DEVELOPMENT OF HEALTH CRITERIA FOR SCHOOL SITE RISK ASSESSMENT PURSUANT TO HEALTH AND SAFETY CODE SECTION 901(g):

PROPOSED CHILD-SPECIFIC REFERENCE DOSE (chRD) FOR SCHOOL SITE RISK ASSESSMENT- Chlorpyrifos

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Executive Summary

A child-specific reference dose (chRD) at 0.0001 mg/kg/day for chlorpyrifos has been established in this document pursuant to Health and Safety Code Section 901(g). Health and Safety Code Section 901(g) requires the Office of Environmental Health Hazard Assessment (OEHHA) to identify chemical contaminants commonly found at school sites to be of greatest concern based on child-specific physiological sensitivities, and to develop numerical health guidance values (HGVs) for these chemical contaminants for use in the assessment of risk at proposed or existing California school sites.

Chlorpyrifos, O,O-diethyl-O-(3, 5, 6-trichloro-2-pyridinyl)-phosphorothioate, is a broad-spectrum organophosphate insecticide. Despite the cancellation of its registration for most home, lawn and garden use by U.S. EPA since 2000, chlorpyrifos continues to be one of the most commonly used pesticides, and the potential risks to children are still of concern to OEHHA.

Inhibition of cholinesterase by its active metabolite chlorpyrifos oxon was once considered the lone mechanism of chlorpyrifos neurotoxicity. However, there is now evidence that chlorpyrifos directly targets events that are specific to the developing brain and that are not related to the inhibition of cholinesterase, including: inhibition of DNA synthesis, impairment of cell acquisition and differentiation, interactions with neurotrophic factors, interruption of cell signaling cascades, and alteration in synaptic function. Based on our review of the existing literature, OEHHA concluded that there are age-related differences in the susceptibility to chlorpyrifos. Young animals are more sensitive to chlorpyrifos than adults. OEHHA also concluded that both cholinesterase and non-cholinesterase related mechanisms contributed to the differential susceptibility between young and adults. The deficits may be manifested immediately after the exposure, or appear later in life. OEHHA proposes a chRD of 0.0001 mg/kg/day for chlorpyrifos based on both cholinesterase inhibitions in dogs and rats and neurobehavioral alterations in rats.

Introduction

Developing a chRD or chRC

Health and Safety Code (HSC), Section 901(g), requires the Office of Environmental Health Hazard Assessment (OEHHA), in consultation with the appropriate entities within the California Environmental Protection Agency, to identify those chemical contaminants commonly found at school sites and determined by OEHHA to be of greatest concern based on child-specific physiological sensitivities. HSC 901(g) also requires OEHHA to annually evaluate and publish, as appropriate, numerical health guidance values (HGVs) for five of those chemical contaminants until the contaminants identified have been exhausted. HGVs established by this mandate are intended for use in the assessment of risk at proposed or existing California school sites. At this time, OEHHA focuses its evaluation on non-cancer effects of the identified chemicals, pending the completion of a new method for developing HGVs based on child-specific carcinogenic effects. Accordingly, current HGVs are in the form of a child-specific reference dose (chRD) or child-specific reference concentration (chRC).

This chapter serves as a background for the technical chRD or chRC reports. For those that are not familiar with this OEHHA program, it is advisable to review this chapter prior to analyzing the individual chRD reports.

Challenge

The use of appropriate HGVs and exposure parameters is essential to provide an unbiased assessment of the health risk at an existing or a proposed school site. Since school children have higher air, food and water intake relative to their body weight compared to adults; and have activity or behavioral patterns that may lead to higher exposure to environmental contaminants than adults, these higher intakes and unique activity patterns need to be considered in developing a set of child-specific exposure parameters for use in the risk assessment. OEHHA has analyzed these exposure parameters in issuing the report, Guidance for Assessing Exposures and Health Risks at Existing and Proposed School Sites (OEHHA, 2004) (http://www.oehha.ca.gov/public_info/public/kids/pdf/SchoolscreenFinal.pdf).

With respect to evaluating non-cancer risk by comparing the potential chemical exposure against the corresponding health criteria in the school setting, HGVs in the form of child-specific reference doses or concentrations should be used. Until the inception of the HSC 901(g) program, these child-specific HGVs were not available. For the most part, existing reference doses or concentrations for non-cancer endpoints, which were based on adult human or animal data, were used. However, a question has been raised that the intraspecies uncertainty factor of 10, the default factor, would not adequately protect children because it was mainly designed to account for genetic variability such as metabolizing isoenzyme variations. The Food Quality and Protection Act of 1996 (http://www.epa.gov/opppsps1/fqpa/) was an attempt to address the issue of children's

sensitivity and susceptibility. It mandated a safety factor of 10 unless data existed to indicate that children were not more sensitive or susceptible than adults.

A case can be made for the development and application of child-specific HGVs based on studies in young animals or epidemiological analysis of pertinent data rather than relying solely on a safety factor or uncertainty factor. While locating the appropriate data is a challenge, OEHHA has strived to do so because children can be more (or less) susceptible to chemical effects due to pharmacokinetic and pharmacodynamic differences between them and adults, and thus empirical data in the young would be preferable. Vulnerability often depends on the organ system in question and its developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life, including adolescence during which a particular structure or function may be more sensitive to disruption due to the action of a toxicant. Damage may not be evident until a later stage of development (DeRosa et al., 1998; Bigsby et al, 1999). The brain, for example, is an organ with distinct neurodevelopmental stages that occur in temporally distinct time frames across different regions, so the specific chemical, dose, and time of exposure during development will determine if a specific function in the brain will be altered (Faustman et al, 2000).

Differences also exist between children and adults with respect to their absorption, distribution, metabolism, and elimination of chemical contaminants. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC, 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water, and their brains and livers are proportionately larger (Altman PL, 1974; Fomon, 1966; Fomon *et al*. 1982; Owen G.M., 1966; Widdowson E.M., 1964). The infant also has an immature blood-brain barrier (Adinolfi, 1985) (Johanson, 1980) and probably an immature blood-testis barrier (Setchell B.P., 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori *et al*. 1990; Leeder and Kearns, 1997; NRC, 1993; Vieira *et al*. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns, who all have a low glomerular filtration rate and have not developed efficient tubular secretion and resorption capacities (Altman PL, 1974; NRC, 1993; West J.R., 1948). Children and adults may differ in their capacity to repair damage from chemical insults.

U.S. EPA and the March of Dimes sponsored a workshop -- Identifying Critical Windows of Exposure for Children's Health -- in September 1999 to systematically review the state of knowledge on prenatal and postnatal exposures and subsequent outcomes (Selevan *et al.* 2000). The workshop focused on the nervous, immune, respiratory, reproductive, and endocrine systems—organ systems that are still undergoing

development and maturation in children and thus deemed to be highly vulnerable to chemical insults. Workshop participants noted that data pertaining to children's sensitivities to environmental contaminants during various critical developmental periods are limited. In particular, little attention has been given to studying peripubertal and adolescent exposures or adult consequences from childhood exposure. Thus, the state of scientific knowledge pertaining to chemical effects on children is and continues to be a limiting factor in OEHHA's ability to develop child-specific HGVs for these contaminants.

In summary, with rare exceptions the use of a study in children or young animals as the basis for a child-specific HGV is preferred, even when studies in adult humans or animals encompassing a greater dose range or a larger experimental population exist and a biological mechanism of action can be established from corroborating studies. If a study in the young does not exist, the challenge is to integrate studies supporting a biological mechanism for greater sensitivity in the young with studies on adults to justify the application of appropriate safety factors.

Process

In June 2002, OEHHA issued a report, "Development of Health Criteria for School Site Risk Assessment Pursuant to Health and Safety Code, Section 901(g): Identification of Potential Chemical Contaminants of Concern at California School Sites," documenting the process by which OEHHA identifies chemicals and presenting a compilation of seventy-eight chemicals. The report can be found at http://www.oehha.ca.gov/public_info/public/kids/schoolsrisk.html. The compilation, whose sole purpose is to provide OEHHA staff with a manageable list of chemicals to work from, has no regulatory status and is a living document – chemicals may be added or removed as new information becomes available.

