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DATE: September 17, 2002

SUBJECT: COMMENTS ON THE DRAFT CHLORPYRIFOS TOXIC AIR CONTAMINANT EVALUATION DOCUMENT PREPARED BY THE DEPARTMENT OF PESTICIDE REGULATION AND SUBMISSION OF OEHHA'S DRAFT FINDINGS ON THE HEALTH EFFECTS OF CHLORPYRIFOS FOR REVIEW

Thank you for the opportunity to review the draft toxic air contaminant (TAC) evaluation document for chlorpyrifos prepared by the Department of Pesticide Regulation (DPR). Pursuant to Food and Agricultural Code Sections 14022 and 14023, the Office of Environmental Health Hazard Assessment (OEHHA) provides review, consultation, and comments to DPR on the evaluation of the health effects of candidate toxic air contaminants. As part of its statutory responsibility, OEHHA also prepares findings on the health effects of the candidate toxic air contaminants. These documents are to be included as part of the TAC document.

Our major comments and concerns on the draft TAC document for chlorpyrifos are briefly summarized in this memorandum and in more detail in the attachment. In addition, we are submitting OEHHA's draft findings for your review.

We acknowledge that at this time, OEHHA and DPR have not completed our joint guidelines for the use of cholinesterase (ChE) inhibition as an endpoint for risk assessment. This impacts both the preparation and review of risk assessments for ChE inhibitors (e.g., azinphos-methyl and chlorpyrifos) generated under Assembly Bill 1807. Therefore, we recommend at this time that any TAC document for a ChE inhibitor include the full range of risk-related results using the various ChE endpoints: brain, plasma, red blood cell (RBC), and other tissues if available. The inclusion of this information will allow for a complete discussion and consideration of all scientific factors involved in assessing risks of these pesticides. Including all information at this time might also facilitate any necessary revision of these documents at a later date, once the guidelines are produced and adopted.

In addition to the draft TAC document for chlorpyrifos, we concurrently reviewed the U.S. Environmental Protection Agency's (U.S. EPA, 2000a) recently revised risk assessment for chlorpyrifos. There are some significant scientific differences between the draft TAC document and U.S. EPA's risk assessment [e.g., acute and chronic no-observed-adverse-effect-levels (NOAELs) and consideration of children's sensitivity and susceptibility]. These differences should be discussed in greater detail in the TAC document. Some of our concerns regarding the draft TAC document for chlorpyrifos are addressed in U.S. EPA's risk assessment and we note this in our attached comments. For example, we consider the dose selected in the draft TAC document from the dog study (0.1 mg/kg-day) for assessment of chronic exposures to be a lowest-observed-adverse-effect-level rather than a NOAEL. We agree with U.S. EPA in selecting 0.03 mg/kg-day as the NOAEL for RBC inhibition from this study. We would use this NOAEL in calculating risk from human chronic exposure to chlorpyrifos. We therefore recommend that the TAC document for chlorpyrifos adopt the lower dose as the NOAEL for estimation of chronic risks and calculation of margins of exposure (MOEs).

We also have some concerns with respect to the selection of the NOAEL for toxicity from acute chlorpyrifos exposure. First, we consider the study used in the draft TAC document (Kisicki *et al*, 1999) to be deficient in design and reporting (see the attached comments for details). We are also concerned about the erratic absorption of the test compound as reported in the study and the fact that plasma ChE measurements were not taken during the study. In addition, there exists a discrepancy in the selection of no effect levels for acute toxicity between the "Summary of Toxicology Data for Chlorpyrifos" (DPR, 2001) and the draft TAC document. We recommend that this discrepancy be explained or corrected in the TAC document.

In general, we found the literature review in the draft chlorpyrifos TAC document to be comprehensive up to about two years ago. However, considerable information has been published in the last two years in the open literature concerning the differential susceptibility of neonatal and young rats compared to adult rats to chlorpyrifos-induced neurobehavioral toxicity.

We have summarized these studies in the attachment and we recommend that a discussion of these studies be included in the TAC document. We also recommend that a complete and updated literature search be conducted as we have identified some key references that were not cited in the draft TAC document. It would also be helpful if more detail were provided in the summaries of a number of the toxicology studies. Specific studies are identified in the attachment.

Following our review of the existing literature on differential susceptibility of neonates and developing young rats, we conclude that the scientific evidence supports an additional uncertainty factor of ten for the particular sensitivity and differential susceptibility of infants and children. Therefore, to account for intra-species (human) variability we recommend that the TAC document for chlorpyrifos apply an uncertainty factor of 100 (includes the standard factor of ten for variation in the human population and a factor of ten for particular sensitivity and differential susceptibility of infants and children) when interpreting MOEs and calculating the corresponding reference exposure level. This use of an additional intra-species uncertainty factor is consistent with U.S. EPA's approach in evaluating chlorpyrifos under the Food Quality Protection Act (FQPA). Under FQPA, U.S. EPA automatically applies an additional safety factor of 10-fold for the protection of infants and children. The safety factor is removed only when there is sufficient evidence demonstrating that infants and children are no more sensitive and/or susceptible than adults to the toxic effects of a particular chemical. The safety factor is retained when there are no data available assessing the toxicity to infants and children (default case). The safety factor is also retained when infants and children have been shown to be more sensitive and/or susceptible. The latter situation also applies in the case of chlorpyrifos as there is ample evidence demonstrating an increased sensitivity and susceptibility of infants and children to the toxic effects of the chemical.

In addition to these comments and concerns, we have included other comments in the attachment. Our staff would be happy to meet with your staff to discuss our comments or our draft findings. If you have any questions, please contact either Dr. Melanie Marty or Dr. Anna Fan at (510) 622-3200 or Dr. David Rice at (916) 324-1277.

Attachments

cc: See next page

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Attachment

Comments on the Draft Toxic Air Contaminant Document For Chlorpyrifos

The Office of Environmental Health Hazard Assessment (OEHHA) has reviewed the draft toxic air contaminant (TAC) document for chlorpyrifos prepared by the Department of Pesticide Regulation (DPR) and has the following comments. In general, the draft TAC includes a substantial analysis of the toxicity of the pesticide active ingredient chlorpyrifos, and provides numerous exposure scenarios on which the risk characterization is based. Nevertheless, we have identified several scientific issues that we have questions or concerns about in the draft TAC document.

OEHHA and DPR are currently developing guidelines for the use of cholinesterase (ChE) inhibition for risk assessment. Although the U.S. Environmental Protection Agency (U.S. EPA) published a draft policy in 2000 (U.S. EPA, 2000b), both departments agree that this document does not provide the essential guidance necessary to address the issues regarding ChE inhibitors. In the interim, we recommend that all TAC documents for pesticides that inhibit ChE include a complete description of all ChE inhibition endpoints, whether from brain, plasma, red blood cell (RBC), or other tissues. In addition, we recommend that the associated risks from ChE inhibition from all sources be calculated and compared.

Database and Citations

1. A wealth of information on the differential susceptibility of neonatal and young rats compared to adult rats to chlorpyrifos-induced neurobehavioral toxicity has been published in the open literature in 2000 and 2001 (including, but not limited to, Moser, 2000; Bushnell *et al.*, 2001; Garcia *et al.*, 2001; Jett *et al.*, 2001; Levin *et al.*, 2001; Slotkin *et al.*, 2001; Won *et al.*, 2001). We understand that a time cutoff point is usually established for a document of this type; however, this information is too important not to be included. This document should be revised to include information presented in these papers. (Note: additional detailed discussion regarding the differential susceptibility of infants and children versus adults is presented below.)

2. In addition to those specifically mentioned in these comments, some relevant references to important toxicological data were not found in the draft TAC document. These specific references can be found in the bibliography at the end of this attachment and are identified by an asterisk (*). We recommend that a thorough and updated literature search be performed to augment the searches already conducted in the preparation of the draft TAC document.

3. We recommend performing a careful review of the references cited in the text and of those listed in the bibliography of the draft TAC document. Several references cited in the text are not found in the Reference section. For example, on page 38, Carr *et al*. (2001); page 39, Hanley *et al*. (1987); page 43, Ellenhorn and Barceloux (1998); page 71, Bolla Wison *et al*. (1998); and page 71, Amundsen *et al*. (1996) are cited in the text but do not appear in the Reference section of the draft TAC document. We also note that a number of the references in the Reference section are not in alphabetical order. In addition, the name "Kisicki" is misspelled in many places in the text (e.g., pages 18, 52, 53, 60, 61, and 70).

4. Descriptions of a number of toxicology studies need to be expanded in the text of the draft TAC document; specific studies are identified in the comments below.

