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

PROPOSED CHILD-SPECIFIC REFERENCE DOSES (chRDs) FOR SCHOOL SITE RISK ASSESSMENT – Cadmium, Chlordane, Heptachlor/Heptachlor Epoxide, Methoxychlor, and Nickel

FINAL DRAFT REPORT

August 2005

Integrated Risk Assessment Branch
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

Final Draft Report

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Office of Environmental Health Hazard Assessment
California Environmental Protection Agency
1001 I Street, 12th Floor
P.O. Box 4010
Sacramento, California 95812-4010

LIST OF CONTRIBUTORS

Authors

David Chan, D.Env., Susan A. Knadle Ph.D., DABT and
Kathleen Stewart

Primary Reviewers

David Siegel, Ph.D., DABT., Chief, Integrated Risk Assessment Section
Jim Carlisle, DVM, Senior Toxicologist, Integrated Risk Assessment Section

Secondary Reviewers

Joseph Brown, Ph.D.
Jim Donald, Ph.D.
Mari Golub, Ph.D., DABT
Poorni Iyer, Ph.D.
Robert Howd, Ph.D.
Lubow Jowa, Ph.D.
Susan Klasing, Ph.D.
David Morry, Ph.D
Tom Parker, M.S.
Karen Randles, M.S.

Library Support

Jerry McGovern

Website Posting

Laurie Monserrat

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Proposed chRDs for School Site Risk Assessment

Executive Summary

As mandated by Part 2 of the Health and Safety Code, section 901(g), the Office of Environmental Health Hazard Assessment (OEHHA) reviewed five chemicals to consider the development of child-specific reference doses (chRDs). This report summarizes OEHHA's review of pertinent scientific studies in proposing these chRDs . Any chRDs established as a result are intended for use in the risk assessment of proposed or existing California school sites.

OEHHA completed Part 1 of that mandate, which called for the identification of chemical contaminants commonly found at school sites and determined to be of greatest concern to children. The 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," was posted on OEHHA's website in June, 2002. In summary, OEHHA identified seventy-eight chemicals that will likely be found as contaminants of California school sites and have the potential for causing adverse effects in children. This should be viewed as a living compilation – chemicals may be added or removed as new information becomes available.

OEHHA chose five chemicals from the compilation for an in-depth evaluation of non-carcinogenic effects: cadmium, chlordane, heptachlor and its metabolite heptachlor epoxide, methoxychlor, and nickel. The criteria used to select these chemicals for the first round of reviews are discussed in Chapter 1.

In reviewing the applicable scientific literature, OEHHA identified relevant quantitative studies from which to propose a chRD for each chemical. The chRD for the non-carcinogenic effects of cadmium is based on a 1990 study by Buchet et al. The authors reported a strong relationship between cadmium body burden and renal tubular dysfunction in adult humans. This study identified a lowest observed adverse effect level (LOAEL) of 1×10^{-3} mg/kg-day. From this LOAEL, OEHHA calculated a chRD of 1×10^{-5} mg/kg-day using an uncertainty factor of 90 (10 to account for human variability, 3 to extrapolate from the LOAEL to the no observed adverse effect level (NOAEL), and a child protective factor of 3 was used to account for the child/adult difference in gastrointestinal absorption of cadmium). A factor of 3 (rather than the usual default of 10) was used for extrapolating from a LOAEL to a NOAEL because the LOAEL was based on the minimal adverse effect observed.

The chRD for the non-carcinogenic effects of chlordane is based on a 1994 study by Cassidy et al. The authors demonstrated changes in sex-steroid mediated behaviors, including increased male-typical spatial abilities in female rats and increased male-typical mating behaviors in male rats, following pre- and postnatal exposure. This study identified a LOAEL of 0.1 mg/kg-day, from which OEHHA calculated a chRD of 3.33 x 10^{-5} mg/kg-day using an uncertainty factor of 3000 (10 for interspecies variability, 10 for human variability, 10 to extrapolate to the LOAEL from the NOAEL, and a modifying factor of 3 to account for an inadequate hematotoxicity/immunotoxicity and neurotoxicity database—toxicities to which children may be particularly sensitive).

The chRD for the non-carcinogenic effects of heptachlor is based on two studies. One is a 2001 study by Moser et al., which shows decreased performance on measures of cognitive function in male rats following pre- and postnatal exposure, through postnatal day 21. The other is a 2001 study by Smialowicz et al., which shows suppression of the primary IgM and secondary IgG antibody responses following exposure during the last half of gestation through puberty. Both studies identified a LOAEL of 0.03 mg/kg-day. OEHHA calculated a chRD of 3 x10^{-5} mg/kg-day using an uncertainty factor of 1000 (10 each for interspecies variability, human variability, and extrapolation from LOAEL to NOAEL). The chRD for the non-carcinogenic effects of heptachlor epoxide utilizes the same study selected by U.S. EPA for its reference dose (RfD) and OEHHA for its Public Health Goal (PHG.) A LOAEL of 0.0125 mg/kg-day for liver-to-body weight ratio was reported when adolescent dogs were fed heptachlor epoxide for 60 weeks (Dow Chemical Co., 1958). Since exposure was to adolescent animals, OEHHA utilized the U.S. EPA RfD for its chRD of 1.3 x 10^{-5} mg/kg-day and utilized the same uncertainty factor of 1000 (10 each for interspecies variability, human variability, and extrapolation from LOAEL to NOAEL).