The chRD development process begins with the prioritization of chemicals from the compilation described in the June 2002 report. OEHHA has employed the following criteria, recognizing that often the availability of health effect data may be the overriding consideration in the selection of chemicals for evaluation.

1. Chemicals having a strong indication of their presence at school sites according to monitoring studies or other reliable sources.

2. Chemicals cited to have possible adverse effects in three or more of the systems that are undergoing critical development during childhood: the nervous, immune, respiratory, reproductive, or endocrine systems.

3. Chemicals that other OEHHA programs have identified as a concern.

In developing health guidance values for children as mandated by Health & Safety Code 901(g) OEHHA has adopted the following: First, in order to protect children from infancy through the time they leave school, HGVs must consider school-aged children up

to age 18, and infants and toddlers in daycare facilities located at school sites. Second, OEHHA considers the most sensitive species and endpoints in our evaluations. When evaluating various studies that use different test parameters to measure the same endpoint such as the nervous system, the lowest LOAEL or NOAEL from these studies would be selected. Third, the paucity of data has underscored the reality that the databases for sensitive endpoints may be incomplete. An uncertainty factor for database deficiency will be considered as appropriate. Fourth, because quantifying differences in susceptibility between a developing organ system and a mature one are hampered by the availability of studies that intentionally compare an effect in young animals with one in adult animals and available data are mainly from developmental toxicity studies that limit dosing to the mother during pregnancy, OEHHA staff have decided that these studies can be used for development of a child-specific health guidance value (chRD or chRC) if it is reasonable to assume that the effect of the chemical on the target organ in the offspring animal is likely to occur on the same target organ undergoing development after birth in humans. If studies that include gestational dosing of the mother and lactational dosing of the offspring (a protocol of the U.S. EPA Developmental Neurotoxicity Health Effects Test) are available, OEHHA will also consider these studies acceptable for establishing a chRD or chRC if the development of the critical organ system continues during childhood.

Finally, these prenatal and perinatal studies are frequently part of a series of studies to elucidate a "mechanism of toxicity". These studies may not have used a large number of animals or dose ranges. However, due to the critical windows in which cell proliferation and differentiation are occurring in specific organ systems during childhood, a study in young animals is usually preferred over one in adults, even adult humans. With corroborating studies showing a mechanism of action and biological plausibility, OEHHA will consider using these studies as appropriate. However, in rare cases, data from adult animals may be used, if they are from high quality studies and if there are data to provide a means of inference to critical windows of development in young animals so that an appropriate uncertainty of safety factor can be applied.

Status

In December 2005, OEHHA issued a final report proposing chRDs for the first six evaluated chemicals: Cadmium, Chlordane, Heptachlor, Heptachlor Epoxide, Methoxychlor, and Nickel, which be found at: http://www.oehha.ca.gov/public_info/public/kids/schools1205.html.

Following the first six chemicals, OEHHA selected 19 chemicals for which literature searches were performed. These chemicals included endosulfan, manganese, pentachlorophenol, toluene, lead, arsenic, aldrin, atrazine, DDE, DDT, dieldrin, endrin, hexachlorobenzene, lindane, malathion, perchloroethylene, permethrin, selenium, and trichloroethylene. The Public Health Library at the University of California at Berkeley assisted in literature search. OEHHA, in turn, reviewed the citations and abstracts; and evaluated relevant qualitative papers and quantitative studies. As a result, OEHHA is proposing chRDs for endosulfan, manganese, pentachlorophenol, toluene, and lead. These chemicals are currently undergoing public or external peer reviews. Chlorpyrifos

is OEHHA's latest addition. This draft report provides a summary on OEHHA's evaluation of chlorpyrifos pursuant to Health and Safety Code Section 901(g).

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Chlorpyrifos

What is Chlorpyrifos?

Chlorpyrifos, O,O-diethyl-O-(3, 5, 6-trichloro-2-pyridinyl)-phosphorothioate, is a broad spectrum organophosphate insecticide. Also known as Dursban, Lorsban, and other trade names, chlorpyrifos was first introduced in 1965 for control of a wide variety of insects on food and feed crops. Chlorpyrifos is one of the most widely used organophosphate insecticides in the U.S. and is the most effective product available for the control of California red scale, a common insect pest of citrus grown in California. On June 8, 2000, the U.S. Environmental Protection Agency (EPA) announced a cancellation of registration for most home, lawn and garden use products containing chlorpyrifos based on human health risks (U.S. EPA, 2000a). Currently, chlorpyrifos is registered for use in orchards, row crops, golf course turf, non-structural wood treatments, greenhouses, etc. Although lower application rates and lower frequencies of treatment are occurring for some agricultural uses of chlorpyrifos since 2001, chlorpyrifos is still being widely used in agriculture (Table 1).

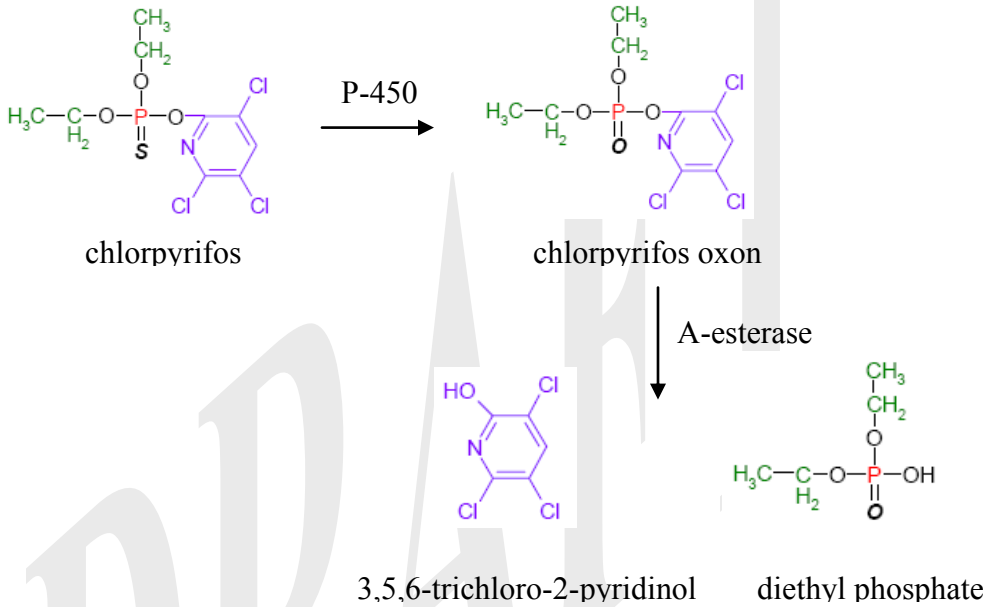
Table 1. Chlorpyrifos Use Trend in California
(Data from California Department of Pesticide Regulation's Pesticide Use Reports)

TOTAL CHLORPYRIFOS APPLIED IN THOUSAND POUNDS (Use includes both agricultural and reportable non-agricultural applications)									
1995	**1996**	**1997**	**1998**	**1999**	**2000**	**2001**	**2002**	**2003**	**2004**
3,385	2,687	3,152	2,355	2,257	2,093	1,674	1,419	1,546	1,775

Chlorpyrifos can be absorbed from the gastrointestinal tract and to a lesser extent through skin or by inhalation. The metabolism of chlorpyrifos is similar in both humans and other mammals. Chlorpyrifos is bioactivated to chlorpyrifos oxon in the liver through cytochrome P450 mediated desulfuration. Chlorpyrifos oxon is subsequently hydrolyzed by A-esterase to diethylphosphate and 3,5,6-trichloro-2-pyridinol (TCP), which is the major biological metabolite and environmental breakdown product of chlorpyrifos (Figure 1). The biological half-life of chlorpyrifos is relatively short, about 18 hours in plasma and 62 hours in fat. Chlorpyrifos is excreted primarily through the kidneys in the urine. Chlorpyrifos oxon is the active metabolite of chlorpyrifos, mediating the toxic effects of chlorpyrifos by binding irreversibly with acetylcholinesterase eliciting cholinergic hyperstimulation in the nervous system and in neuro-muscular junctions. Chlorpyrifos is a category II pesticide with an oral LD50 in rats ranging from 82 to 270 mg/kg. Clinical signs of acute poisoning associated with cholinergic hyperstimulation may include dizziness, vomiting, nausea, diarrhea, headache, blurred vision, salivation,

sweating, slurred speech, anxiety, respiratory failure and cardiac arrest. The major effects of chronic exposure are cholinergic signs and decrease in plasma, red blood cell (RBC), and brain cholinesterase activity. Some studies suggest that chlorpyrifos may be genotoxic, while no chronic studies have indicated chlorpyrifos is carcinogenic to this point. Chlorpyrifos is moderately persistent in the environment. The soil half-life of chlorpyrifos is usually between 30 and 120 days. The half-life of chlorpyrifos in water is relatively short, from a few days to two weeks.

