one of the several studies that have been conducted. The value that OEHHA chose does not represent actual study data on absorption, nor does it represent the weight of evidence or the best science, and is not consistent with the

Proposition 65 statute which stipulates the estimation of reasonably anticipated exposures, i.e., average exposure and dose values. An overall grand mean value of 2.5% dermal absorption, which is less than one-third of the 8% value used by OEHHA, can be estimated from all the available studies, and would reflect a reasonably anticipated exposure and absorbed dose for the average deposition used in the various studies.

#### Executive Summary References

Dow AgroSciences LLC. 2017. 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.

California Environmental Protection Agency Office of Environmental Health Hazard Assessment (OEHHA). 2019. Proposed Amendments to Section 25805(b), Specific Regulatory Levels: Chemicals Causing Reproductive Toxicity. Maximum Allowable Dose Levels for Chlorpyrifos (Oral, Inhalation and Dermal Exposures). Published May 24, 2019.  
<https://oehha.ca.gov/proposition-65/crn/amendment-section-25805-specific-regulatory-levels-chemicals-causing-0>

## II. Background on Prop 65 Statute and Requirements

As set forth is Cal. Health & Safety Code sec. 25249.5, et seq.

“(b) A chemical is known to the state to cause cancer or reproductive toxicity within the meaning of this chapter 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 cancer or reproductive toxicity, or if a body considered to be authoritative by such experts has formally identified it as causing cancer or reproductive toxicity, or if an agency of the state or federal government has formally required it to be labeled or identified as causing cancer or reproductive toxicity.”

The DARTIC, through its opinion as “the state’s qualified experts,” concluded on November 29, 2017, that chlorpyrifos was “clearly shown through scientifically valid testing according to generally accepted principles to cause developmental toxicity.” The developmental endpoint was not identified. Nonetheless, chlorpyrifos was added to the Proposition 65 list as a developmental toxicant on December 15, 2017.

In efforts to establish a MADL pursuant to the Proposition 65 Regulations under Section 25803 (Assessment), it is stated that, “[o]nly studies producing the reproductive effect which provides the basis for the determination that a chemical is known to the state to cause reproductive toxicity shall be utilized for the determination of the NOEL.” Since chlorpyrifos was listed as a developmental toxicant, this means that the MADL must be based on an endpoint of developmental toxicity.

The challenge here is that the DARTIC did not identify a specific developmental effect as the basis for their decision to list chlorpyrifos under Proposition 65. While the DARTIC may not be required technically to specify a particular toxicological endpoint of concern, the principles of chemical risk assessment typically require the most sensitive effect from the most sensitive species

to be identified and utilized as a point of departure for protection of human health. The consideration of study quality is specifically called out in the regulations (Section 25803 Assessment), as noted by the following:

(5) “The NOEL shall be based on the most sensitive study deemed to be of sufficient quality.”

(6) “The results obtained for the most sensitive study deemed to be of sufficient quality shall be applicable to all routes of exposure for which the results are relevant” (The Statute, 2012).

Importantly, the distinction between effects caused by prenatal exposure and those caused by postnatal exposure is vitally important in identifying an appropriate study to determine a MADL for developmental toxicity. An adverse developmental effect does not come within the purview of Proposition 65 unless it is attributable to prenatal exposure. This principle, established from the outset in the implementation of Proposition 65, was explained and confirmed at a public meeting of the DARTIC on December 4, 1996. At that meeting, OEHHA’s then-Chief Counsel William Soo Hoo confirmed on the record the long-standing principle that, for developmental toxicity, “postnatal exposures [are] not encompassed by Proposition 65.” Mr. Soo Hoo’s testimony on this point appears in the public meeting minutes of the December 4, 1996 hearing (California EPA, OEHHA, 1996).

#### References for Section II

OEHHA (OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT; California EPA). 1996. Statement and Testimony of Mr. William Soo Hoo. DARTIC Meeting, December 4, 1996.

### III. Evaluation of Studies Cited by OEHHA as the Basis for the Proposed MADL Within the Context of Proposition 65

As the basis for proposing a MADL for chlorpyrifos, OEHHA has included four studies, the primary one being Silva et al. (2017). Table 1 of the OEHHA proposal is reproduced below.