5. We have identified some important discrepancies between the Summary of Toxicology Data for Chlorpyrifos (DPR, 2001) and the draft TAC. For example, the Summary identifies the administered dose level of 1.0 mg/kg (0.3 mg/kg absorbed dose as per the draft TAC document) in the study of Kisicki *et al*. (1999) as the "no-observed-effect-level" (NOEL), based on RBC ChE inhibition. The next higher absorbed dose of 0.7 mg/kg was identified in the draft TAC, however, as the "NOEL" based on clinical signs and symptoms. Another example is given below in the Developmental Toxicity section. These discrepancies need to be corrected.

A. Exposure Assessment

1. Data from an application on oranges are used for the estimation of acute exposures from application sites. The application rate was not provided in the draft TAC document, so it is unknown if the scenario represents a maximum rate application. We recommend including the application rate used for this study and the rationale for the selection of this particular application for monitoring.

2. Dermal exposure from airborne chlorpyrifos is not addressed in the draft TAC document. This potential exposure route should be discussed in the Exposure Assessment and Human Health Assessment section of the draft TAC document even if it is assumed that exposure by this route does not contribute significantly to total exposure.

3. In the draft TAC document, no seasonal or chronic exposure scenarios for individuals living on farms surrounded by chlorpyrifos-treated orchards/crops are developed. We recommend developing scenarios and estimating exposures for these hypothetical receptors. Exposure could be estimated as a combination or composite of ambient and application air concentrations of chlorpyrifos. We recommend estimating both seasonal and chronic exposures of these receptors to airborne chlorpyrifos.

4. A study of chlorpyrifos in ambient air in Kern County (Seiber *et al*., 1987) is available. The results from this study are not summarized or utilized in the draft TAC document. We recommend that the results of this study be briefly summarized in the draft TAC document, and if appropriate, utilized for exposure assessment.

5. Although ambient air monitoring was conducted in the citrus growing regions of Tulare County, more chlorpyrifos is actually used on cotton crops than citrus crops. Therefore, the monitoring data used for exposure assessment in the draft TAC document might underestimate actual ambient exposures. It would be helpful if the draft TAC document addressed this issue of concern.

C. Acute Toxicity

1. We recommend that the discussion of the Kisicki *et al*. (1999) study in the draft TAC document be expanded to identify all of the strengths and weaknesses of using such a study as a replacement for the required and adequate animal data, to include an appropriate statistical analysis of the results (focusing on the power of the study to detect a negative effect), and to identify additional factors of uncertainty that should be considered in the risk appraisal. However, in our opinion the Kisicki *et al*. (1999) study should not be used for risk assessment for the following reasons:

    a. The mean absorbed doses for the Kisicki *et al*. (1999) human study ranged from 30 to 36 percent of the administered doses. This level of absorption of orally administered chlorpyrifos is about half that measured in the human and rodent studies discussed in the Toxicokinetics section(s) of the draft TAC document. Since the average value for an absorbed dose in the Kisicki *et al*. (1999) study (0.7 mg/kg) is used calculating "reference doses" and margins of exposure (MOEs) later in the draft document, possible reasons for this relatively low level of absorption should be discussed. Given the extreme range of absorbed dose levels (14 to 94 percent) there should be appropriate statistical adjustment (using the confidence intervals) to the value used to account for the uncertainty in the actual absorbed dose.

b. It is stated in Kisicki *et al*. (1999) that no "treatment-related signs or symptoms of chlorpyrifos toxicity" were observed. This is restated on page 54 of the draft TAC document. The Toxicology Summary for Chlorpyrifos (DPR, 2001) states that anorexia, diarrhea, nausea, vomiting, dizziness, dyspnea, and headache were reported, but none showed a dose-response. Since the dose range was only 4-fold (for absorbed dose), the lack of a dose-response is not surprising. If these symptoms are not treatment-related, their incidences in the treated groups should be similar to controls. These data should be presented and discussed in the draft TAC document. In particular, studies that claim to be "negative" should meet the same standards for study design (e.g., sample size, statistical power, scientific rigor) as studies that claim to be "positive."

c. The Kisicki *et al*. (1999) study did not present meaningful statistical analysis. Furthermore, the general design of human volunteer exposure studies limits the dosing levels to those that do not result in obvious clinical signs or serious toxicity. This is in contrast to studies using experimental animals where doses are employed that achieve frank toxicity from which a dose-response relationship may be established. This key difference in study design between an experimental study using animals and a volunteer study in humans is important to consider in risk assessment and the identification of no effect levels.

d. The study of Nolan *et al*. (1984) reported that plasma ChE activity was inhibited 85 percent at 12 to 24 hours following an oral dose of 0.5 mg/kg (0.35 mg/kg absorbed dose) in human volunteers.

2. The summary of the critical study used in the draft TAC document for assessing acute risks of plasma ChE inhibition (Mendrala and Brzak, 1998) is a rat study and is presented in the Metabolism section and not the Toxicology section. We recommend moving the discussion of the Mendrala and Brzak (1998) study into the Toxicology section for the proper context. In addition, a NOAEL of 0.5 mg/kg for the inhibition of plasma ChE was identified in this study. Therefore, we recommend expanding the justification for its selection as a critical study to allow comparison with the other toxicology studies, in particular the human study (Nolan *et al*., 1984) that reported an apparently lower NOAEL for the inhibition of plasma ChE in humans.

3. Because of the important limitations of the Kisicki *et al*. (1999) study including low and variable absorption, the fact that the most sensitive endpoint (plasma ChE inhibition) was not measured in the study and that the NOAEL identified in this study is larger than a dose associated with 85 percent inhibition of plasma ChE in a previous human study (Nolan *et al*., 1984), we consider the animal data to be more reliable and scientifically defensible for assessment of acute exposures to chlorpyrifos.

D. Chronic Toxicity

In reference to the discussion of McCollister *et al*. (1971) on page 27, we note that the female dogs exhibited a significant ($p<0.01$) decrease in RBC ChE activity at 24 months at the dose (0.1 mg/kg-day) selected in the draft TAC document as the NOAEL. We identify this latter dose as a lowest-observed-adverse-effect-level (LOAEL) and the next lower dose of 0.03 mg/kg-day as the NOAEL based on this statistically significant level of inhibition of RBC ChE activity. Our analysis of the data is in agreement with U.S EPA, which also selected 0.03 mg/kg-day as a NOAEL for the inhibition of RBC ChE activity from the same study in its chlorpyrifos risk assessment (U.S. EPA, 2000). We recommend adopting the dose level of 0.03 mg/kg-day as the NOAEL for use in the risk assessment. Since this is the critical study selected for the development of the chronic reference exposure level (REL) and for MOE calculations, selection of this lower NOAEL requires that MOEs and RELs be changed accordingly.

E. Reproductive Toxicity

1. Additional discussion should be added on page 32 of the draft TAC document in the presentation of Breslin *et al*. (1991). In the draft TAC document, it is stated "that F_1 pup weights were significantly reduced at the high dose." It should be added that pup survival was also reduced at the high dose level. Later in the discussion, it is stated that high dose pups were "cold to the touch and unresponsive, indicating a lack of maternal interaction with the pups." Hypothermia and reduced movement are also consistent with cholinergic toxicity in the pups. Therefore, the data showing that high dose dams failed to nurture their pups should be presented in order to support this conclusion.

2. The same study identifies a parental and pup NOAEL of 1.0 mg/kg-day. This was based on inhibition of brain ChE activity and other effects in parental animals and reduced F_1 pup weights at the highest dose level of 5.0 mg/kg-day. We would select the dose of 0.1 mg/kg-day as the NOAEL for both pups and dams based on statistically significant inhibition of plasma and RBC ChE activity at the next higher dose level of 1.0 mg/kg-day (data shown in Table 12 of the draft TAC document).

F. Developmental Toxicity

1. In the discussion of Hoberman (1998), additional effects were noted in the Toxicology Summary for Chlorpyrifos (DPR, 2001) that were not mentioned in the draft TAC document. Specifically, high dose female pups at 66 days postpartum showed reduced dimensions of the parietal cortex and hippocampal gyrus. This is not discussed in the draft TAC document and this information should be added to the description of the study results. Also, this study is listed as "acceptable" in the Summary while it is characterized as "unacceptable" in the draft TAC document. This discrepancy should also be corrected.

2. In reference to the Hoberman (1998) study, the U.S. EPA Safety Factor Committee expressed concern over what it considered to be a toxicologically significant effect of chlorpyrifos on the developing brains of offspring at the mid dose level, a dose which also caused statistically significant brain ChE inhibition in the dams and not the pups (U.S. EPA, 1999). It should be noted that the low dose level in the Hoberman (1998) study was not evaluated by the study investigators for the same developmental toxicity endpoints that were evaluated for the mid and high dose levels. However, at the request of U.S. EPA a similar analysis of the low-dose pups is underway. The Committee suggested that the inability to identify a developmental NOAEL from this study represents significant uncertainty that bears on the selection of an additional safety factor under the federal Food Quality Protection Act (FQPA). This is an important scientific issue that should be addressed and accounted for in the TAC document.