Figure 1. The Metabolism of Chlorpyrifos



Modified from Biomarkers of Exposure: Organophosphates (National Pesticide Information Center)

What characteristics make chlorpyrifos of concern pursuant to Health & Safety Code Section 901 (g)?

Although the June 2000 Memorandum of Agreement between the US EPA and the technical registrants prohibited all the domestic use of chlorpyrifos, it continues to be one of the most commonly used organophosphate pesticides, and the risks to children are still of concern to OEHHA. Because of its extensive use, the metabolites of chlorpyrifos are frequently found in human tissue. The chlorpyrifos metabolite TCP has been found in the urine of 82 percent of adults sampled from all regions of the country (CDC 2001). A second report released two years later showed similar levels of TCP in urine samples (CDC 2003). In California, a joint study conducted by the California Air Resource Board and the California Department of Health Services between 2001 and 2002 showed that chlorpyrifos residue was present in 80 percent of all floor dust samples in California's portable classrooms. Additional problems have now surfaced with chlorpyrifos, as it has been found at National Priorities List (NPL) sites.

The half-life of chlorpyrifos indoors is estimated to be 30 days, but some studies show chlorpyrifos present in ambient air up to eight years post application. The half-life of chlorpyrifos in water is relatively short, from a few days to two weeks. However, a study done in Chesapeake Bay showed that the hydrolysis half-lives of chlorpyrifos varied from 24 days in the Patuxent River to 126 days in the Susquehanna River, and the author indicates that there might be a potentially long environmental half-life for this chemical (Liu et al, 2001). The soil half-life of chlorpyrifos is usually between 30 and 120 days, but can vary from 2 weeks to over one year, depending on the climate, soil type and other conditions. Reports from the USDA Forest Service showed that the termiticide formulation of chlorpyrifos can be effective against termites for more than 15 years (Wagner, 2003).

Existing Health Criteria for Chlorpyrifos

U.S. EPA (IRIS) Reference Dose (RfD). U.S. EPA's Integrated Risk Information System has established an RfD of 0.003 mg/kg/day for chronic oral exposure of chlorpyrifos (U.S. EPA, 1988). The RfD is based on a 1972 human study conducted by Dow Chemical Company (Coulson *et al*, 1972). Sixteen healthy adult male volunteers were separated into four experimental groups and treated (4 per dose group) with 0, 0.014 or 0.03 mg/kg/day of chlorpyrifos by tablet for 20 days, and at 0.10 mg/kg/day for 9 days. The 0.10 mg/kg/day treatment was terminated after 9 days because of the runny nose and blurred vision in one of the subjects. The plasma cholinesterase in this group was reduced by about 65 percent compared to the controls. No reduction in plasma cholinesterase was seen at the lower doses. The RBC cholinesterase activity was unaffected at any dose examined. Based on the decreased plasma cholinesterase activity at 0.10 mg/kg/day, the NOEL for plasma cholinesterase inhibition is 0.03 mg/kg/day. The RfD of 0.003 mg/kg/day was calculated based on the NOEL of 0.03 mg/kg/day and an uncertainty factor of 10 (human variability). The U.S. EPA's RfD was established in 1988 based on the 20-day human study, which did not measure chronic chlorpyrifos toxicity because of the insufficient exposure duration. The human study is also limited because it only included 4 test subjects in each treatment group, none of which were children.

ATSDR Minimal Risk Level (MRL). The Agency of Toxic Substances and Disease Registry (ATSDR) has established a MRL of 0.001 mg/kg/day for chronic oral exposure of chlorpyrifos (ATSDR, 1997). The MRL is based on a 2-year rat study conducted by Dow Chemical Company (McCollister et al, 1974). Sherman rats (25 rats/sex/dose) were treated with chlorpyrifos at 0, 0.01, 0.03, 0.1, 1, or 3 mg/kg/day for 2 years starting at 7 weeks of age. Supplementary groups (5-7 rats/sex/dose) were included in the study to provide interim pathological examination and cholinesterase (ChE) determinations. Brain ChE was inhibited by 56 percent in 3 mg/kg/day treatment group during the 2-year study. No reduction in brain ChE was seen at the lower doses. Plasma and RBC ChE activity were reduced at 1 and 3 mg/kg/day. Neither plasma nor RBC cholinesterase was affected by the treatment at 0.1 mg/kg/day or below. A NOEL of 0.1

mg/kg/day was established based on the reduced plasma and RBC ChE activity. ATSDR applied an uncertainty factor of 100 (10 for intra-species variation, and 10 for extrapolation from animals to human) to the NOEL and a MRL of 0.001 mg/kg-day was derived. The OEHHA analysis, discussed below, also relies in part on the McCollister rat study and the cholinesterase inhibitory effects of chlorpyrifos.

U.S. EPA Reference Dose (RfD) and Population Adjusted Dose (PAD). The Office of Pesticide Programs (OPP) at U.S. EPA has established two health guidance values for chronic dietary assessment in support of the reregistration eligibility decision for chlorpyrifos (U.S. EPA, 1999; U.S. EPA, 2000b; U.S. EPA, 2002). These health guidance values are based on 5 animal studies: a 2-year dog study (McCollister et al, 1974), a 90-day dog study (Barker, 1989), a 90-day rat study (Crown, et al, 1985), a 2-year rat study (Crown et al, 1990) and a developmental neurotoxicity study in rats (Hoberman et al, 1998a, b). McCollister's 2-year dog study is a key study from which a NOEL was derived. This study was conducted in two separate phases. In phase A, 11-month old dogs (3 males and 3 females per group) were treated with chlorpyrifos at 0, 0.01, 0.03, 0.1, 1.0, and 3.0 mg/kg/day by diets for 1 year. In phase B, 10-month old dogs (4 males and 4 females per group) were treated with chlorpyrifos at 0, 0.01, 0.03, 0.1, 1.0, and 3.0 mg/kg/day by diets for 2 years. The cholinesterase activity was decreased at 0.03, 0.1, 1.0 and 3.0 mg/kg/day in plasma, 0.1, 1.0 and 3.0 mg/kg/day in red blood cells, and 3.0 mg/kg/day in brain. A NOEL of 0.03 mg/kg/day was established based on reduced plasma and RBC ChE activity. McCollister's 2-year dog study and the cholinesterase inhibitory effects of chlorpyrifos has also been used by OEHHA as part of the basis for deriving a chRD, and it is further discussed below.

OPP also considered the qualitative differences between F_0 and F_1 females in a developmental neurotoxicity study as part of the basis to retain the 10X FQPA safety factor. Hoberman et al. (1998a, b) observed a qualitative difference in response to chlorpyrifos between the F_0 and F_1 female rats (cholinesterase inhibition in F_0 female rats vs. morphologic alterations in the brain of F_1 females).