Table 1. Key developmental neurotoxicity studies of chlorpyrifos

Study	Species Exposure Period, Route	Doses (mg/kg-day) <sup>a</sup>	POD (mg/kg-day)	Animals Tested and Key Effects
<b>Critical Study</b>				
Silva et al., 2017 <sup>15</sup>	Rat dam GD 14 - 20 Gavage	0, 0.01, 0.1, 1, 10	0.01 (NOEL)	PND 21 male rat pups ↑ Anxiety ↑ locomotor activity
<b>Supportive Studies</b>				
Gómez-Giménez et al., 2017 <sup>16</sup>	Rat dam GD 7 - PND 21 Food/diet	0, 0.1, 0.3, 1	0.1 (LOEL)	2-3-month-old male offspring ↓ Spatial learning in Morris water maze
Gómez-Giménez et al., 2018 <sup>17</sup>	Rat dam GD 7 - PND 21 Food/diet	0, 0.1, 0.3, 1	0.1 (LOEL)	2-3-month-old male and female offspring ↑ Locomotor activity
Hoberman, 1998a,b <sup>18</sup>	Rat dam GD 6 - PND 11 Gavage	0, 0.3, 1, 5	1 (LOEL)	PND 66 pups ↓ parietal cortex thickness

<sup>a</sup> Chlorpyrifos was administered in corn oil via oral gavage in the studies by Silva et al. (2017) and Hoberman (1998a,b), and in corn oil mixed in a sweet jelly via diet in the studies by Gómez-Giménez et al. (2017; 2018).

Abbreviations: GD, gestation day; PND, postnatal day; NOEL, no observable effect level; LOEL, lowest observable effect level

The Proposition 65 regulations call for the evaluation of studies to determine if they are deemed to be “of sufficient quality” and therefore suitable for MADL derivation. The regulations provide a description of the numerous factors that must be considered, although the regulations are not specific in defining what satisfies each factor. Generally speaking, the Regulations state that: **“Animal bioassay studies for assessment shall meet generally accepted scientific principles including the thoroughness of experiment protocol, the temporal exposure pattern, the duration of study, the purity of test material, the number and size of exposed**

**groups, and the route of exposure and the extent of occurrence of effects.”** DAS has carefully evaluated each of these studies in accordance with these Proposition 65 criteria:

1. Silva, et al. (2017), the primary study cited by OEHHA to derive the proposed MADL, is not a study of “sufficient quality,” and should not be used to establish a MADL for chlorpyrifos. This study reported on anxiety-like behavior in rat offspring following exposure to chlorpyrifos during pregnancy (i.e., GD14-20). The offspring were not exposed postnatally. The investigators employed doses ranging from 0.01 to 10 mg/kg/day, but failed to report on purity of the test material or whether dose concentration verification was conducted. Statistical power was generally recorded as  $p < 0.05$ . The group size ranged from 11 to 14 pregnant females per group. The actual number of offspring tested for behavioral effects on PND 21 and PND 70 is not stated. It is not clear whether testing included littermates, and if so, how the study controlled for the presence of littermates. Furthermore, it is not clear if animals were randomly assigned to dose groups.

Silva, et al. (2017) reported effects at 0.1, 1.0, and 10.0 mg/kg/day, citing induction of anxiogenic-like, but not depressive-like behavior at PND21. The effect was reversed by PND 70. This begs the question whether increased or decreased anxiety-like behavior is biologically significant and whether both are adverse, or whether one is adverse while the other is not (particularly since other investigators have reported decreased anxiety related to chlorpyrifos exposure (Carr, et al. 2017)). In addition, there was a lack of dose-response for this reported observation, given that the dose-spacing was quite wide (0.1-10.0 mg/kg for reported statistically significant observations), which may infer that this endpoint (anxiogenic behavior) is not a particularly sensitive one. While locomotor activity was reported as statistically significant, the increased (relative to control) motor activity at 0.1 mg/kg/day was virtually the same as that reported following exposure to 10 mg/kg/day (i.e., no dose response for this observation).

CDPR commented on this study specifically, claiming that “the most important implication of this study is that the threshold for CPF-induced neurobehavioral effects in young rats following gestational exposure may be as much as 10-fold lower than the reported threshold of 1 mg/kg/day established for RBC AChE inhibition in adult rats.” This statement is factually incorrect as there

is clear evidence that the threshold for RBC ChEI is well below 1 mg/kg/day (Marty, et al, 2012). While DAS is not asserting that cholinesterase inhibition represents the only or optimal point of departure, failure to measure it concomitantly in studies which are evaluating potential developmental toxicity should preclude any objective investigator from declaring that observed and reported developmental effects are or did occur below the threshold for cholinesterase inhibition. Silva et al. (2017) did not measure either brain or RBC ChEI.