3. Additional information would be helpful regarding the description on page 35, third paragraph of the draft TAC of the study by Rubin *et al*. (1987). Specifically, the strain of rat used for the study should be noted. Additionally, we are unable to determine if this is the same study as "342-695-153117" found in the Toxicology Summary on Chlorpyrifos (DPR, 2001). If it is indeed the same study, we would consider the "slight increase" (as reported in the Toxicology Summary) in early resorptions/increased post implantation loss at 15 mg/kg an adverse effect, and would therefore identify the next lower dose of 2.5 mg/kg-day as the developmental NOAEL. We recommend a more detailed discussion of this study and, if appropriate, why the observed effect was not considered adverse. We note that this study was "acceptable" under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (DPR, 2001).

4. We recommend providing additional discussion to the presentation of the Mattson *et al*. (1998) study. For example, the data on brain ChE activity (Tables 13 and 14) showing an increased inhibition of ChE activity in dams versus pups at the high dose, which roughly correlates with the level of chemical in the blood of these two groups, should be pointed out in the TAC document. Additionally, relatively high levels of chlorpyrifos are found in the milk versus the pup's blood, suggesting that relatively little of the parent compound found in the mother's milk reaches the blood and brain in pups. It should also be mentioned that the level of chlorpyrifos detected in the blood of female pups from the high dose group was much lower than that found in male pups on gestation day 20 and post-natal day one. Including this additional discussion serves to support the conclusions reached in the draft TAC document and to more fully utilize the data from the paper.

5. Hanley *et al*. (1987) administered the metabolite 3,5,6-trichloro-2-pyridinol (3,5,6-TCP) to pregnant rabbits and observed adverse developmental effects at the two high dose groups of 100 and 250 mg/kg-day, but not at 25 mg/kg-day. Therefore, the developmental NOAEL from this study is 25 mg/kg-day. We consider this to be an indication of a possible adverse developmental outcome. Accordingly, we suggest including additional discussion of this potential outcome in the Risk Appraisal section.

6. Additional description needs to be added to the Moser and Padilla (1998) study where 17- and 70-day old rats were dosed orally with chlorpyrifos. Specifically, it is important to know if the two doses of 15 mg/kg for the 17-day old and 80 mg/kg for the 70-day old rats exert a similar effect on ChE inhibition. Upon correspondence with the author of the study (Moser, 2001) it appears that the same amount of maximal brain ChE inhibition was produced in the two age groups at the different doses, which provided the basis for dose selection. In other words, the 15 mg/kg in pups was as effective as 80 mg/kg in adults in inhibiting brain ChE activity. The study authors' rationale for the choice of these doses should be included in the description of the study. This type of information is needed to arrive at any conclusions from the findings of this research. We recommend adding this information to the discussion of the study.

7. Age and gender-related differences in Long-Evans rats were observed in the study by Moser *et al*. (1998). Specifically, age-related differences in the results of the functional observed battery (FOB) were reported in females, but not in males; the difference being influenced by the amount of time post-dose (3.5 versus 6.5 hours) the FOB was performed. No details were provided in the text regarding which FOB parameters were age-sensitive. In any event, the differences were explained in terms of age-related differences in detoxification enzymes, citing a reference by Chanda *et al*. (1997). We find this explanation confusing since the reference cited (Chanda *et al*., 1997) was a study in male rats, while the differences in FOB sensitivity were observed in female rats.

We recommend providing additional justification/explanation for the conclusions provided in the draft TAC document.

8. A pivotal study published subsequent to the preparation of this draft TAC document by Jett *et al*. (2001) in Long-Evans rats reports alteration in the cognitive function in juvenile rats as measured in the Morris swim test through a mechanism not involving the inhibition of brain ChE activity. The responses observed in this study appear to be classic effects on cognition as evidenced by a diminished spatial navigation ability in rats administered chlorpyrifos at 7 mg/kg subcutaneously on postnatal days 7, 11 and 15 (i.e., pre-weaning study) and in rats administered chlorpyrifos at 0.3 mg/kg and 7 mg/kg subcutaneously on postnatal days 22 and 26 (i.e., post-weaning study) and tested on postnatal days 24 through 28. However, the lack of brain ChE inhibition observed in this study may be a result of the timing when the animals were tested (3 hours, 24 hours or 5 days after subcutaneous injection). Other limitations of the study include lack of analysis of variance of group effect by time to evaluate rate of learning and fewer animals in the post-weaning study. Testing on the first day (day 24) revealed a significant difference between the controls and 0.3 mg/kg as well as the 7 mg/kg group in the pre-weaning study and it appears that in the post-weaning study, the day effect was not significant because both treatment groups had stable latencies higher than the controls throughout the study. Despite the variability associated with the test, it appears that even at 0.3 mg/kg the animals are demonstrating an impaired ability to learn the spatial navigation over the entire testing period. The probe test (built-in confirmatory test within the experimental protocol) conducted one day after the last day of testing demonstrated a significant difference between both treatment groups (7 mg/kg and 0.3 mg/kg) and control in the post-weaning study and a significant difference only at the 7 mg/kg in the pre-weaning study. These findings indicate either a loss in memory (i.e., recall), or diminished learning, either of which would constitute an effect on cognition. This study needs to be discussed in this draft TAC document, especially since it is the only study that, despite its limitations, is suggestive of cognitive impairment in weanling rats at low dose levels. The authors of the study suggest that alteration in cognitive function in juvenile rats is an important functional correlate of the cellular and molecular effects (cholinesterase inhibition and structural effects) of chlorpyrifos in the immature brain.

   We consider the dose of 0.3 mg/kg a possible LOAEL based on the cognitive effects observed at this dose level. At this point, however, we are concerned about the statistical analysis applied to the data and the reproducibility of the results. Accordingly, at this time we consider the results of this study to be qualitative, adding to the weight of evidence that suggests a differential susceptibility of infants and children to chlorpyrifos. This topic is discussed in greater detail below. We have requested additional information from the study authors (Jett *et al*.) for a more detailed evaluation.

G. Hazard Identification/Dose Response Assessment

1. Acute Toxicity

Several points raised earlier are repeated here in less detail:

a. We recommend that the discrepancy for the different NOAELs from the Kisicki *et al*. (1999) study reported in the draft TAC document and the Summary of Toxicology Data (DPR, 2001) be corrected.

b. We recommend that a more detailed discussion of the critical study of Mendrala and Brzak (1998) be included in the draft TAC document along with a justification for the selection of this as the critical study for assessing acute risk of plasma ChE inhibition.

c. We recommend that the impact of selecting a NOAEL (0.5 mg/kg) from a study in rats (Mendrala and Brzak, 1988) that would result in the potential for about 85 percent inhibition of human plasma ChE (at 0.5 mg/kg from Nolan *et al*, 1984), assuming comparable absorption of chlorpyrifos in the rats and humans, be discussed in this section.

d. A comparison of the suitability of these two studies to other "acute" studies, such as the developmental study of Hoberman (1998), which identifies a maternal NOAEL of 0.3 mg/kg-day for inhibition of maternal brain ChE should be presented in this section.

2. Chronic Toxicity

As we mentioned earlier, we would select the NOAEL of 0.03 mg/kg-day for inhibition of RBC cholinesterase in dogs (McCollister *et al*., 1971) as the key endpoint for the evaluation of chronic exposures to chlorpyrifos. The use of this NOAEL would alter the results of the MOE and REL calculations.

In the same study, 0.01 mg/kg-day is identified as the NOAEL for inhibition of plasma ChE (page 27), however, later in the document (Hazard Identification section, page 57 and the Risk Characterization section, Table 22, page 63), the NOAEL is identified as 0.03 mg/kg-day. It is assumed that 0.03 mg/kg-day was selected in the draft TAC document as the NOAEL for the inhibition of plasma ChE since that is the value used in the MOE calculations. This apparent discrepancy should be corrected or explained. Note also that we identify the dose of 0.01 mg/kg-day as the NOAEL for the inhibition of plasma ChE

based on a significant inhibition of the enzyme at the next higher dose of 0.03 mg/kg-day. The use of this NOAEL would also alter the results of the MOE and REL calculations.