Based on the weight of evidence consideration from the 5 studies in dogs and rats, OPP used the NOEL of 0.03 mg/kg/day as the basis for the chronic RfD. They applied an uncertainty factor of 100 (10 for intra-species variation, and 10 for extrapolation from animals to human) to the NOEL to derive a RfD of 0.0003 mg/kg/day. OPP includes an additional FQPA safety factor of 10 for children and women 13-50 due to 1) age-related difference in cholinesterase inhibition (Zheng et al, 2000; Moser and Padilla 1998), 2) qualitative difference between dams and adult offspring in the developmental neurotoxicity study (Hoberman et al, 1998a,b), and 3) uncertainties regarding the potential non-cholinergic adverse effects of chlorpyrifos, which are further discussed below. This additional FQPA Safety Factor results in a Population Adjusted Dose (PAD) of 0.00003 mg/kg/day for children and women ages 13 to 50. The U.S. EPA's RfD and Population Adjusted Dose are the most current and health-protective among existing health criteria for chlorpyrifos.

Current Evaluation Results

Human epidemiological studies have indicated that chlorpyrifos exposure early during development is associated with the deficits developed in infants. Reduction in birth weight, decrease in birth length, and birth defects were observed in infants exposed to chlorpyrifos during pregnancy (Perera et al, 2003; Sherman, 1995; Whyatt et al, 2005; Rull et al, 2004; Rauh et al, 2004). A definitive evaluation with a focus on the age-related difference in chlorpyrifos toxicity is thus becoming necessary and important.

I. Age-Related Differences in the Detoxification of Chlorpyrifos.

A-esterase (e.g., chlorpyrifos oxonase and paraoxonase) and carboxylesterase are known to play an important role in the detoxification of chlorpyrifos. Berkowitz et al. (2004) studied the correlation between the paraoxonase activity and chlorpyrifos neurotoxicity. Significant reduction in head circumference, which is an indicator for neurodevelopmental disorders, was seen only in infants born to mothers with low paraoxonase-1 (PON1) activity (Berkowitz et al, 2004). Animal studies indicated that paraoxonase pretreatment provides protection in rats challenged with chlorpyrifos oxon (Costa et al, 1990). Paraoxonase-1 knockout mice are more susceptible to chlorpyrifos and its metabolite chlorpyrifos oxon (Shih et al, 1998).

Some human studies showed that young children have less serum paraoxonase activity than adults. A-esterase activity is 3-fold lower in infants than adults (Augustinsson and Brody, 1962; Ecobichon and Stephens, 1973). Paraoxonase activity in newborn cord blood is 2.4-fold lower than those in adults, suggesting that its activity is not fully developed at birth (Mueller et al, 1983). A multiethnic cohort study including both adults and neonates at Mount Sinai Hospital in New York City demonstrated that neonates have lower paraoxonase-1 (PON1) activity than adults. The differences are 2.6, 3.6, and 4.6 times for African Americans, Caribbean Hispanics, and Caucasians, respectively. In addition, the differences in the activity between different PON1 genotypes are also larger in neonates compared to adults (Chen et al, 2003). Some animal studies also showed that levels of A-esterase and carboxylesterase were much lower in newborn and juvenile rats than in adults. A study in Long-Evans rats showed liver and plasma carboxylesterases are 6-fold lower in newborn compared to adults, and 2-fold lower in juvenile than in adults. Chlorpyrifos oxonase, the A-esterase that hydrolyzes chlorpyrifos oxon showed a 30-fold difference between newborn and adults (Moser et al, 1998). A separate study in Long-Evans rats showed 11-fold difference between 4 days of age and adult in plasma chlorpyrifos oxonase activity and 2-fold difference in liver chlorpyrifos oxonase (Mortensen et al, 1996). The levels of liver microsomal carboxylesterases are also low in young rats. Comparing to the adults, the levels are 6-fold and 2-fold lower in one-week-old and four-week-old rats respectively (Morgan et al, 1994). A study in Sprague-Dawley rats also showed lower levels of carboxylesterase activity in young rats compared to adults. The enzyme activity in plasma, liver and lung were 5-, 11- and 4-fold lower at 7 days of age compared to adults. The differences were 2.7-, 2- and 1.7- fold respectively at 21 days of age compared to adults (Karanth and Pope, 2000). Another study in Sprague-Dawley rats showed a 4-fold difference between 1-day-old and 80-day-old for

liver carboxylesterase activity (Atterberry et al, 1997). The lack of enzymes to detoxify chlorpyrifos in young vs. adults would make children more sensitive to chlorpyrifos toxicity compared to adults.

II. Age-Related Differences in Chlorpyrifos-Induced Cholinesterase Inhibition.

Some studies have shown that immature organisms are more sensitive to chlorpyrifos-induced cholinesterase inhibition following acute high dose exposure. Chlorpyrifos, given by oral gavage to young rats (17 days of age) at 15 mg/kg, produced cholinesterase inhibition and behavioral changes similar to those in adult rats (70 days of age) at 80 mg/kg. The same degree of cholinesterase inhibition can be achieved in postnatal day 17 rats at a 5-fold lower dose compared to adults (Moser and Padilla 1998). The maximum tolerated dose (MTD) of chlorpyrifos following subcutaneous injections was 45 mg/kg in neonatal rats at 7 days of age compared to 279 mg/kg in adult rats at 80-100 days of age (Pope et al, 1991). Pope and Chakraborti (1992) also studied the dose that would cause 50 percent inhibition of cholinesterase activity (ED50) following subcutaneous injections. The ED50 for brain cholinesterase inhibition was 19.8 mg/kg in neonatal rats at 7 days of age compared to 44 mg/kg in adult rats at 3 months of age (Pope and Chakraborti 1992).

Zheng et al. (2000) compared chlorpyrifos-induced cholinesterase inhibition in neonatal and adult rats following single or repeated oral exposure at non-lethal doses (0.15-15 mg/kg/day). Despite the fact that immature rats still show greater sensitivity to single oral exposure (NOELs for cholinesterase inhibition in plasma, RBC and brain are 0.15-1.5 in neonates vs. 1.5-15 mg/kg/day in adults), no apparent age-related differences were seen following repeated exposure for 14 days (NOELs for cholinesterase inhibition in plasma, RBC and brain are 0.75 in neonates vs. 0.15-1.5 mg/kg/day in adults) (Zheng et al, 2000). Zheng's repeated exposure results were consistent with other studies showing that while young animals are more sensitive to the acute toxicity of chlorpyrifos than adults, the difference is not evident following repeated exposure (Zheng et al, 2000; Liu et al, 1999; Chakraborti et al, 1993). It is uncertain whether there is likely to be a similar age-related difference in sensitivity to chronic low dose exposure to chlorpyrifos, which is more relevant to the environmental chlorpyrifos exposure of general human population.

III. Non-Cholinesterase Mechanisms of Chlorpyrifos Neurotoxicity.

Inhibition of cholinesterase by its active metabolite chlorpyrifos oxon was once considered the lone mechanism of chlorpyrifos neurotoxicity. Studies from the past decade helped us to better understand the mechanism of chlorpyrifos. There is evidence that chlorpyrifos directly targets events that are specific to the developing brain and that are not necessarily related to the inhibition of cholinesterase (Qiao et al, 2001; Qiao et al, 2003b; Qiao et al, 2004; Qiao et al, 2005; Whitney et al, 1995; Dam et al, 1998; Song et al, 1997). Indeed, the greater toxicity of chlorpyrifos in juvenile animals cannot be explained solely by developmental differences in cholinesterase-mediated events, nor do

age-related increments in chlorpyrifos metabolism account for differential toxicity. Immature animals actually recover more rapidly from cholinesterase inhibition, so measurements of cholinesterase activity alone may not be sufficient for the assessment of adverse effects. Chlorpyrifos-induced neurochemical and neurobehavioral changes unrelated to ChE inhibition, such as those listed below, are of equal concern for human health risk assessment:

1. Chlorpyrifos affects the developing brain during cell division. Chlorpyrifos exerts antimitotic actions on developing neural cells independently of cholinesterase inhibition (Qiao et al, 2001; Qiao et al, 2003a; Dam et al, 1998; Whitney et al, 1995, Campbell et al, 1997). Administration of chlorpyrifos by subcutaneous injections to neonatal rats at doses that were devoid of any overt toxicity showed significant inhibition of DNA synthesis and subsequent cell loss in brain regions examined. For example, single dose (2mg/kg) subcutaneous administration of chlorpyrifos on postnatal day 1 and day 8 showed acute inhibition of DNA synthesis in rat brain. Repeated chlorpyrifos administration on postnatal day 1 through day 4 at 1 mg/kg/day showed persistent inhibition of DNA synthesis. Chlorpyrifos treatment on postnatal day 11 through day 14 at 1 or 5 mg/kg/day leads to deficits in cell number in forebrain, which were seen between 15 and 20 days of age rather than during the chlorpyrifos treatment. The results thus indicate that, with postnatal exposure, cell loss and deterioration of cell function continue well after the end of the exposure period and after cholinesterase activity returns to normal. Additional experiments also demonstrated that the effects are not cholinesterase related. For example, Qiao et al (2001) showed that chlorpyrifos can inhibit DNA synthesis in cultured neural cell lines to a much greater extent than the oxon despite the fact that chlorpyrifos is a weaker cholinesterase inhibitor. The results therefore indicate that the effects of chlorpyrifos on DNA synthesis may not be mediated through cholinesterase inhibition by chlorpyrifos oxon.