While the inferred NOEL for this study would be 0.01 mg/kg/day, the absence of a well-defined dose-response at the top three dose levels calls into question whether these reported effects are treatment-related, and if so, how sensitive they are as markers of exposure to chlorpyrifos. If certainty in a clearly defined point of departure for risk assessment (in this case MADL derivation by use of a NOEL) is of such importance, then one would have to question whether the reported outcome (increased anxiety-like behavior) is beneficial or adverse, particularly when others (Carr et al., 2017) have reported that CPF decreases anxiety-like behavior (whether beneficial or adverse). Certainty in clarifying and determining what is adverse (versus perhaps adaptive or even beneficial) is the first step in establishing a POD and NOEL, and this study fails to provide sufficient evidence or quality to be considered for underpinning a MADL. Thus, for all the above stated reasons, the Silva study cannot be considered “of sufficient quality.”

Two additional points are worth noting. First, during the DARTIC November 29, 2017 deliberations over the listing of chlorpyrifos as a developmental toxicant, based on transcripts of the hearing, neither the lead discussant on the animal behavioral studies, nor any other of the discussants cited Silva as a study that served as a basis for potential listing. While one of the Gomez-Gimenez (2017, not specified) studies as well as the Maurissen (2000) study (i.e., this is the Hoberman (1998) study) were cited, Silva was not but is now being cited as the primary basis for the proposed MADLs. It should be noted that the Silva study was available online 24 January 2017, 10 months before the DARTIC meeting.

Secondly, an independent review (Li, et al. 2012) assessed the available studies at that time involving the EPM (elevated plus maze) which was the specific test used by Silva, et al. (2017) and for which they concluded anxiogenic-like behavior and which OEHHA has cited as the basis

for the MADL. Li, et al. (2012) evaluated five studies involving the EPM and none of the studies reported any treatment-related effects below 1.0 mg/kg. Only one study used a dose lower than 1.0 mg/kg and no effects were noted by Li, et al. (2012). Li, et al. (2012) concluded that, “[i]n summary, there are no consistent patterns of adverse effect on novelty-induced activity, plus maze...especially at 1 mg/kg-d.”

2. Gomez-Gimenez, et al. (2017a) evaluated sex-dependent effects of developmental exposure to different pesticides on spatial learning. As the following discussion indicates, there are multiple reasons why this study is unsuitable for determining a MADL for chlorpyrifos. The investigators tested chlorpyrifos at 0.1, 0.3 and 1.0 mg/kg/day from GD7 through PND 21. Neither purity of the test substance nor dose level verification was noted as having been conducted. The authors stated that p values lower than 0.05 were considered statistically significant. The pups were exposed postnatally through the mothers’ milk (and perhaps from ingesting the test material directly (see below)). Spatial learning and memory tests were performed when the pups were 2 to 3 months of age. Because this study involved both pre and post-natal exposure, one cannot determine if the effects reported were due to prenatal exposure vs. postnatal exposure, and the authors do not state otherwise. Developmental effects due to postnatal exposure are excluded by Proposition 65. Therefore, this study is unsuitable for determining a MADL for chlorpyrifos.

Gomez-Gimenez, et al. (2017a) reported on a number of parameters/measurements including spatial learning, reference errors, working memory, learning indices, and hippocampal content of pro- and anti-inflammatory cytokines, but do not appear to have established an overall NOEL considering the number of parameters evaluated. Results were varied with some statistically significant effects reported, but in a scattered fashion where dose-responsiveness was rare and outcomes were gender-specific (i.e., reported decreased learning in males, but not females).