The chRD for the non-carcinogenic effects of methoxychlor is based on two studies as well. One is a 1995 study by vom Saal et al., which demonstrates increased urine marking in male mice, an index of territorial behavior, subsequent to prenatal exposure. The other is a 1999 study by Welshons et al., which shows an increase in adult prostate size following prenatal exposure. The LOAEL identified from these studies is 0.02 mg/kg-day. OEHHA calculated a chRD of 2 x10^{-5} mg/kg-day using an uncertainty factor of 1000 (10 each for interspecies variability, human variability, and extrapolation from LOAEL to NOAEL).

The chRD for the non-carcinogenic effects of nickel is based on the observed pup mortality in three reproductive studies – Smith et al., 1993, and Springborn Laboratories, 2000 a and b. In reviewing these three studies in totality, OEHHA concludes that the 1.1 mg nickel/kg-day (5 mg nickel sulfate hexahydrate/kg-day) dose constitutes the appropriate NOAEL. From this NOAEL, OEHHA calculated a chRD of 11 x 10^{-3} mg/kg-day, using an uncertainty factor of 100 (10 each for interspecies extrapolation and human variability).

Table ES 1 below compares the chRDs and U.S. EPA's RfD, which are based on studies in adult animals.

Table ES 1 OEHHA's chRD and U.S. EPA's RfD

	OEHHA's Proposed chRD (mg/kg-day)	U.S. EPA's RfD (mg/kg-day)
Cadmium	1×10^{-5}	5×10^{-4}
Chlordane	3.3×10^{-5}	5×10^{-4}
Heptachlor	3×10^{-5}	5×10^{-4}
Heptachlor epoxide	1.3×10^{-5}	1.3×10^{-5}
Methoxychlor	2×10^{-5}	5×10^{-3}
Nickel	11×10^{-3}	2×10^{-2}

These proposed chRDs were reviewed internally. OEHHA is currently releasing this draft report for external peer review and public comment. Any chRDs established by this process are intended for use in risk assessment of proposed or existing school sites in California.

1. Introduction

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).

1.1 Developing a chRD or chRC

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 (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 most part existing reference doses or concentrations for non-cancer endpoints, which were based on adult human or animal data, were used. The Food Quality and Protection Act of 1996 (http://www.epa.gov/opppsps1/fqpa/) was an attempt to address the issue of children sensitivity. It mandated a safety factor of 10 unless data existed to indicate that children were not more sensitive than adults. Moreover, 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.

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 are a challenge, OEHHA has strived to do so because children can be more (or less) susceptible to chemical effects due to pharmacodynamic and pharmacokinetic differences between them and adults, and thus empirical data in the young would be preferable. 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/adolescent exposures or adult consequences from childhood exposure. Thus, the state of scientific knowledge pertaining to chemical effects on children is and will continue to be a limiting factor in OEHHA's ability to develop child-specific HGVs for these contaminants.

The evaluation of empirical data in the young can be a complex task. Vulnerability of the young 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 its critical period(s), a particular structure or function is most sensitive to disruption due to interactions between a toxicant and target tissues that are undergoing biochemical changes. 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 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.

OEHHA faces an additional challenge when evaluating chemicals that are potential endocrine disruptors. The topic of endocrine disruption during development has been the subject of much scientific and regulatory debate (Colborn *et al*. 1993a; Colborn *et al*. 1993b; Cranmer *et al*. 1984; US EPA, 1998). While not all chemicals selected for the OEHHA review are endocrine disruptors, the endocrine disruptors do pose a greater concern because not only could they directly impact the maturation and proper functioning of the endocrine system, they could also interfere with hormonal signal transduction that leads to abnormal growth and functioning of other target organs (e.g., immune and nervous systems) in school children. Exposure to endocrine disruptors during critical "programming" periods in development, in contrast to exposure during adulthood, may produce irreversible effects on the reproductive, nervous, and/or immune systems (Bigsby *et al*. 1999). In adulthood, these endocrine disruptors might only produce reversible effects by participating in the "seesaw" process of stimulation and feedback inhibition.