2. Chlorpyrifos interferes with RNA synthesis during differentiation. Neonatal rats treated with chlorpyrifos on postnatal days 1 through 4 (1 mg/kg/day) and postnatal days 11 through 14 (5 mg/kg/day) showed a significant reduction in total cellular RNA in brain as one of the earliest-detectable events (Johnson et al, 1998). Alterations in RNA concentration and content were seen in the developing brain when tested 1 or 6 days after chlorpyrifos exposure. The results indicate that chlorpyrifos targets pivotal macromolecules that control cell differentiation during brain cell development. The lower threshold for these subcellular effects compared to that for systemic toxicity demonstrate that the developing brain is a selective target for chlorpyrifos, a factor that should be fully considered in the risk assessment process.

3. Chlorpyrifos interrupts cell signaling. The adenylyl cyclase signaling transduction pathway is involved in cell replication and differentiation in virtually all prokaryotic and eukaryotic cells. Therefore interference with this pathway during development would be expected to have a significant impact on brain cell development. When the effects of otherwise subtoxic doses of chlorpyrifos on adenylyl cyclase activity were examined in the developing brain, profound effects were found (Song et al, 1997). Importantly, low doses (1 mg/kg/day) of chlorpyrifos given early in development

(postnatal days 1-4), with minimal cholinesterase inhibition, had a much greater effect on the adenylyl cyclase pathway than did larger doses (5 mg/kg/day) administered later in development (postnatal days 11-14), even though the latter exposure produced a much greater inhibition of cholinesterase. The effects on adenylyl cyclase were not evident during the immediate period of chlorpyrifos treatment. The largest effects on signaling appeared after several days of delay, at a time point when cholinesterase activity had returned to normal values. The results demonstrated that non-cholinergic mechanisms play a key role in the adverse effects of chlorpyrifos on brain development. Thus, conversion of chlorpyrifos to its metabolite chlorpyrifos oxon, and the subsequent inhibition of cholinesterase, might not be the only factor in determining developmental neurotoxicity of this chemical.

4. Chlorpyrifos interferes with important nuclear transcription factors involved in cell differentiation. The ability of chlorpyrifos to affect nuclear transcription factors involved in cell replication and differentiation was also studied (Crumpton et al, 2000). Apparently subtoxic doses (e.g., 1mg/kg daily) of postnatal chlorpyrifos treatment (postnatal days 1-4 or postnatal days 11-14) interfered directly with the binding activity of AP-1 and SP-1 transcription factors, which are involved in activation of many genes required in differentiation. The changes were present in both forebrain and cerebellum. Unlike the forebrain, cerebellum is a brain region with sparse cholinergic innervation. Again, this study indicates the direct actions of chlorpyrifos on brain cell development, effects not related to cholinesterase inhibition.

5. Chlorpyrifos impairs cholinergic synaptic function during development. Effects of chlorpyrifos on cholinergic synaptic function were also studied. Choline acetyltransferase (ChAT), the enzyme responsible for the synthesis of acetylcholine, is a constitutive marker for cholinergic nerve terminals. Hemicholinium-3 (HC-3) binding to the presynaptic choline transporter, which is responsive to neuronal activity, is widely used as an index of nerve impulse activity. Choline acetyltransferase (ChAT) activity and hemicholinium-3 (HC-3) binding were thus studied as indices of synaptic proliferation and synaptic function. Low doses of chlorpyrifos exposure (1 or 5 mg/kg) at different postnatal stages caused reduction in both synaptic proliferation and synaptic activity; deficits appear almost immediately after the exposure (Dam et al, 1999).

6. Chlorpyrifos affects the catecholamine system in the developing brain. Effects of chlorpyrifos were not limited to the cholinergic system. Catecholamine pathways were also involved (Dam et al, 1999). Postnatal chlorpyrifos (1 or 5 mg/kg) was shown to augment the release of both dopamine and norepinephrine within the central nervous system in experimental rats. Notably, the cerebellum, a region with sparse cholinergic innervation, was affected the most. The results also suggest that non-cholinergic mechanisms may play a key role in the adverse effects of chlorpyrifos on brain development.

7. Chlorpyrifos elicits oxidative stress in the developing brain. Reactive oxygen species are thought to be involved in the toxicity of many neurotoxicants. Investigators (Qiao et al, 2005; Bagchi et al, 1995) also evaluated the ability of

chlorpyrifos to produce lipid peroxidation, an index of oxidative stress. Their results indicate that chlorpyrifos elicits oxidative damage as demonstrated by the increased lipid peroxidation after chlorpyrifos exposure to developing neural cells both in vitro (1 nmol/ml) and in vivo (41 mg/kg). Therefore the production of reactive oxygen species and resulting tissue damage may also contribute to the toxic manifestations of chlorpyrifos.

8. Chlorpyrifos interferes with gliogenesis and axonogenesis. Neurons are not the only target of chlorpyrifos in the CNS. Chlorpyrifos also targets glia during gliogenesis and axonogenesis. Both prenatal (1 through 40 mg/kg) and postnatal (1 or 5 mg/kg) chlorpyrifos exposures cause alterations in neuroprotein markers for oligodendrocytes, neuronal cell bodies, and developing axons. The deficiencies occur both in the immediate post-treatment period and later during development (Garcia et al, 2002; Garcia et al, 2003). Morphological changes such as a decrease in the number of glial cells were also observed in juvenile rat brain after neonatal chlorpyrifos exposure (Roy et al, 2004). Gliogenesis and axonogenesis are late events in brain development. These findings thus indicate that chlorpyrifos targets developing organisms over a wide developmental period. Roy et al (2004) state that "the vulnerable period for adverse effects of chlorpyrifos is likely to extend into childhood or adolescence."

9. Behavioral abnormalities after chlorpyrifos exposure. There is evidence that chlorpyrifos may be especially damaging to the developing brain, targeting diverse events in neural development. Effects that are unique to the developing brain include inhibition of DNA synthesis, impaired cell acquisition and differentiation, interactions with neurotrophic factors, effects on cell signaling cascades involved in cell differentiation and alteration in synaptic function. To determine whether these biochemical changes elicit behavioral abnormalities, behavioral studies were also conducted in developing rats. Postnatal days 1-4 rats administered chlorpyrifos at 1.0 mg/kg exhibited decreased locomotor activity and deficits in coordination skills (Dam et al, 2000). The deficits occurred both during chlorpyrifos exposure and for days after the treatment, indicating both immediate and delayed behavioral abnormalities induced by chlorpyrifos. Another study further confirmed chlorpyrifos induced behavior alterations (Jett et al, 2001). Two groups of rats were given chlorpyrifos by subcutaneous injection at different developmental stages. The early treatment group was given chlorpyrifos on postnatal days 7, 11 and 15 at 0, 0.3, or 7 mg/kg (17-20 rats per dose group). The late treatment group was treated on postnatal days 22 and 26 at 0, 0.3, or 7 mg/kg (7-8 rats per dose group). The two treatments covered key periods during development, from postnatal day 7 through postnatal day 26 including both preweaning and postweaning stages. Behavior tests were conducted from postnatal day 24 through day 28 for rats from both groups. Rats treated with 7 mg/kg in the early group and 0.3 or 7 mg/kg in the late group showed chlorpyrifos-induced alteration in cognitive function as measured in the Morris swim test. These effects did not appear to be related to cholinesterase inhibition, as there were no cholinergic signs, brain cholinesterase inhibition, or growth impairment in any treatment group. The authors indicate that a deficit in cognitive function in juvenile rats is thus an important functional correlate of the molecular and biochemical effects of chlorpyrifos in the immature brain. OEHHA has used this study

and the neurobehavioral effects of chlorpyrifos as the basis for deriving a chRD, as further discussed below.