The test material was administered by mixing chlorpyrifos in an undefined “sweet jelly.” According to the authors, “[w]e confirmed that all rats ate all the sweet jelly and, therefore, the dose of pesticide.” This is an unusual method of administering a test material orally. Further, it is not clear how the pups were prevented from ingesting the test material in the sweet jelly since the dams and pups were presumably housed together until weaning. No information was provided

on the housing conditions of the animals. The group size was small, with the offspring from only six dams per dose group used. The publication states, “[t]he litter effects were controlled by using pups from different litters per treatment group in each experiment.” However, the authors do not explain how this was done. The actual number of pups used per dose group, which were provided in Figure 1, ranged from 6 to 13 per dose. This means that some of the pups must have been littermates since there were only six dams per group. It is not clear how littermates were selected for testing. The authors do not state whether the litter or the pup was considered to be the statistical unit. Neither brain nor RBC ChEI was measured. For these various reasons, Gomez-Gimenez, et al. (2017a) is not a study “of sufficient quality.”

3. Gomez-Gimenez, et al. (2017b – Note: This is cited in Table 1 above as 2018, but the publication date is cited as published online 03 October 2017; hence we are referring to it as 2017b)) also conducted a study evaluating the motor activity and coordination of rats exposed to several pesticides, including chlorpyrifos. Like the other study by these authors, pregnant rats and their offspring were exposed to chlorpyrifos from GD 7 to PND 21. Neither purity of the test material nor dose level verification was conducted. Based on the various statistical approaches used, the authors stated that p values less than 0.025 were considered statistically significant. Motor activity and coordination were evaluated when the offspring were adults. Therefore, it is not possible to distinguish between effects due to prenatal exposure vs. postnatal exposure. Accordingly, this study is not suitable for establishing a MADL for chlorpyrifos, which must be based on effects caused solely by prenatal exposure.

The Gomez-Gimenez, et al. (2017b) study is not “of sufficient quality.” It suffers from many of the same scientific limitations as the first Gomez-Gimenez study, including lack of information on purity/source of test material, no analytical verification of doses, no dose-response observed, and small number of animals per group. Even if the study had not included postnatal exposure, it would still have been unsuitable for establishing a MADL for chlorpyrifos due to these scientific limitations.

4. Also cited by OEHHA as supportive of Silva, et al. (2017) is the Hoberman (1998) study. In fact, this is the GLP guideline chlorpyrifos developmental neurotoxicity (DNT) study in rats that

was subsequently published by Maurissen, et al. (2000). This study meets the study-design requirements of regulatory agencies world-wide and is the EPA guideline compliant study for evaluating neurodevelopmental toxicity.

Most importantly for consideration in setting a MADL under the Regulation, this study meets all requirements of experimental conditions and parameters that would be considered “of sufficient quality” for identification of a clear NOEL related to developmental toxicity (i.e., given that the study was specifically designed by EPA to evaluate developmental neurotoxicity) including (a) thoroughness of the experimental protocol (as agreed by EPA); (b) degree to which dosing resembles the expected manner of human exposure (i.e., oral); (c) temporal exposure pattern (i.e., this study involved both pre and post-natal exposure, but given that there is a clear NOEL at 1.0 mg/kg/day for developmental neurotoxicity in the pups, this obviates the need to specifically denote whether an effect was due to pre- or post-natal exposure because there were no effects reported); (d) duration of study – in this case appropriate based on the exposure duration and subsequent behavioral testing at PND22-24 and PND 61-90 (more extensive than most other reported studies); (e) purity of test material (reported as 99.8%, higher purity than any other reported material used in other studies); (f) dose-concentration verification, homogeneity documentation, and test material stability including dose formulation stability over the range used in the study); and (g) number and size of exposed groups (this study reported using 24 litters with average liveborn litter size of around 13, while Gomez-Gimenez, et al. (2017a) used from 7-12 males and 5-10 females per group and Silva, et al. (2017) used from 8-10 animals per group for their open-field locomotor activity measurements

The maternal doses were 0, 0.3, 1 or 5 mg/kg/day. The route of exposure was oral gavage to dams (chlorpyrifos in vegetable oil) from gestation day 6 to lactation day 10 (birth = lactation day 0). Although this study had both prenatal and postnatal exposure, it is relevant if no effects were observed at a particular dose because it shows that neither prenatal nor postnatal exposure cause an effect at that dose. Purity of the test substance was provided as 99.8% and dose level verification was conducted. Additionally, the type-1 error rate was set at 0.02. All p values were subsequently reported as uncorrected based on this alpha level of 0.02. Maternal plasma, brain,

and RBC cholinesterase activity were measured and reported.