H. Hazard Identification/Human Exposure Assessment

As discussed in greater detail in our comments on the Exposure Assessment, we recommend adjusting the estimated Annual Absorbed Daily Doses to account for year-round exposure to airborne chlorpyrifos as a result of structural pest control applications. We also recommend estimating seasonal and chronic chlorpyrifos exposure for individuals residing in close proximity to orchards and/or other treated crops.

I. Risk Characterization

MOEs for acute and chronic exposures should be recalculated and presented based upon any changes in the selection of NOAELs as described above.

J. Risk Appraisal

1. Acute Toxicity

Due to the limitations of the Kisicki *et al*. (1999) study: low and highly variable absorption and no measurement of plasma cholinesterase inhibition, we strongly recommend that the authors of the draft TAC document consider selecting a different study or studies for the evaluation of acute exposures to chlorpyrifos.

2. Increased Sensitivity and Differential Susceptibility of Infants and Children

In discussing the FQPA safety factor of 10-fold for the protection of infants and children, it is stated in the draft TAC document (last paragraph on page 77) "There is no evidence that infants or small children are more susceptible to the toxicity of chlorpyrifos than adults." We disagree with this statement for the following reasons:

a. Several studies are available in the open literature that demonstrate that young rats are more susceptible to chlorpyrifos toxicity than adults. This includes differences at the LD_{50} as well as more subtle sensitivities such as ChE inhibition (brain and blood), behavioral changes and effects on cognition at lower dose levels. Some of these studies are cited on page 77 of the draft TAC document while others (Dam *et al*., 2000, Jett *et al*., 2001, Whitney *et al*., 1995; Padilla *et al*., 2000, Slotkin *et al*., 2001; Zheng *et al*., 2001) can be found in the references we provide at the end of this attachment. These results are not

b. necessarily at odds with the FIFRA guideline studies of reproductive and developmental toxicity summarized in the draft document, which typically show no differences in sensitivity between adult and younger animals. First, these non-guideline studies involved direct dosing of the neonates in contrast to developmental and developmental neurotoxicity guideline studies, where exposure of offspring is via the mother during gestation and lactation. Since direct exposure of human infants to chlorpyrifos via inhalation, ingestion and dermal contact is possible, direct dosing of neonate rats provides data that is relevant to this risk assessment. Secondly, endpoints in the non-guideline studies included neurobehavioral testing (Dam *et al*., 2000; Jett *et al*., 2001, Moser *et al*., 1998), which is not typically performed in guideline reproductive toxicity testing, where young animals are dosed directly through the feed.

c. The developmental neurotoxicity study discussed on page 35 of the draft TAC document and on page 5 of this appendix has raised concern that chlorpyrifos may inhibit brain development at dose levels causing only cholinesterase inhibition in dams. This finding was cited by U.S. EPA as part of its rational for retaining a 10-fold FQPA safety factor for its chlorpyrifos risk assessment. Developmental effects were also seen following exposure to the chlorpyrifos metabolite, TCP (page 30 of the draft TAC document). Developmental effects (central nervous system and lung malformations) were observed at a lower dose (100 mg/kg-day) than maternal effects (250 mg/kg-day) such as minor body weight decrement). Furthermore, the recent study by Jett *et al*. (2001) is suggestive of cognitive impairment in juvenile rats at low dose levels.

d. We find that the preponderance of evidence indicates that young rats are more sensitive to chlorpyrifos toxicity than adults. Comparable studies cannot be conducted in humans. Furthermore, the increased sensitivity in young rats is associated with decreased activities of the detoxification enzymes, A-esterase (Atterberry *et al*., 1997; Karanth and Pope, 2000; Moser *et al*., 1998; Padilla *et al*., 2000; Li *et al*., 1997; Mortensen *et al*., 1996) and carboxylesterase (Atterberry *et al*., 1997; Karanth and Pope, 2000; Moser *et al*., 1998; Padilla *et al*., 2000). In young rats, A-esterase activity in plasma ranged from 10 to 30-fold less than that of older animals. In liver, the range was from 42 percent less to 30-fold less in young rats compared to older animals. Two human studies are available, both of which detected an approximate 3-fold lower level of A-esterase activity in infants compared to older subjects (Augustinsson and Barr, 1963; Ecobichon and Stephens, 1973). Carboxylesterase activity in neonate rat liver ranged from 2.5 to 10-fold less than that in liver from older animals. No human data on age-related changes in carboxylesterase levels were located. This

e. represents another possible data gap for considering the potential increased sensitivity of human infants to chlorpyrifos.

f. In conclusion, we consider the scientific evidence for increased sensitivity and differential susceptibility of infants and children to be sufficiently robust so that it should be considered when evaluating MOEs and when calculating RELs. Our conclusion is based on the following lines of evidence. Young animals exhibit increased sensitivity to chlorpyrifos as reflected in acute lethality (approximate 9-fold difference in LD_{10}s), ChE inhibition (3 to 5-fold difference in RBC, plasma and brain ChE inhibition) and behavioral effects (approximately a 5-fold difference in FOB performance). Furthermore, young animals are differentially susceptible to the effects of chlorpyrifos as evidenced by the effects of the chemical on the developing brain and on cognition. The susceptibility is not differentially quantifiable because these effects are seen only in juvenile animals and have no adult counterpart and because no NOAEL has been set for the effects of chlorpyrifos on the developing brain, thus no quantitative comparison can be made between maternal and developmental NOAELs. It is important to note that the effects observed on brain development may be occurring at exposures that do not significantly inhibit brain ChE and therefore the effects may be occurring through a mechanism different from ChE inhibition. Levels of detoxification enzymes are significantly lower in juvenile rats compared to adults; A-esterase activity is approximately 30-fold less and carboxylesterase activity is approximately 10-fold less in juvenile rats compared to adult rats. In two human studies, infants exhibit an approximate 3-fold lower level of A-esterase activity compared to older subjects. No human data on age-related differences in either chlorpyrifos sensitivity or carboxylesterase levels have been found in the literature. OEHHA feels that due to the defined quantitative differences is sensitivity of juvenile rats, the documented differential susceptibility of juvenile rats and the differences in detoxification enzymes, an additional uncertainty factor of 10-fold is justified and should be applied to the calculation of RELs for chlorpyrifos.

e. U.S. EPA has retained the 10-fold FQPA safety factor[1] for the protection of infants and children based on its concerns regarding the potential consequences of chlorpyrifos exposure to infants and children. The scientific bases for retaining the 10-fold factor are: 1) increased sensitivity of juvenile rats to ChE inhibition following single and repeated dosing with chlorpyrifos as reported in literature studies, 2) qualitative differences in maternal and developmental responses in the developmental neurotoxicity study, 3) no offspring NOAEL for structural alterations in brain development has been demonstrated in the developmental neurotoxicity study, however, the lowest dose has not yet been evaluated, and 4) a suggestion that cholinesterase inhibition may not be essential for effects on brain development (U.S. EPA 1999, 2000b,c). Therefore, our conclusion, to apply an additional 10-fold uncertainty factor in the calculation of RELs, is in agreement with U.S. EPA.

3. Cumulative Exposure

In addition to the differential susceptibility and increased sensitivity of infants and children to the toxic effects of chlorpyrifos, there is the potential for cumulative exposure with other ChE inhibitors as well as aggregate exposure to other sources of chlorpyrifos, such as structural fumigations and residential applications. We acknowledge the inclusion of these issues in the draft TAC document. However, there is no discussion of the implications of these considerations in interpreting the risk estimates from exposure to airborne residues of chlorpyrifos. Specifically, we recommend including a discussion of, in the context of these FQPA issues, their impact on the establishment of a suitable MOE.

K. Reference Exposure Levels

1. A total of 21 different RELs are calculated in the body of this section. While we applaud the attempt to include as many different exposed populations as possible, we find this number of RELs (called RfCs in the text) to be potentially confusing to risk managers and other readers of this document. Selecting and presenting a single value that is

[1] OEHHA notes that the FQPA requires an additional safety factor of 10-fold for the protection of infants and children. The safety factor is removed or reduced by U.S. EPA when there is sufficient evidence demonstrating that infants and children are no more sensitive and/or susceptible than adults to the toxic effects of a particular chemical. The safety factor is retained when there is no data available assessing the toxicity to infants and children (default case). The safety factor is also retained when infants and children have been shown to be more sensitive and/or susceptible. The latter situation applies in the case of chlorpyrifos as there is ample evidence demonstrating an increased sensitivity and susceptibility of infants and children to the toxic effects of the chemical.

2. appropriately health protective for the most susceptible subpopulation for each exposure duration would be easier to understand.

3. The table of contents identifies this section as "Ambient Air Reference Concentrations" and in the in the body of the document the section is titled "Reference Exposure Levels" while the text of the sections discusses "Reference Air Concentrations." We suggest utilizing one term (we suggest using "reference exposure level").