IV. Late Arising Deficits in Young Animals After Brief Subtoxic Exposure to Chlorpyrifos During Development.

As discussed above, it is increasingly evident that the developmental neurotoxicity of chlorpyrifos may depend on a variety of mechanisms, rather than reflecting simply the inhibition of cholinesterase. Accordingly, their impact is evident over a wide developmental period. It must be noted that, with postnatal chlorpyrifos exposure, many of the neurotoxic effects appear after a delay. Therefore, there is increasing concern over the long-term neurobehavioral consequences of fetal and neonatal exposure to chlorpyrifos, since the damage may not be evident until a later stage of development. Accordingly, a definitive evaluation of the consequences of fetal and postnatal exposure will require a longitudinal study from early development through adulthood. Some recent studies discussed below addressed this concern.

1. Late arising deficits after postnatal chlorpyrifos exposure. Animals exposed to chlorpyrifos postnatally were examined in the early postnatal period and into adolescence and adulthood. They showed later-emerging, persistent deficits in cholinergic synaptic function and related cognitive behavioral performance. Defects emerge in adolescence or adulthood even in situations where normative values are initially restored in the immediate post-exposure period. For example, chlorpyrifos was given at 1 mg/kg/day on postnatal days 1-4 or at 5 mg/kg/day on postnatal days 11-14, treatments that were devoid of overt toxicity. Spontaneous alternation in the T-maze, locomotor activity in the Figure-8 apparatus and learning in the 16-arm radial maze were tested throughout adolescence and adulthood. Both early and late postnatal chlorpyrifos exposure caused long-term changes in cognitive performance (Levin et al, 2001). The late-arising behavioral deficits in animals exposed to chlorpyrifos postnatally were accompanied by delayed neurotoxic changes in neurochemical indices of cholinergic synaptic activity and in other neurotransmitter systems regulated by cholinergic input (Slotkin et al, 2001, 2002). Animals exposed during the same postnatal stages showed deficits in cholinergic synaptic function as reflected by the changes on choline acetyltransferase (ChAT) activity and hemicholinium-3 (HC-3) binding. The deficits in cholinergic synaptic function persist into adolescence and adulthood, long after the termination of exposure and well after the restoration of cholinesterase activity. The same postnatal chlorpyrifos exposure also elicits widespread alterations in the catecholaminergic system that continue into adulthood. The content and utilization rates of both dopamine and norepinephrine were altered in multiple brain regions examined.

Developmental exposure to chlorpyrifos also causes long-lasting changes in the serotonergic (5HT) system (Aldridge et al, 2004, 2005a, 2005b). Young rats briefly exposed to chlorpyrifos at an early postnatal stage (postnatal days 1-4, 1 mg/kg/day) showed anhedonia and decreased anxiety in adulthood as evidenced by alterations both in elevated plus maze test and anhedonia test. These effects involve serotonergic mechanisms and resemble animal models of depression. The long-term alterations in

behaviors were accompanied by alterations in 5HT function, as early postnatal exposure to chlorpyrifos triggered long-term increases in 5HT turnover across multiple brain regions in adulthood. Chlorpyrifos exposure during different developmental stages also elicits long-lasting alterations in 5HT receptors, the presynaptic 5HT transporter and 5-HT mediated signaling pathway. Exposures to chlorpyrifos during development that are not overtly toxic thus elicit lasting alteration of the 5HT system in association with 5HT-related behavioral changes.

As discussed above, alterations in adenylyl cyclase signaling were observed in the immediate post-treatment period of chlorpyrifos. Animals exposed during different prenatal or postnatal periods also showed impairment of adenylyl cyclase signaling in adulthood, significant changes in adenylyl cyclase signaling can be seen in a wide variety of brain regions studied (Meyer et al, 2004).

2. Late arising deficits after prenatal chlorpyrifos exposure. The same dose of chlorpyrifos given prenatally did not produce the same deficiencies in cholinergic synapses as we have seen following postnatal treatment. However, despite the initial sparing, animals exposed prenatally still developed behavioral deficits in adolescence and adulthood, associated with impaired cholinergic function (Qiao et al, 2002, 2003b, 2004; Levin et al, 2002; Icenogle et al, 2004).

Using treatment regimens that lie below the threshold for fetal growth impairment, Qiao et al (2002, 2003b, 2004) identified postnatal deficits in cholinergic activity that persisted into adulthood. Chlorpyrifos was given to pregnant rats on gestational days 9-12 or gestational days 17-20 at 1, 2 or 5 mg/kg/day. Subsequent development of acetylcholine systems was examined and the effects were compared to those on general biomarkers of cell development. Hemicholinium-3 (HC-3) binding to the presynaptic choline transporter, which is responsive to neuronal activity, was markedly impaired. Deficits were again apparent in adolescence and adulthood. Chlorpyrifos also causes late-emerging abnormalities of neural cell packing density, cell number, cell size and neuritic extensions that may represent a contributory factor for cholinergic synaptic dysfunction. Accordingly, the major change elicited by prenatal chlorpyrifos administration appears to be a reduction in cholinergic synaptic function, effects that were demonstrable even at exposure to 1 mg/kg/day, a dose that lies below the threshold for maternal and fetal growth impairment and for inhibition of fetal brain cholinesterase.

Prenatal chlorpyrifos exposure also impaired working and reference memory in adolescence and adulthood (Levin et al, 2002; Icenogle et al, 2004). Although chlorpyrifos has no effects on growth and viability, offspring showed behavioral impairment when tested in adolescence and adulthood. For example, locomotor hyperactivity was discovered in early T-maze and in the elevated plus-maze trials. Changes in the rate of habituation were identified. Impairment in learning and working memory was also demonstrated with the 16-arm radial maze. The results indicate that otherwise nontoxic prenatal exposures to chlorpyrifos elicit deficits in cholinergic function that influence cognitive performance in adolescence and adulthood.

These findings indicate that the developing brain is adversely affected by chlorpyrifos regardless of whether exposure occurs early or late in brain development, and that defects emerge in adolescence or adulthood even in situations where normative values are initially restored in the immediate post-exposure period. Accordingly, developmental neurotoxicity consequent to fetal or childhood chlorpyrifos exposure may occur in settings in which immediate symptoms of intoxication are absent.

V. Potential Adverse Effect of TCP in Developing Brain.

Trichloropyridinol (TCP), the major catabolic product of chlorpyrifos, was once considered the inactive metabolite of chlorpyrifos. However, Qiao et al, 2001 showed that TCP inhibits DNA synthesis in vitro. The effect of TCP was seen in both neuronotypic PC12 cells and gliotypic C6 cells, indicating that TCP may affect both neurons and glia. TCP has also been shown to inhibit neurite outgrowth, a morphological marker of neural cell differentiation (Das et al, 1999). Most importantly, TCP accumulates in high concentrations in the fetal brain after maternal chlorpyrifos administration and is also found as the major chlorpyrifos residue in children. TCP concentration is about 3-fold higher in the fetal brain compared to adults (Hunter et al, 1999). Thus, additional effects may be contributed by the supposedly "inactive" metabolite TCP to the age-related differences in the toxicity of chlorpyrifos, and considering the higher concentration and longer half-life of TCP compared to chlorpyrifos and chlorpyrifos oxon, even a relatively small effect of TCP in vivo would be dangerous to the developing organisms.