High-dose dams had clinically-evident toxicity 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 controls that were attributed to treatment. Small but statistically significantly 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.

The DNT study authors concluded that the developmental NOEL 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 selective developmental neurotoxicity following exposure to chlorpyrifos in the DNT study (Maurissen *et al.*, 2000).

As previously described, Maurissen *et al.* (2000) evaluated learning and memory including three phases of testing involving maze acclimation, acquisition training, and delay testing. It is important to note that this DNT study employed an automated T-maze model (in contrast to the EPM approaches used by Silva, *et al.* 2017) and the paradigm used was spatial alternation. This particular test is designed to assess hippocampal function. Engin and Treit (2007) evaluated the role of the hippocampus in anxiety and concluded that, “[t]he data clearly suggest that the hippocampus is importantly and directly involved in the mediation of untrained anxiety reactions in animals.” The DNT study evaluated learning and memory. Anxiety, as mediated in the

hippocampus, is one component that may affect learning and/or memory, although no effects on these parameters were seen at any treatment level in pups. Motor activity and auditory startle in addition to extensive brain weight, histopathology and morphometrics were also measured. Cognitive function (learning, short-term memory, and habituation in two different tasks) were not impaired in the pups, at any time at any dosage. The clear NOEL is 1 mg/kg/day in pups owing to effects observed in pups at 5 mg/kg/day and also observed in the presence of maternal toxicity and consistent with delayed maturation.

In light of these data, DAS disagrees with OEHHA's contention that 1 mg/kg/day from Hoberman (1998) is a LOEL based on parietal cortex effects in PND 66 rats. 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 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. The importance and relevance of the additional available control morphometric data help to place the results of the chlorpyrifos DNT study results into context and demonstrate that the changes observed in the Hoberman study cannot be attributed to chlorpyrifos.

As important, 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.

It is also important to point out that CDPR has reviewed and evaluated this study and concluded its suitability and acceptability in the CHLORPYRIFOS RISK CHARACTERIZATION DOCUMENT - Spray Drift, Dietary and Aggregate Exposures to Residential Bystanders - Chlorpyrifos RCD: Draft 12-31-2015. A review of CDPR's analysis and conclusions concerning

this study shows that CDPR considers it fully acceptable, does not dispute any of the findings reported in the study or the subsequent peer-reviewed publication (Maurissen et al. 2000), and does not challenge either the NOEL reported in the study or question the "sufficient quality" of the study.

### Summary

The study selection for a MADL derivation hinges upon two central tenets related to Proposition 65 regulations – that the NOEL is derived from the most sensitive study of sufficient quality and that "[a]nimal bioassay studies for assessment shall meet generally accepted scientific principles including the thoroughness of experiment protocol, the temporal exposure pattern, the duration of study, the purity of test material, the number and size of exposed groups, and the route of exposure and the extent of occurrence of effects." For multiple reasons, the Silva, et al. (2017) study, which is proposed as the primary study to determine the MADL for chlorpyrifos, is not a study "of sufficient quality," as required in the regulations. Moreover, the two studies by Gomez-Gimenez are not appropriate to establish a MADL for chlorpyrifos since the basis for the listing is developmental toxicity and the effects reported in these two studies may be due to postnatal exposure, which is inconsistent with the Proposition 65 definition of developmental toxicity. Thus, the Gomez-Gimenez studies also do not meet the criteria for "sufficient quality". The "most sensitive study of sufficient quality" is the Hoberman et al. (1998) study, which OEHHA employed as one of the supporting studies in its proposed MADL.

For purposes of establishing a MADL, comprehensive review of the studies used by OEHHA discussed above, indicates the most relevant, scientifically defensible NOEL from a study of "sufficient quality" is 1 mg/kg/day derived from Maurissen et al (2000).

### References for Section III:

Carr, R.L. et al. 2017. Decreased anxiety in juvenile rats following exposure to low levels of chlorpyrifos during development. *Neurotoxicology*. 59:183-190.

Engin, E. and Treit, D. 2007. The role of hippocampus in anxiety: intracerebral infusion studies. *Behavioral Pharmacology*. 18:365-374.

Gomez-Gimenez, B. et al. 2017a. Sex-dependent effects of developmental exposure to different pesticides on spatial learning. The role of induced neuroinflammation in the hippocampus. *Food and Chemical Toxicology*. 99:135-148.