4. If different and/or additional NOAELs are adopted for evaluating acute and chronic exposures and/or additional uncertainty factors are applied to account for an increased sensitivity and differential susceptibility of infants and children, the corresponding REL(s) will need to be recalculated.

5. We recommend applying an additional uncertainty factor of ten to the REL calculations to account for the increased sensitivity and differential susceptibility of infants and children. This means that for intra-species variability in the human population we recommend a 100-fold uncertainty factor, which is a combination of the 10-fold standard factor with a second 10-fold factor the differences between adults and children.

L. Specific Comments

Part B

Page 8, second paragraph; specify the application rate.

Part C

Page 3, first paragraph, ADD is used as an acronym for Average Daily Dose in this Summary while in the Exposure Assessment document (Part B) it is used for Absorbed Daily Dose. The documents should be modified to be consistent.

Page 3, a number of values for ADDs do not agree with those in the Exposure Assessment document; these inconsistencies should be corrected.

Page 7, last paragraph, "No nascent chlorpyrifos was found in urine." It is unclear what is meant by "nascent" chlorpyrifos and this should be defined in the TAC document.

Page 14, last paragraph, state what the inhibitor is.

Page 18, first paragraph, the volunteers were fasted overnight, not for 48 hours. This should be corrected.

Page 20, Table 4, the text should state that the dermal rabbit study is toxicity category III rather than IV.

Page 22, last paragraph, the dermal rat study needs to be referenced.

Page 23, last paragraph, "Examination of the data in Table 6" should read Table 5.

Page 27, referring to the statement in reference to the study of McCollister *et al*. (1971): "However, the author of a detailed, statistical re-analysis of the data concluded the 2-year NOEL for inhibition of RBC cholinesterase was actually 0.1 mg/kg-day (Mattsson *et al*., 2001)," the reanalysis was actually for a one year time period. Accordingly, the above quoted sentence should read "1-year NOEL." Page 33, Table 12, the title of the table states that cholinesterase inhibition is being presented while the sub-headings are labeled (ChE) activity. This is confusing and potentially misleading. Since ChE inhibition is being presented, the sub-heading should be corrected.

Page 42, second paragraph, it states that the maternal "NOEL" for red blood cell inhibition in mice was 0.1 mg/kg-day, but on page 39 (second paragraph) that "NOEL" is given as 1.0 mg/kg-day. This error should be corrected.

Page 42, second paragraph, "Indirect evidence suggested that chlorpyrifos can partition into milk." It is not clear why the evidence is considered to be "indirect," since chlorpyrifos was measured in the milk. This apparent error should be clarified.

Page 45, second paragraph, in reference to the statements "Age-related differences in brain AChE activity" and "observed difference in the levels of brain cholinesterase activity," it would be helpful if these effects were described in quantitative terms, especially with regard to the relationship between age and ChE inhibition.

Page 48, second paragraph, please provide the route of exposure in text.

Page 53, second paragraph, last sentence 1.0 mg/kg should read 5.0 mg/kg.

Page 58, last paragraph in reference to the statement "The calculated ADDs for people residing in towns ranged from 0.04 µg/kg-day for adult females at the University of California Lingrove station to 0.25 µg/kg-day for children residing in Lindsay (Table 19)." This sentence should read "0.05 µg/kg-day for adult females at Lingrove to 0.27 µg/kg-day for children residing in Strathmore."

Page 63, Table 22, the "NOEL" for plasma ChE in the McCollister *et al*. (1971) study is 0.01 mg/kg-day, not 0.03 mg/kg-day and should be corrected.

Page 66, second paragraph, "They noted that the ratio of the apparent Michaelis constant for desulfuration to the maximum velocity (V_{max}) for the dearylation reaction ($Km_{app}s/V_{max}$) was three times greater in males than in females." This is not correct. Ma and Chambers (1994) show that "the clearance factor for dearylation was about three-fold greater in males than in females." This should be corrected.

Page 69, third paragraph, "Indeed, the whole thesis that there are age-related differences in human susceptibility to the toxicity of chlorpyrifos rests on rodent data because such a relationship has never been shown in humans." This is not surprising, since testing such a hypothesis would require dosing children and infants with chlorpyrifos. We recommend deleting this statement.

Page 75, second paragraph, "When the NOEL is derived from a human study, an MOE is considered adequate." This should this read: "… an MOE of 100."

Page 77, top paragraph, the descriptions of these studies are vague and not very informative. It would be useful to describe the effects quantitatively in the text.

M. References

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* an asterisk denotes additional toxicology-related references not cited in these comments but suggested for inclusion in the TAC document

Office of Environmental Health Hazard Assessment's Draft Findings On the Health Effects of Chlorpyrifos

Pursuant to Food and Agricultural Code Sections 14022 and 14023, the Office of Environmental Health Hazard Assessment (OEHHA) of the California Environmental Protection Agency has reviewed and commented on the Department of Pesticide Regulation's (DPR) draft documents on the evaluation of human health risk associated with potential exposure to chlorpyrifos for consideration of the identification of chlorpyrifos as a toxic air contaminant (TAC). In addition, as part of its statutory responsibility, OEHHA has prepared these findings on the health effects of chlorpyrifos which are to be included as part of DPR's TAC document.

Environmental Fate and Exposure

1. Chlorpyrifos is a broad-spectrum insecticide used to control a variety of pests on a wide variety of agricultural commodities, structures and animals. Following an application chlorpyrifos volatilizes from leaf and soil surfaces, and may be transformed by various chemical processes as well as plant and animal metabolism. The primary transformation product in the environment is 3,5,6-trichloro-2-pyridinol (TCP). In soil, TCP is further degraded. Chlorpyrifos oxon and desethyl chlorpyrifos are minor transformation products found in the environment. Humans, animals and insects metabolize chlorpyrifos to the oxon, which is the form of the chemical responsible for its cholinergic toxicity.

2. Ambient air monitoring data for chlorpyrifos and its oxon are available from four towns in citrus growing regions of Tulare County: Lindsay, Exeter, Strathmore, and Lindcove. The monitoring was conducted from May 28 through June 30, 1996. Values less than the limit of quantitation of 9.4 ng/m^3 were not reported. These monitoring data were used in the TAC document for estimation of acute, seasonal and chronic human exposure to chlorpyrifos in ambient air and also used by OEHHA in preparing these findings.

3. Air concentrations of chlorpyrifos and its oxon during and after an application on an orange grove were also measured and the data used in the TAC document for estimating human exposure at application sites. Residential applications (lawn and structural) of chlorpyrifos were also monitored and reported, but these data were not used for human dose estimation.

4. Exposure values presented in the TAC document were based on the sum of the air concentrations of chlorpyrifos plus chlorpyrifos oxon and estimated as follows: a) average daily doses were calculated for acute exposures in ambient air based on the sum of the 95^{th} percentile air concentration of chlorpyrifos and chlorpyrifos oxon for all locations (see findings number 2 and 3); b) seasonal average daily doses were calculated for seasonal exposures for each site from the average air concentration at the site; and c) annual average daily doses, based on a four-month annual use period, were calculated for chronic exposures. Seasonal and chronic dose estimates were calculated from ambient air concentrations only and not for individuals living adjacent to an application site. Human doses were estimated for adult men, adult women and for one to six-year old children and were based on generally accepted default values for body weights and

breathing rates. OEHHA also used these exposure estimates in the preparation of its findings.

5. Human exposure to atmospheric chlorpyrifos can occur by both inhalation and dermal routes, but the predominant exposure route for systemic doses is inhalation. Inhalation uptake was assumed in the TAC document to be 100 percent for these estimates, based on the physical properties of chlorpyrifos. Dermal uptake of chlorpyrifos has not been quantitatively estimated in these studies but it is expected to provide less than 1 percent of the systemic dose received by inhalation.

Health Effects Studies

Humans

6. Numerous reports of pesticide illness involving chlorpyrifos have been reported over the past several years. Between 1982 and 1995, a total of 786 incidents were reported associated with the use of chlorpyrifos as the sole active ingredient. Of these cases, 146 were incidents of localized dermal and eye irritation (104 probable and 42 possible associations) and 640 cases were associated with systemic effects (333 probable cases and 307 possible cases). During the same time period, chlorpyrifos in combination with other active ingredients was associated with 89 episodes of local irritation (31 probable and 58 possible) and 640 cases of systemic toxicity (386 probable and 254 possible cases). Most of the cases were multiple exposures to agricultural workers as a result of equipment failure. Most non-occupational incidents were premature re-entry into treated areas.