Recommendation of Child-Specific Reference Dose (chRD) for Chlorpyrifos

Based on our review of the existing literature, OEHHA concluded that there are age-related differences in the susceptibility to chlorpyrifos. OEHHA also concluded that both cholinesterase and non-cholinesterase related mechanisms contributed to the differential susceptibility between young and adults. The deficits may be manifested immediately after the exposure, or appear later in life. Young animals are more sensitive to chlorpyrifos compared to adults based on the following findings:

- Quantitative differences in the detoxification of both chlorpyrifos and its metabolite chlorpyrifos oxon between young and adults. The slower removal of the toxic forms of chlorpyrifos in young vs. adults would make children more vulnerable, a factor that should be fully considered in the risk assessment process.
- Chlorpyrifos targets diverse events that are specific to the **developing brain**. The developing brain is a selective target for chlorpyrifos, a factor that should be fully considered in the risk assessment process. Although it is difficult to quantify neurodevelopmental impairment, numerous research articles provided clear evidence of increased susceptibility of neonates to chlorpyrifos.

- Brief exposure to a subtoxic dose of chlorpyrifos early **during development** elicits long-term deficits later in life. The low threshold for the adverse effects, the lack of immediate symptoms of intoxication and the long lasting damage make childhood exposure even more dangerous, a factor that should also be fully considered.

I. Calculation of the chRD: Neurobehavioral Endpoint

As indicated above, the many neurochemical or neurobehavioral studies have limited dose selections and small sample size. It is difficult to identify a LOAEL or NOAEL from these studies. Among studies available, Jett et al (2001) studied cognitive impairment after chlorpyrifos exposure at the dose level of 0.3 mg/kg. Two groups of rats were given the same subcutaneous doses of chlorpyrifos at two different developmental stages (preweaning or postweaning stages). The first group of rats was given chlorpyrifos at 0, 0.3, or 7 mg/kg on postnatal days 7, 11 and 15 (17-20 rats per dose group), while the second group of rats was given chlorpyrifos at 0, 0.3, or 7 mg/kg on postnatal days 22 and 26 (7-8 rats per dose group). The two treatments covered key periods during development, from postnatal day 7 through postnatal day 26 including both preweaning and postweaning stages. Behavior tests were conducted from postnatal day 24 through day 28 for rats from both groups. Rats treated with 7 mg/kg in both groups showed chlorpyrifos-induced alteration in cognitive function as measured in the Morris swim test. Rats treated with 0.3 mg/kg of chlorpyrifos in the postweaning group also showed behavior alterations. The dose of 0.3 mg/kg was therefore considered as the lowest effective dose for the exposure of chlorpyrifos based on the behavioral endpoints. OEHHA's chRD is based in part on Jett's cognitive study in developing rats because it covers the vulnerable developmental windows and fits the purpose of school site risk assessment. The LOAEL of 0.3 mg/kg/day for cognitive alteration in this study was used. Since the two treatments covered key periods during development, including both preweaning and postweaning stages, OEHHA did not add an uncertainty factor to adjust from acute to chronic exposure. However, since the treatments were conducted once every four days instead of daily dosing, an extrapolation factor of 3 was added to compensate for uncertainty surrounding the target tissue dose at the critical time during development. The calculation of the non-cancer chRD for chlorpyrifos is as follows:

$$\text{chRD} = \frac{\text{LOAEL}}{\text{UF}} = \frac{0.3\,\text{mg/kg/day}}{3000} = 0.0001\,\text{mg/kg/day}$$

Where, UF = Uncertainty factor of 3000 (10 for intra-species variation, 10 for extrapolation from rats to humans, 10 for extrapolation from LOAEL to NOAEL, and 3 to compensate for uncertainty surrounding the target tissue dose at the critical time during development).

Many factors may contribute to the uncertainty of the presumed LOAEL from the Jett study. First, the authors indicate that the deficit in cognitive function is an important functional correlate of the molecular and biochemical effects of chlorpyrifos; however,

the molecular and biochemical deficits may not always be accompanied by behavior alterations. Some molecular or biochemical changes may happen at lower doses where behavior deficits have not been developed or observed. Second, the real LOAEL could be lower even with the behavioral endpoints used in the study since chlorpyrifos was only given once every four days instead of once a day.

Chlorpyrifos was given by subcutaneous injection in the study. U.S. EPA's Office of Pesticide Program (OPP) stated in their chlorpyrifos reevaluation document that subcutaneous injection "is not a pathway of human exposure" and "can not be reliably compared to the oral route given the lack of pharmacokinetic data on this dosing regime". OPP also suggested that "these studies still provide important qualitative information" (U.S. EPA, 2000b). In comparing routes of exposure, one must consider the difference in absorption, first pass clearance and bioavailability. Absorption is the extent of what the chemical is absorbed from the gastrointestinal tract into the portal blood. First pass clearance is the extent to which chemical is metabolized by the liver when it passes through the liver from the portal circulation into the systemic circulation. Bioavailability, the percent of the intact chemical that reaches the systemic circulation, depends on both the percent of chemical that has been absorbed and the percent of the chemical that has passed through the liver without being metabolized. Intravascular injection was considered to achieve 100 percent bioavailability. To evaluate the bioavailability of oral exposure, parallel studies are conducted for the same chemical by using both the dietary and intravascular injection approaches, the area under the plasma concentration versus time curve (AUC) from dietary and intravascular administration are compared. The bioavailability of oral exposure is calculated based on the 100 percent bioavailability from the intravascular injection. However, many research articles confuse absorption with bioavailability, and this makes the extrapolation of chlorpyrifos between routes even more difficult.

Based on the current literatures available, the route difference in the administration of chlorpyrifos does not seem to be a concern in terms of the absorption of chlorpyrifos since studies showed that chlorpyrifos is rapidly and well absorbed after oral administration. Human studies showed a range of 70 percent to 93 percent of the administered doses being absorbed after oral administration of chlorpyrifos (Nolan et al, 1984; Griffin et al, 1999). In rats, an average of 90 percent of the orally applied doses was absorbed (Bakke et al, 1976). However, a significant first-pass conversion of chlorpyrifos to its metabolites in the liver may contribute to the incomplete bioavailability of chlorpyrifos following oral exposure. Oral bioavailability was 41 percent in catfish, whereas it is believed to be substantially lower in mammals (Barron et al, 1991). The first-pass metabolism of chlorpyrifos transforms a large amount of chlorpyrifos into its metabolites. If the toxicity of chlorpyrifos is only mediated through its active metabolite chlorpyrifos oxon, then the first-pass metabolism should make chlorpyrifos appear more potent since it makes the oxon available earlier and in higher concentration than subcutaneous injection. However, as discussed earlier, recent studies showed that chlorpyrifos itself can also cause adverse effects. For some endpoints identified, such as the inhibition of DNA synthesis, chlorpyrifos seems to have a greater effect than chlorpyrifos oxon. The antimitotic effect of chlorpyrifos may lead to the loss

of neural cells, deterioration of brain function, and eventually, behavioral changes. Therefore, the first-pass metabolism of chlorpyrifos after oral administration may lead to a relatively lower toxicity and higher LOAEL for neurobehavioral effects compared to the subcutaneous injection.

In summary, despite its limitations, the current study still provides pivotal information on the developmental neurotoxicity of chlorpyrifos, although due to insufficient data, the current evaluation results may at some point need to be reevaluated.

II. Calculation of the chRD: Cholinesterase Inhibition Endpoint

As indicated above, both cholinesterase and non-cholinesterase related mechanisms contribute to the differential susceptibility between young and adults. To fully justify the proposed chRD, it is necessary to have separate calculations using different approaches. Plasma and RBC cholinesterase inhibition as an endpoint is widely used by agencies such as U.S. EPA and ATSDR to develop their reference dose for chlorpyrifos.

OEHHA considers the 2-year dog study conducted by Dow Chemical Company (McCollister et al, 1974) as the basis for derivation of chRD. This study is widely accepted by many agencies such as U.S. EPA and California Department of Pesticide Regulation (CDPR) and is a critical study used by U.S. EPA's Office of Pesticide Program to develop their RfD and Population Adjusted Dose, described above. A NOEL of 0.03 mg/kg/day was established based on reduced plasma and RBC ChE activity in the 0.1 mg/kg/day group. The calculation of the non-cancer chRD for chlorpyrifos is as follows:

$$\text{chRD} = \frac{\text{NOEL}}{\text{UF}} = \frac{0.03\,\text{mg/kg/day}}{300} = 0.0001\,\text{mg/kg/day}$$

Where, UF = Uncertainty factor of 300 (10 for intra-species variation, 3 for extrapolation from dogs to humans, and 10 as an additional uncertainty factor for children, since young animals were not tested).