Given the complexity of hormone signaling processes, it is also not surprising to find the evaluation of the dose and response relationship to be another challenge. The shape of the dose response curve may not be linear, but rather shaped like an upright U or an inverted U (Markowski *et al*. 2001; vom Saal *et al*. 1997). This makes data interpretation difficult when the study does not include sufficient treatment doses to span the entire range of interest.

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.

From a public health protection standpoint, the OEHHA scientists working on health guidance values for children as mandated by Health & Safety Code 901(g) have adopted the following procedures in evaluating and developing chRDs or chRCs. First, in order to protect children from infancy through the time they leave school, chRDs must consider school-aged children up to age 18, and infants and toddlers in daycare facilities located at school sites. Second, OEHHA opts to consider 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 deemed 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 would likely 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 pups (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 to occur 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 vulnerability of development in young animals so that an appropriate uncertainty or safety factor can be applied.

2. Evaluation of Five Chemicals

This chapter is divided into five sections, one for each chemical reviewed in 2002. Each section provides information specific to the particular chemical, including background and exposure information; how the chemical meets our criteria for evaluation; existing pertinent health guidance values; findings from our literature review; and OEHHA's recommendation. The recommendation includes a discussion of the study (or studies) used in developing the chRD, the uncertainty and modifying factors used, the calculation, and the proposed chRD.

2.1 Cadmium

Cadmium is an important industrial chemical with diverse applications. It is used for the production of nickel-cadmium batteries, pigments, alloys, plastics, and synthetics. It is also used in metal plating. The Toxic Chemical Release Inventory of 2000 shows that 2,292 pounds of cadmium were emitted into the air, 792 pounds were discharged into surface water, 69,000 pounds were injected underground, and 663,895 pounds were released to land in the U.S. In comparison, 16 pounds of cadmium entered the air and 36,104 pounds were disposed of on land in California during 2000 (U.S. EPA, TRI2000).

Given its indestructible nature, cadmium persists in the environment, and can enter the food chain. OEHHA identified air, drinking water, soil, and food as the primary pathways for human exposure to cadmium (OEHHA, 1999a). U.S. EPA and ARB/DHS have deemed cadmium as a chemical of interest in their NHEXAS and Portable Classroom Study, respectively (OEHHA, 2002). ARB reported the occurrence of cadmium in California air and DTSC reported the presence of cadmium at 10 percent of the potential school sites reviewed by the Department, making it a relatively frequently observed contaminant.

In reviewing literature for establishing a Public Health Goal (PHG) for cadmium in drinking water (OEHHA, 1999a), OEHHA found some evidence that cadmium may elevate blood pressure in both animals and humans. Renal toxicity of cadmium is well known. Cadmium tested positive in several mutagenic assays and in an epidemiological study; it was observed that individuals with higher levels of cadmium in their urine (>3 μg/L) had more frequent chromosomal aberrations in their lymphocytes. A number of studies in rats and mice indicated the developmental and reproductive toxicity of cadmium. Neurological and immune effects were also reported. Finally, tumors of the prostate, testes and hematopoietic system in the rat were associated with oral cadmium exposure; and human lung and prostate cancers had been associated with inhalation exposure. Thus, it is regarded as a potential human carcinogen by the oral route and a human carcinogen by the inhalation route.

Recent studies on metal-responsive transcription factor-1 (MTF-1) and its role in regulating the expression of metallothionein genes suggested that these proteins are essential to cadmium detoxification and thus they may affect cadmium toxicity (Masters et al., 1994; Wang, Y. et al., 2004; Gunes, C. et al., 1998). It appears that MTF-1 is highly conserved; whereas, a number of metallothionein isoforms has been identified. Genetic variability of metallothioneins in individuals may cause different susceptibilities.

OEHHA selected cadmium for an in-depth review in this first cycle not only from the standpoint of its exposure potential at school sites, but also because of its adverse effects on various organ systems; some of which are still undergoing development in school children.

Pertinent Guidance Values

U.S. EPA RfD: 0.5 µg/kg-day (water) and 1.0 µg/kg-day (food)

U.S. EPA's RfD is based on cadmium's effect on the kidney. A concentration of 200 µg cadmium (Cd)/g wet human renal cortex is the highest renal level not associated with significant proteinuria (U.S. EPA, 1985). A toxicokinetic model is available to determine the level of chronic human oral exposure (NOAEL) which results in 200 µg Cd/g wet human renal cortex; the model assumes that 0.01percent day of the Cd body burden is eliminated per day (U.S. EPA, 1985). Assuming 2.5 percent absorption of Cd from food or 5 percent from water, the toxicokinetic model predicts that the NOAEL for chronic Cd exposure is 0.005 and 0.01 mg Cd/kg-day from water and food, respectively (i.e., levels which would result in 200 µg Cd/g wet weight human renal cortex). Thus, based on an estimated NOAEL of 0.005 mg Cd/kg-day for Cd in drinking water and an uncertainty factor (UF) of 10 that accounts for intra-human variability, an RfD of 0.0005 mg Cd/kg-day (water) was calculated; an equivalent RfD for Cd in food is 0.001 mg Cd/kg-day.