7. Human volunteers (six/sex/dose) were administered chlorpyrifos in gel capsules at nominal doses of 0, 0.5, 1.0, or 2.0 mg/kg and were examined for up to 168 hours post-dosing (Kisicki et al., 1999). Absorption ranged from 19 to 94 percent, with a mean absorption of 34.7 percent. One female high-dose volunteer had red blood cell (RBC) cholinesterase (ChE) inhibited by 30 percent. Her absorbed dose was determined to be 1.7 mg/kg, more than twice the mean value of 0.7 mg/kg. An absorbed dose no-observed-adverse-effect level (NOAEL) of 0.7 mg/kg was identified based on clinical signs and symptoms. In a single dose study in six adult men volunteers (Nolan, 1984), plasma ChE was inhibited by 85 percent at 12 to 23 hours following exposure to a nominal dose of 0.5 mg/kg. Absorption was estimated at 70 percent in this study, yielding an absorbed dose of 0.35 mg/kg.

8. One subchronic oral exposure study in adult men is available (Coulston et al., 1972). Volunteers (four/dose) were exposed orally to chlorpyrifos tablets at dose levels of 0, 0.014, 0.03, or 0.1 mg/kg-day for up to 28 days. A NOAEL of 0.03 mg/kg-day was established for the inhibition of plasma ChE.

Animals

9. The acute toxicity of chlorpyrifos has been evaluated in a variety of animal species including rats, guinea pigs, rabbits, dogs and sheep. Signs of acute intoxication with chlorpyrifos are cholinergic in nature and consist of watery eyes, loose stools, vomiting, rough coats, labored breathing and tremors. Oral LD_{50}s range from 69 mg/kg in the rat to 2,000 mg/kg in the rabbit. From one acute study (Mendrala and Brzak, 1998), NOAELs for inhibition of brain and plasma ChE were identified as 5 and 0.5 mg/kg, respectively. A NOAEL for inhibition of RBC ChE of 1 mg/kg-day was identified in a four-day oral exposure study (Calhoun and Johnson, 1998). Chlorpyrifos is also a weak dermal irritant in the rabbit.

10. Subchronic toxicity studies in laboratory animals provide information on adverse effects following dietary, dermal and inhalation exposure. In dogs, dietary exposure to levels of 8 mg/kg-day and greater led to gross cholinergic signs of loose stools, vomiting, labored breathing and tremors. The NOAELs identified from subchronic studies in rats (28 to 90 days in duration) are fairly consistent. From these rat studies, NOAELs of 1 mg/kg-day for cholinergic signs and inhibition of brain ChE and 0.1 mg/kg-day for the inhibition of RBC and plasma ChE were identified.

11. Seven chronic feeding studies are available for chlorpyrifos, three in rats, two in mice, one in dogs and one in the rhesus monkeys. No histopathological changes associated with chlorpyrifos exposure were noted in any of the studies. The primary exposure-related effects observed were inhibition of brain, RBC, and plasma ChE activities. From the dog study (McCollister et al., 1971), NOAELs of 1.0, 0.1 and 0.03 mg/kg-day were identified in the TAC document for the inhibition of brain, RBC, and plasma ChE, respectively.

13. OEHHA identifies the dose of 0.01 mg/kg-day as the NOAEL for the inhibition of plasma ChE based on a significant inhibition of the enzyme at the next higher dose of 0.03 mg/kg-day in the dog study (McCollister et al., 1971). We also identify 0.1 mg/kg-day as a lowest-observed-adverse-effect level (LOAEL) for the inhibition of RBC ChE and select the next lower dose of 0.03 mg/kg-day as the NOAEL based on a statistically significant level of RBC ChE inhibition in female dogs at the LOAEL. This is in agreement with U.S. Environmental Protection Agency's (U.S. EPA) analysis of the same data (U.S EPA, 2000; Toxicology Chapter for Chlorpyrifos).

14. There is no evidence of oncogenicity in any of the oral studies with chlorpyrifos. No long-term study via inhalation is available for chlorpyrifos.

15. Five reproductive toxicity studies are available in Sprague-Dawley rats, including two two-generation feeding studies. There are two single generation studies and one single-dose study in lactating dams. No effects on reproduction were observed at doses lower than those resulting in maternal toxicity. In the TAC document a parental NOAEL of 1.0 mg/kg-day is identified based on inhibition of brain ChE activity and histological lesions of the adrenal gland (vacuolation of the cells of the zona fasiculata) at the next higher dose of 5.0 mg/kg-day from one of the two-generation studies (Breslin et al.,

1991). The reproductive NOAEL was identified to be 1.0 mg/kg-day based on reduced pup weights and survival at the next higher dose of 5.0 mg/kg-day. From the same study, OEHHA identifies 0.1 mg/kg-day as the parental NOAEL based on inhibition of plasma and RBC ChE at the next higher dose of 1.0 mg/kg-day.

16. Several developmental toxicity studies in rats, mice and rabbits are available for chlorpyrifos. Increased post-implantation loss was noted in one rat study (but not in another) at the highest dose level tested (15 mg/kg-day). In mice, at higher dose levels (25 mg/kg-day), minor skeletal variations, delayed ossification and reduced fetal weight and length were observed; similar effects were also observed in rabbits (140 mg/kg-day). In these studies, maternal effects such as decreased body weights (described as minor) and food consumption (magnitude not provided) and increased mortality (in mice – magnitude not provided) were also observed. Maternal effects were observed at doses equal to or less than those associated with developmental effects.

17. A number of neurotoxicity studies have been performed in the rat. In the classic delayed-neuropathy study used for registration, chlorpyrifos did not cause delayed neuropathy in hens at single doses of up to 110 mg/kg. In more recent single dose studies, oral exposure of hens to doses of 60 to 150 mg/kg caused 50 to 87 percent inhibition of neurotoxic esterase (NTE) four to six days after exposure. Delayed neurotoxicity was reported at 60 to 90 mg/kg, which are four to six times the LD_{50} and required aggressive antidotal treatment. In rats, chlorpyrifos did not inhibit NTE at single doses up to 100 mg/kg and there was no evidence of histopathology at doses of up to 15 mg/kg-day for 13 weeks. Except under extreme conditions (attempted suicides), chlorpyrifos is not expected to cause organophosphate-induced delayed neuropathy.

18. Developmental neurotoxicity studies with chlorpyrifos have demonstrated a number of developmental effects associated with chlorpyrifos exposure. In the principal rat developmental neurotoxicity study (Hoberman, 1998), delayed alterations in brain development were observed in the offspring of exposed dams. Pups in the two highest dose groups (1 and 5 mg/kg-day) displayed significant dose-related reduced dimensions of the parietal cortex and hippocampal gyrus. A NOAEL has not been established for the developmental toxicity observed in the pups in this study because the study investigators have not yet analyzed the morphometric data at the low dose. In the highest dose group, pups also exhibited decreased body weight gain and food consumption, reductions in viability, delays in development, and decreased brain weight. Significant decreases in maternal body weights and signs of cholinergic toxicity (tremors and fasciculations) were observed in the dams at the highest dose. Statistically significant inhibition of RBC and plasma ChE was observed in the dams at all three doses; statistically significant inhibition of brain ChE was observed in the dams at the two highest doses. In the 1 mg/kg-day group (mid-dose), the only maternal effect was brain, plasma and RBC ChE inhibition. The maternal LOAEL was established as the lowest dose, 0.3 mg/kg-day, based on the inhibition of plasma and RBC ChE. The results of this study demonstrate a clear qualitative difference between the response of the fetus and of the adults to chlorpyrifos exposure.

19. Results of neurobehavioral studies are consistent with the morphological and biochemical effects of chlorpyrifos. Impairment of cognitive function in both adult and juvenile rats has been demonstrated following moderate to high level chlorpyrifos exposures. In a recent study (Jett et al., 2001), low doses of chlorpyrifos were demonstrated to alter cognitive function in juvenile rats as measured in the Morris swim test. The responses in the study appeared to be classic effects on cognition as evidenced by diminished spatial navigation ability. The lowest administered dose, 0.3 mg/kg, was identified as a LOAEL. It is suggested that these effects are independent of ChE inhibition as no significant inhibition of regional brain ChE was measured at any of the time points studied at this dose. Muscarinic receptor density also remained unaffected. The findings of this recent study underscore the concerns regarding the unique susceptibility of the fetus or neonate to potential developmental effects of chlorpyrifos.