Gomez-Gimenez, B. et al. 2017b. Developmental exposure to pesticides alters motor activity and coordination in rats: sex differences and underlying mechanisms. *Neurotox Res*. DOI 10.1007/s12640-017-9823-9.

Hoberman, A.M., 1998. Developmental neurotoxicity study of chlorpyrifos administered orally via gavage to Crl:CD®BR VAF/Plus® presumed pregnant rats. Argus Laboratories, May 1, 1998.

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Marty, M.S., et al. 2012. Cholinesterase inhibition and toxicokinetics in immature and adult rats after acute or repeated exposures to chlorpyrifos or chlorpyrifos-oxon. *Reg. Toxicol. Pharmacology*. 63:209-224.

Maurissen, JP, Hoberman AH, Garman RH, Harvey TR. 2000. Lack of Selective Developmental Neurotoxicity in Rat Pups from Dams Treated by Gavage with Chlorpyrifos. *Toxicological Sciences* 57:250-263.

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#### **IV. OEHHA Value for Human Dermal Absorption of Chlorpyrifos is Overestimated and Does Not Consider Full Scientific Weight-of-Evidence**

OEHHA's derivation of a dermal absorption value from one study is inappropriate and results in a dermal MADL that is incorrect. OEHHA relied upon a single dermal absorption value (the highest) from one of the several studies that have been conducted (see Table 1 below). The value that OEHHA chose does not represent actual study data on absorption, nor does it represent the weight of evidence or the best science, and is not consistent with the Proposition 65 statute which stipulates the estimation of reasonably anticipated exposures, i.e., average exposure and dose values.

Specifically, OEHHA estimated a dermal absorption factor of 8% based on a dermal pharmacokinetic study of chlorpyrifos in adult humans by Meuling, et al. (2005). In this study, application of approximately 5 mg chlorpyrifos (in ethanol) to approximately 100 square centimeters (cm<sup>2</sup>) of the volar surface of the forearm (of three adult males) resulted in a mean absorption value of 4.3%, with a highest individual absorption value of 5.8%, as calculated from urinary excretion of the chlorpyrifos metabolite TCP (3,5,6-trichloro-pyridinol) over a 120-hour period. However, OEHHA interpreted the study differently. Since the authors reported that approximately 50% of the applied dose was washed off the skin with water four hours after application, OEHHA estimates that when the potentially absorbed dose (the applied dose minus the washed-off dose) was used instead of the applied dose, the average dermal absorption was 7.9% (rounded to 8%). OEHHA incorrectly claims that these findings are consistent with those of two other studies in humans.

In contrast to OEHHA's evaluation, dermal absorption can be based on consideration of available data, across multiple studies, that represent four orders of magnitude with respect to dermal loading or dose density on the skin. Dermal absorption is a key factor in estimating absorbed dose from dermal contact with chlorpyrifos. Three human dermal absorption studies have been conducted with chlorpyrifos (Nolan, et al., 1984; Griffin, et al., 1999 and Meuling, et al., 2005). The kinetics of chlorpyrifos, and its principal metabolite, TCP, were investigated in six healthy male volunteers given a single 0.5 mg/kg oral and, two weeks later, a 0.5 or 5.0 mg/kg dermal dose (390 and 4,200 µg/cm<sup>2</sup>) of chlorpyrifos (Nolan, et al., 1984). Griffin, et al. (1999) conducted an absorption study

in five human volunteers with chlorpyrifos applied at the rate of 370  $\mu\text{g}/\text{cm}^2$  using alkyl phosphate metabolites to quantify absorbed dose via urinary excretion. They estimated dermal absorption of 1% and an excretion half-life of 41 hours. Most recently, Meuling, et al. (2005) reported a pharmacokinetic study with dermally-applied chlorpyrifos in six humans applied at the rate of 50 and 160  $\mu\text{g}/\text{cm}^2$  resulting in a dermal absorption of 4.3 and 1.2%, respectively, and an excretion half-life of 41 hours.