As indicated above, there are age-related differences in the detoxification of chlorpyrifos; and the lack of enzymes to detoxify chlorpyrifos in young vs. adults raises concerns regarding possible increased sensitivity in children compared to adults. Although available studies only demonstrated age-related differences in chlorpyrifos toxicity after acute exposure, there is uncertainty surrounding chronic low dose exposure to chlorpyrifos. An additional safety factor for children is therefore necessary in terms of deriving a chRD. Therefore, OEHHA applied a 10x safety factor based on the age-related differences in the detoxification of chlorpyrifos and the uncertainty regarding the age-related differences in chlorpyrifos-induced cholinesterase inhibition [U.S. EPA's Office of Pesticide Program (OPP) applied a 10x safety factor for their Population Adjusted Dose in order to protect children and women who are at the child-bearing age].

Selected studies on the effects of chlorpyrifos on cholinesterase inhibition are listed in Table 2. Human studies are currently under review by U.S. EPA. Over 10 guideline studies were conducted in rats, dogs and mice. Among them, the dog is a sensitive indicator species for cholinesterase inhibition by chlorpyrifos (Zhao et al, 2006; U.S. EPA, 2000b; CDPR, 2000). U.S. EPA indicated in their chlorpyrifos reevaluation document that *dogs appear to be the most sensitive species for cholinesterase inhibition* (U.S. EPA, 2000b). California Department of Pesticide Regulation stated in their risk characterization document for chlorpyrifos that the dog *appeared to be more sensitive to chlorpyrifos than the rat* (CDPR, 2000). The NOEL in 90-day and 2-year dog studies was 1/3 that in the human male as shown in Table 2. While the chronic human NOEL is uncertain due to the small number of volunteer subjects and the short duration of this single human study, OEHHA believes that these results suggest that humans are unlikely to be significantly more sensitive than dogs to the cholinesterase-inhibiting effect of chlorpyrifos. OEHHA recommends an interspecies uncertainty factor of 3 rather than the default value of 10, based on a comparison of the NOELs for blood cholinesterase inhibition in dogs and humans.

OEHHA also considered two rat studies as the basis for derivation of the chRD. The first study is a 2-year rat study (Young and Grandjean, 1988). Fischer-344 rats (60 rats/sex/dose) were treated with chlorpyrifos by diets at 0, 0.05, 0.1, 1, or 10 mg/kg/day for 2 years starting at 6 weeks of age. Plasma and RBC cholinesterase activity was studied (10 rats/sex/dose) at 6, 12, 18, and 24 months, brain cholinesterase was studied at 12 months (10 rats/sex/dose) and 24 months (20 rats/sex/dose). Chlorpyrifos treatment at 10 mg/kg/day for up to 2 years decreased cholinesterase activity in plasma, RBC, and brain, while 1 mg/kg/day of chlorpyrifos only decreased cholinesterase activity in plasma and RBC. A NOEL of 0.1 mg/kg/day was established based on the reduced plasma and RBC cholinesterase activity. These results are consistent with those of the McCollister et al (1974) rat study as discussed above.

OEHHA used the NOEL of 0.1 mg/kg/day for plasma and RBC cholinesterase inhibition (McCollister et al, 1974; Young and Grandjean, 1988) to develop a chRD. The calculation based on the rat studies is as follows:

$$\text{chRD} = \frac{\text{NOEL}}{\text{UF}} = \frac{0.1\,\text{mg/kg/day}}{1000} = 0.0001\,\text{mg/kg/day}$$

Where, UF = Uncertainty factor of 1000 (10 for intra-species variation, 10 for extrapolation from rats to humans, and 10 as an additional safety factor for children, since young animals were not tested).

Table 2. Chlorpyrifos cholinesterase inhibition studies

Study	Species	Route	Duration	Endpoint	NOEL (mg/kg/day)	LOEL (mg/kg/day)
Coulston et al., 1972	human	oral	20 days	plasma	0.03	0.1
				RBC	not determined	not determined
McCollister et al., 1974	dog	oral	2 years	plasma	0.01	0.03
				RBC	0.03	0.1
Barker, 1989	dog	oral	90 days	plasma	0.01	0.22
				RBC	0.01	0.22
Crown et al., 1985	rat	oral	90 days	plasma	not determined	0.025
Crown et al., 1990	rat	oral	2 years	plasma	0.014	0.35
				RBC	not determined	not determined
Hoberman et al., 1998a,b	rat	oral	developmental neurotoxicity	plasma	not determined	0.3
				RBC	not determined	0.3
Young and Grandjean, 1988	rat	oral	2 years	plasma	0.1	1
				RBC	0.1	1
McCollister et al., 1974	rat	oral	2 years	plasma	0.1	1
				RBC	0.1	1
Szabo et al., 1988	rat	oral	90 days	plasma	0.1	1
				RBC	0.1	1

III. Conclusion

By this document, OEHHA establishes a child-specific reference dose of 0.0001 mg/kg/day for use in the assessment of risk at proposed or existing California school sites. This benchmark is based on cognitive deficiencies in young rats and cholinesterase inhibition in adult dogs and rats. Table 3 compares the proposed chRD for chlorpyrifos with existing health guidance values.

Table 3. Comparison of the proposed chRD with existing health criteria for chlorpyrifos

Organization	Endpoint	Study	Duration & Species	NOEL or LOAEL (mg/kg/day)	Uncertainty factor	Health Criterion (mg/kg/day)
U.S. EPA (IRIS) RfD	plasma ChE	Coulston et al., 1972	20 days human	0.03 (NOEL)	10	0.003
ATSDR MRL	plasma and RBC ChE	McCollister et al., 1974	2 years rat	0.1 (NOEL)	100	0.001
OEHHA chRD	RBC ChE	McCollister et al., 1974	2 years dog	0.03 (NOEL)	300	0.0001
	plasma and RBC ChE	Young and Grandjean, 1988; McCollister et al., 1974	2 years rat	0.1 (NOEL)	1000	0.0001
	Cognitive alterations	Jett et al., 2001	4-8 days rat	0.3 (LOAEL)	3000	0.0001
U.S. EPA (OPP[1]) RfD & PAD[2]	plasma and RBC ChE	McCollister et al., 1974; Barker, 1989; Crown, et al, 1985; Crown et al, 1990; Hoberman et al, 1998a, b	2 years dog; 90 days dog; 90 days rat; 2 years rat; developmental neurotoxicity rat	0.03 (NOEL)	100 (RfD)	0.0003 (RfD)
					1000 (PAD)	0.00003 (PAD)

1. OPP: Office of Pesticide Programs, U.S. EPA
2. PAD: Population Adjusted Dose (including additional FQPA safety factor = 10 for children and females 13-50 based on age-related difference in cholinesterase inhibition, qualitative difference between dams and adult offspring in the developmental neurotoxicity study, and uncertainties regarding the potential non-cholinergic adverse effects of chlorpyrifos, as described above).

As indicated in Table 3, the current chRD proposed by OEHHA is 1/3 of OPP's RfD but 3 times OPP's Population Adjusted Dose. OEHHA recommends a factor of 3 for the extrapolation from dogs to humans since dogs appear to be more sensitive than rats and

possibly as sensitive as humans for cholinesterase inhibition. OEHHA also considered other rat studies (Young and Grandjean, 1988; McCollister et al, 1974; Szabo et al, 1988) as the basis for deriving a chRD because of the consistent results observed in these studies.

As with all toxicity benchmarks, the chRD is subject to change if future studies indicate that changes are needed. Since a reference concentration or reference exposure level has not been established, OEHHA recommends that the chRD be used for comparison with exposures from all routes. The fact that studies using different exposure routes yielded the same chRD increases our confidence that such cross-route extrapolation is justified.

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