20. Most chlorpyrifos genotoxicity data are negative. No chromosomal effects were seen in rat lymphocytes *in vitro* or mice *in vivo.* No mutagenic activity in bacterial or mammalian systems with or without metabolic activation has been reported for chlorpyrifos. No DNA damage was reported in human embryo fibroblasts or rat primary hepatocytes *in vitro.* Genotoxicity was reported in yeast cells *in vitro.*

Differential Toxicity

21. Studies are available in the literature that, when considered together, demonstrate young animals respond to the ChE inhibitory effects of chlorpyrifos at lower doses than do adult animals. This quantitative difference in response is reflected in the LD_{10}, which is 15 mg/kg for neonates and 136 mg/kg for adult rats (Zheng et al., 2000). At doses ranging from 0.15 to 15 mg/kg, RBC and plasma ChE is inhibited in rat pups three to five-fold more than in adults (Moser and Padilla, 1998; Zheng et al., 2000). OEHHA notes that the Zheng et al., 2000 study demonstrates the special sensitivity of young animals to chlorpyrifos at relatively low doses (0.15 mg/kg). Muscarinic receptor down-regulation is also more extensive in rat pups versus adults following a single 15 mg/kg dose of chlorpyrifos (Moser and Padilla, 1998). Effects of chlorpyrifos exposure on performance in a functional observational battery (FOB) and on motor activity are seen at lowered doses in young animals versus adults (Moser and Padilla, 1998). In this study, similar results were observed in the FOB and on motor activity in juvenile rats (post natal day 17) exposed to single doses of 15 mg/kg as compared to adult rats exposed to 80 mg/kg chlorpyrifos. In addition to the well-documented effects of chlorpyrifos on the developing animal, there is evidence in animal toxicity studies that suggest the increased sensitivity of juvenile animals is due to a difference in detoxification enzymes. In experimental studies, we note that A-esterase enzymatic activity is approximately 30-fold less and carboxylesterase activity approximately 10-fold less in younger rats compared to adult animals (Karanth and Pope, 2000; Moser et al., 1998). In the two human studies available, A-esterase activity varied three-fold between infants and adults (Augustinsson and Barr, 1963; Ecobichon and Stevens, 1973). No human studies comparing the differential activities of carboxylesterase in children versus adults are available.

22. Age-related differential susceptibility has also been observed with the chlorpyrifos metabolite, TCP. A developmental NOAEL of 25 mg/kg-day was observed in New

Zealand White rabbits based on hydrocephaly and dilated cerebral ventricles in fetuses at the next higher dose of 100 mg/kg-day (Hanley et al., 1987). The dose of 100 mg/kg-day was identified as the maternal NOAEL, based on body weight decrements at the next higher dose of 250 mg/kg-day. There was no evidence of hydrocephaly and/or dilated cerebral ventricles in the maternal animals. This study provides quantitative and qualitative evidence for the increased sensitivity and susceptibility of young animals to the primary chlorpyrifos metabolite, TCP. Additionally, it is important to note that there is demonstrable human exposure to this metabolite, as it was found in 100 percent of the urine samples obtained from 416 children (new born to six years) in North and South Carolina who were exposed to chlorpyrifos.

23. It is important to note that the effects observed on brain development may be occurring at exposures that do not significantly inhibit brain ChE and therefore the effects may be occurring through a mechanism different from ChE inhibition. Reports available in the open literature provide mechanistic support for the observed effects on brain development in young animals (see finding number 22). Key cellular processes (DNA synthesis, cell to cell communication) that are necessary for normal brain development have been shown to be effected by chlorpyrifos.

24. OEHHA concludes that the use of 10 or 100 as the benchmark value for margins of exposure (MOEs) based on human and animal studies, respectively, are too low because young animals are differentially sensitive and susceptible to the toxic effects of chlorpyrifos compared to adults. We also conclude that an additional 10-fold uncertainty factor should be considered when evaluating MOEs or in calculating reference exposure levels (RELs) when evaluating risks to infants, children and women of childbearing age from chlorpyrifos exposure. Our conclusion is based on the following lines of evidence:

    a. Young animals exhibit increased sensitivity to chlorpyrifos as reflected in acute lethality (approximate 9-fold difference in LD_{10}s), ChE inhibition (3 to 5-fold difference in RBC, plasma and brain ChE inhibition) and behavioral effects (approximately a 5-fold difference in FOB performance).

    b. Young animals are differentially susceptible to the effects of chlorpyrifos as evidenced by the effects of the chemical on the developing brain and on cognition. The susceptibility is not differentially quantifiable because these effects are seen only in juvenile animals and have no adult counterpart and because no NOAEL has been set for the effects of chlorpyrifos on the developing brain. Therefore, no quantitative comparison can be made between maternal and developmental NOAELs.

    c. Levels of detoxification enzymes are significantly lower in juvenile rats compared to adults; A-esterase activity is approximately 30-fold less and carboxylesterase activity is approximately 10-fold less in juvenile rats compared to adult rats. In two human studies, infants exhibit an approximate 3-fold lower level of A-esterase activity compared to older subjects. No human data on age-related differences in either chlorpyrifos sensitivity or carboxylesterase levels have been found in the literature.

25. U.S. EPA retained the 10-fold Food Quality Protection Act (FQPA) safety factor[1] for the protection of infants and children in the calculation of its reference dose (U.S. EPA, 2000; HED Doc. No. 014077; Re-evaluation Report of the FQPA Safety Factor Committee). Our determination is consistent with U.S. EPA's approach in evaluating chlorpyrifos.

U.S. EPA based on the following considerations retained the 10-fold safety factor under FQPA:

a. Increased sensitivity following a single oral exposure to neonates was seen at substantially lower doses i.e., the new data by Zheng at al. (2000) demonstrated that this was not a high dose phenomenon.

b. A clear qualitative difference in response (i.e., susceptibility) between adult rats and their offspring was demonstrated in the developmental neurotoxicity study in rats.

c. Uncertainties on the mechanism of action on brain development (data suggesting that the inhibition of ChE may not be essential for adverse effects on brain development).

d. Uncertainties resulting from the lack of an offspring NOAEL in the DNT due to insufficient data on the toxicity endpoint of concern (i.e., structural alterations in brain development on day 66).

Basis, Potency, and Range of Health Risks to Humans

26. Human health risks for acute exposures to chlorpyrifos are estimated in the TAC document based on the absorbed-dose NOAEL of 0.7 mg/kg for cholinergic signs and symptoms in the human study of Kisicki et al. (1999) and on the oral NOAEL of 0.5 mg/kg for inhibition of plasma ChE activity in rats (Mendrala and Brzak, 1998).

27. OEHHA selects the Mendrala and Brzak (1998) study as the sole critical study for the evaluation of acute exposures to chlorpyrifos because of the limitations of the Kisicki et al. (1999) study (e.g., low and variable absorption and no measurement of plasma ChE activity). Therefore, we consider the animal data to be more reliable and scientifically defensible for risk assessment purposes. The NOAEL of 0.5 mg/kg-day identified in the rat study was based on significant inhibition (28 to 40 percent) of plasma ChE at the next higher dose of 1.0 mg/kg. We note that this is the only single dose animal study that measured ChE activity at the peak time of inhibition, three to six hours post-dosing. Although RBC ChE was not measured in this study, we assume it would be significantly

[1] Under FQPA, an additional safety factor of 10-fold is required for the protection of infants and children. U.S. EPA removes the safety factor only when there is sufficient evidence demonstrating that infants and children are no more sensitive and/or susceptible than adults to the toxic effects of a particular chemical. The safety factor is retained when there are no data available assessing the toxicity to infants and children (default case). The safety factor is also retained when infants and children have been shown to be more sensitive and/or susceptible. The latter situation applies in the case of chlorpyrifos as there is ample evidence demonstrating an increased sensitivity and susceptibility of infants and children to the toxic effects of the chemical.

inhibited at the dose of 1.0 mg/kg (at the three to six hour time point) based on dose-response information obtained from other studies. Accordingly, we consider the NOAEL of 0.5 mg/kg for plasma ChE inhibition to also be applicable for inhibition of RBC ChE.

28. Human health risks from seasonal exposure to chlorpyrifos are estimated in the TAC document based on NOAELs of 0.1, 0.1, and 1.0 mg/kg-day for the inhibition of plasma, RBC and brain ChE, respectively, identified from rat subchronic studies. Risks to human health from chronic exposure to chlorpyrifos are estimated in the TAC document based on NOAELs of 0.03, 0.1, and 1.0 mg/kg-day for the inhibition of plasma, RBC, and brain ChE, respectively, as observed in the dog study of McCollister et al. (1971).

29. For assessment of human health risks from seasonal exposure to chlorpyrifos, OEHHA selects the NOAEL of 0.1 mg/kg-day for the inhibition of plasma and RBC ChE identified from the subchronic toxicity studies in rats. For evaluation of chronic risks, OEHHA identifies the NOAEL of 0.01 mg/kg-day for plasma ChE inhibition from the dog study (McCollister et al., 1971). The NOAELs selection between the TAC document and OEHHA are summarized in Table 1.