In addition, a number of studies have been conducted with human subjects exposed environmentally to chlorpyrifos. From those studies it is possible to estimate human dermal absorption by knowing the dermal dose and concurrently determined absorbed dose measured with urinary biomonitoring. Geer, et al. (2004) estimated that over the range of dermal doses in a variety of work tasks, the dermal absorption was approximately 5%. Krieger, et al. (2000) and Bernard, et al. (2001) each determined absorbed dose in a cohort of college students exposed to a known deposition of chlorpyrifos on carpet or turf while wearing bathing suits with another concurrent cohort wearing full body dosimeters. In those studies, the average dermal load was 0.1-0.9  $\mu\text{g}/\text{cm}^2$  based on a body surface area of 16,000  $\text{cm}^2$  (entire body minus head). At that dose, the dermal absorption can be estimated at 1.3-6%. While many chemicals exhibit an inverse correlation of dermal load and percent absorption, the absorption of chlorpyrifos appears to range from 1-6% over a dermal load range of 0.1- 4,000  $\mu\text{g}/\text{cm}^2$ .

Thongsinthusak (1991) concluded that the regulatory dermal absorption of chlorpyrifos should be 9.6%. This estimate was based upon the results of the human absorption studies of Nolan, et al. (1984) and two unpublished measurements obtained from one individual in which excretion was near the limit of detection. However, measured absorption in humans at dermal loads of 0.1-4,000  $\mu\text{g}/\text{cm}^2$  indicates that an upper bound of 6% is an extremely high estimate for absorption at low dermal loading (see Table 1 below).

Table 1. Chlorpyrifos dermal absorption estimated from various sources.

<b>Study</b>	<b>Dermal Dose (µg)</b>	<b>Dermal Dose Rate (µg/cm<sup>2</sup>)</b>	<b>Absorbed Dose (µg)</b>	<b>Estimated Absorption (%)</b>
Krieger et al., 2000	13,800	0.9	180	1.3
Bernard et al., 2001	1,600	0.1	98	6.0
Nolan et al., 1984	416,000	4,200	5,400	1.0
Nolan et al., 1984	38,900	390	1,000	2.6
Griffin et al., 1999	28,600	370	290	1.0
Meuling et al., 2005	16,200	160	194	1.2
Meuling et al., 2005	5,390	50	232	4.3

Based on the summary of dermal absorption data specifically for chlorpyrifos, for example, if the skin received a loading density of 1 µg/cm<sup>2</sup>, then a dermal absorption rate of approximately 1% (0.01), would be relevant, not the 9.6% value that CDPR has used, or the value of 3% dermal absorption used by EPA for chlorpyrifos in its risk assessments. An overall grand mean value of 2.5% dermal absorption can be estimated from all the available data (see table), which would reflect a reasonably anticipated exposure and absorbed dose to the average deposition used in the various studies.

This overall mean value is further supported by physiologically-based pharmacokinetic and pharmacodynamic modeling (PBPK-PD) of chlorpyrifos for the dermal route of exposure (Timchalk, et al. 2002). Unlike simple one-compartmental estimation methods that describe the fractional absorption of chlorpyrifos (CPF) in humans (see Table 1 above; e.g., Nolan, et al. 1984), the PBPK-PD model available for CPF can be used to calculate flux through human skin as a function of CPF permeability, concentration, area of exposed skin surface and exposure duration. Hence, the CPF PBPK-PD model was used to calculate permeability coefficient (Kp) of 4.81 x 10<sup>-5</sup> cm/h, and an assumed dermal exposure duration not exceeding 20 hours post-application (post-exposure). The estimated fractional absorption was 2.32% (Timchalk, et al. 2002). This result is similar to the approximately 1% dermal absorption based on the recovery of trichloropyridinol

(TCP) and dialkylphosphate metabolites in the urine of adults participating in studies conducted by Nolan (1984) and Griffin, et al. (1990).

Recently, the European Union (EU 2017) reviewed data (rat and human) for chlorpyrifos and concluded that dermal absorption values for chlorpyrifos to assess potential exposure from EF-1551 concentrate (484 g a.s./L) and spray dilutions, at concentrations of 1.80 g a.s./L and 0.48 g a.s./L, are 0.9 %, 5% and 7 %, respectively, based on data from *in vivo* and *in vitro* dermal absorption studies (triple pack approach). This range is consistent with values from the studies listed in Table 1. Moreover, with the plethora of human data available, there is no justification to rely upon results from a single study.

In conclusion, as noted above, an overall grand mean value of 2.5% dermal absorption can be estimated from all the available data (see table). This would reflect a reasonably anticipated exposure and absorbed dose for the average deposition used in the various studies.

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