30. In the TAC document, MOE calculations for acute human exposures were based on the NOAELs from both the animal and the human studies described in findings number 8, 10 and 27. For exposure to residents adjacent to an application site, all MOEs for adults were greater than 100, but the MOEs for children were less than 100. Acute MOEs for ambient exposure were all greater than 100 and ranged from 2,000 to 14,000. MOEs exceeding 10 when based on NOAELs from human studies and 100 when based on NOAELs from animal studies are generally considered by DPR to be sufficiently protective of human health.

31. MOEs for seasonal exposures to chlorpyrifos presented in the TAC document ranged from 1,000 to 50,000 depending upon the scenario and the endpoint evaluated. MOEs for chronic exposures ranged from 1,000 to 100,000 depending upon the scenario and the endpoint evaluated. MOEs exceeding 100 when based on NOAELs from animal studies are generally considered by DPR to be sufficiently protective of human health.

32. For acute exposures to residents living adjacent to application sites, OEHHA's MOE calculations (based only on the NOAEL from the rat study) range from 43 to 172. For ambient air exposures, MOEs range from 2,000 to 12,000. Therefore, OEHHA believes that residential exposures to chlorpyrifos adjacent to application sites present a public health concern.

33. MOEs calculated by OEHHA for seasonal exposures in children are 1,000 at three of the four monitored locations whereas all of the MOEs calculated by OEHHA for seasonal exposure in adults are greater than 1,000. MOEs calculated by OEHHA for chronic exposures to chlorpyrifos in ambient air are 300 or greater depending on the scenario, but for children, all MOEs are less than 1,000 (includes standard factors of ten for inter- and intra-species variation and an additional factor of ten for particular sensitivity and differential susceptibility of infants and children). Therefore, children's seasonal and chronic exposure to chlorpyrifos in ambient air presents a potential public health concern.

Table 1. Comparison of the NOAELs Selected by DPR and OEHHA for the Three Different Exposure Periods: Acute, Seasonal and Chronic

Exposure Duration	DPR NOAEL (endpoint)	OEHHA NOAEL (endpoint)
Acute	**0.7 mg/kg** [1] (cholinergic signs & symptoms in humans) **0.5 mg/kg**[2] (plasma ChE inhibition in rats)	**0.5 mg/kg**[2] (plasma and RBC ChE inhibition in rats)
Seasonal	**0.1 mg/kg-day**[3] (plasma and RBC ChE inhibition in rats) **1.0 mg/kg-day**[4] (brain ChE inhibition in rats)	**0.1 mg/kg-day**[3] (plasma and RBC ChE inhibition in rats)
Chronic	**0.03 mg/kg-day**[5] (plasma ChE inhibition in dogs) **0.1 mg/kg-day**[5] (RBC ChE inhibition in dogs) **1.0 mg/kg-day**[5] (brain ChE inhibition in dogs)	**0.01 mg/kg-day**[5] (plasma ChE inhibition in dogs)

1. Kisicki et al. (1999).
2. Mendrala and Brzak (1998).
3. Szabo et al. (1988); Breslin et al. (1991).
4. Szabo et al. (1988); Breslin et al. (1991); Shankar and Crissman (1993).
5. McCollister et al. (1971).

34. A total of 21 RELs are calculated in the TAC document for adults and children (aged one to six years) under acute, seasonal and chronic exposure scenarios for a number of different endpoints. Oral doses were converted to "human equivalent inhalation NOAELs" by dividing the oral "NOEL" by the age and gender-specific inhalation rate.

Acute, subchronic and chronic RELs were calculated by dividing the human equivalent inhalation NOAELs by an uncertainty factor of 100 if the NOAEL was from an animal study (ten for inter-species variability and ten for intra-species variability), or by ten if the NOAEL was derived from a human study (ten for intra-species variability). RELs were calculated from all NOAELs (or estimated NOAELs) used in the MOE calculations. The RELs were calculated from the NOAELs described in findings 27 and 29 by converting the oral NOAELs to inhalation NOAELs by dividing the oral NOAELs by the age and gender-specific inhalation rate. Acute RELs ranged from 7 $\mu g/m^3$ for children based on the plasma ChE inhibition endpoint to 389 $\mu g/m^3$ for adult women based on cholinergic signs and symptoms in humans. Seasonal RELs ranged from 2.3 $\mu g/m^3$ for children based on inhibition of plasma ChE in the rat to 56 $\mu g/m^3$ for adult women based on inhibition of brain ChE from in the rat. Chronic RELs ranged from 0.7 $\mu g/m^3$ for children based on inhibition of plasma ChE in a chronic dog study to 56 $\mu g/m^3$ based on inhibition of brain ChE in the same dog study.

35. OEHHA calculated a single REL for each exposure duration: acute, seasonal, and chronic by dividing the oral NOAEL (mg/kg-day) by the breathing rate (m^3/kg-day) and uncertainty factor (unitless). All NOAELs were derived from experimental studies in animals. Children's breathing rates were used for the calculations since children have higher breathing rate(s) per unit of body weight than do adults; hence, they experience the greatest exposure on a per-weight basis. Acute and seasonal RELs were calculated using the upper 95^{th} percentile breathing rate for children of 0.581 m^3/kg-day. The chronic REL was based on a child's mean breathing rate of 0.452 m^3/kg-day. The distribution of children's breathing rates is described in OEHHA's Technical Support Document for Exposure Assessment and Stochastic Analysis (September, 2000). Uncertainty factors of 1,000 were applied to the NOAELs in consideration of the variability between and within species (100) and an additional ten-fold uncertainty factor in consideration of the differential sensitivity and susceptibility of infants and children to chlorpyrifos toxicity. This results in RELs of 0.86, 0.17 and 0.02 $\mu g/m^3$ for acute, subchronic (seasonal) and chronic exposures, respectively. A comparison of the RELs calculated by DPR and OEHHA is shown in Table 2.

Other Relevant Findings

36. Although ambient air monitoring was conducted in the citrus growing regions of Tulare County, more chlorpyrifos is actually used on cotton crops than citrus crops. Therefore, the monitoring data used for exposure assessment in the TAC may underestimate actual ambient exposures.

Table 2. Comparison of the RELs Calculated by DPR[1] and OEHHA[2] for the Three Different Exposure Periods: Acute, Seasonal and Chronic

Exposure Duration receptor	DPR REL ($\mu g/m^3$)	OEHHA REL ($\mu g/m^3$)
Acute		
adult males	250[3], 18[4]	
adult females	389[3], 28[4]	
children	95[3], 74[4]	0.86[4]
Seasonal		
adult males	50[5], 5.0[6]	
adult females	56[5], 5.6[6]	
children	23[5], 2.3[6]	0.17[6]
Chronic		
adult males	50[7], 5.0[8], 1.5[9]	
adult females	56[7], 5.6[8], 1.7[9]	
children	23[7], 2.3[8], 0.7[9]	0.02[10]

1. Based on acute breathing rates of 0.28, 0.18 and 0.74 m^3/kg-day for human adult men, women and children, respectively and on breathing rates for repetitive exposures of 0.20, 0.18 and 0.44 m^3/kg-day for human adult men, women and children, respectively. Uncertainty factors of 10 and 100 were applied to NOAELs from human and chronic studies, respectively.
2. Acute and seasonal RELs were calculated using the upper 95th percentile breathing rate for children of 0.581 m^3/kg-day. The acute REL was based on a child's mean breathing rate of 0.452 m^3/kg-day. An uncertainty factor of 1,000 was applied to all calculations.
3. Kisicki et al. (1999); NOAEL of 0.7 mg/kg, cholinergic signs and symptoms in humans.
4. Mendrala and Brzak (1998); NOAEL of 0.5 mg/kg for inhibition of plasma ChE in rats.
5. Szabo et al. (1988); Breslin et al. (1991); Shankar and Crissman (1993); NOAEL of 1.0 mg/kg-day for inhibition of brain ChE in rats.
6. Szabo et al. (1988); Breslin et al. (1991); NOAEL of 0.1 mg/kg-day for the inhibition of RBC and p-plasma ChE in rats.
7. McCollister et al. (1971); NOAEL of 1.0 mg/kg-day for inhibition of brain ChE in dogs.
8. McCollister et al. (1971); NOAEL of 0.1 mg/kg-day for inhibition of RBC ChE in dogs.
9. McCollister et al. (1971); NOAEL of 0.03 mg/kg-day for inhibition of plasma ChE in dogs.
10. McCollister et al. (1971); NOAEL of 0.01 mg/kg-day for inhibition of plasma ChE in dogs.