U.S. EPA gives a high confidence to its cadmium RfD. The choice of NOAEL does not reflect the information from any single study. Rather, it reflects the data obtained from many studies on the toxicity of cadmium in both humans and laboratory animals. These data also permit calculation of pharmacokinetic parameters of cadmium absorption, distribution, metabolism and elimination.

OEHHA PHG: 0.07 µg/L (a safe dose of 1 x 10^{-5} mg/kg-day)

OEHHA deemed the Buchet investigation (Buchet et al., 1990) as the best study for use in developing a Public Health Goal (PHG) for cadmium in drinking water. The study avoided the healthy worker effect by performing a cross-sectional examination on 1699 Belgian subjects between the ages of 20 and 80 years. The investigators found a strong relationship between cadmium body burden and renal tubular dysfunction. They observed a risk of renal effects at or above the urinary excretion rate of 2 µg cadmium/24 hours. Assuming an oral absorption rate of 5 percent and a daily excretion rate of 0.005 percent of body burden, Buchet estimated that this excretion rate corresponded to a mean renal cortex concentration of about 50 ppm or 50 µg/g (wet weight). In non-smokers

(investigators' design to subtract a major source of cadmium from tobacco smoke), this level is reached after 50 years of an oral daily intake of 1.0 μg/kg body weight. As such, a LOAEL of 1.0 μg/kg-day was established.

OEHHA (1999a) applied this LOAEL in conjunction with an aggregated uncertainty factor (UF) of 100 (10 for intra-human variability, 3 for LOAEL to NOAEL extrapolation, and 3 for uncertainty in applying adult biokinetics to the entire age range from infancy to adulthood) for calculating a safe dose of 0.01 μg/kg-day. A factor of 3 (rather than the usual default of 10) was used for extrapolating from a LOAEL to a NOAEL because the LOAEL was based on the minimal adverse effect observed. The safe dose in turn was used to derive the PHG.

Current Evaluation Results

Because the cadmium data have recently been reviewed under the PHG process, we used the PHG review as a baseline for the current evaluation. Accordingly, we focused our literature search and review on the information that was not covered by the PHG evaluation. An attempt was also made to target literature pertaining to cadmium's effect on sensitive organ systems that are still undergoing postnatal development.

Based on the above search criteria, we compiled a list of references. From that list, we identified a number of papers relevant to cadmium's effects on testes and semen of rabbits exposed before and after puberty (Foote, 1999); pubertal and postpubertal cadmium exposure on the hypothalamic-pituitary-testicular axis function in rat (Lafuente et al., 2000); cadmium induction of apoptosis in the immune system (Tsangaris et al., 1998); postnatal cadmium exposure and long-term behavioral changes in rat (Smith et al., 1982); effects of cadmium and lead on cognitive functioning in children (Thatcher et al., 1982); and neurotoxic effects of cadmium in young rats (Wong et al., 1982). However, these were either qualitative/mechanistic, semi-quantitative, or quantitative studies with a LOAEL higher than that on which the PHG was based.

Recommendation

The renal effect of cadmium seems to be the most sensitive endpoint (with the lowest LOAEL), even when it is compared with developmental and reproductive study endpoints identified in the OEHHA PHG document or those identified by OEHHA's current evaluation. The rate of cadmium absorption affects its rate of accumulation in the kidney, and in turn its toxicity. Thus, available data suggest the PHG LOAEL should be retained for our current consideration. This LOAEL is lower than the NOAEL used by U.S. EPA in developing its RfD. Both the safe dose from which the PHG was derived and U.S. EPA's RfD are based on cadmium's effect on renal function. In developing these two health criteria, long-term cumulative exposure data were used. Both U.S. EPA and Buchet et al. applied a 5 percent absorption in their respective biokinetic modeling, based on adult human absorption of 4.7 to 7 percent of the cadmium intake (Rahola et al., 1972, cited in Mahaffey, 1983). In proposing the PHG, OEHHA applied a factor of 3 to

account for the uncertainty associated with Buchet's modeling in which he applied adult biokinetics to the entire age range from infancy to adulthood. According to Alexander et al. (1974), the absorption of cadmium by children, from early infancy through 8 years of age, averages 55 percent.

To illustrate the appropriateness for applying the factor of three for developing the chRD, OEHHA ran a model similar to Buchet, using his modeling assumptions. Using a daily dose of 1 µg/kg, an absorption rate of 5 percent, and a daily clearance rate of 0.005 percent of body burden, OEHHA estimated that the urine excretion of 2 µg cadmium /24 hour (LOAEL biomarker) would be reached by age 53, which was in good agreement with Buchet's study. However, by changing the absorption rate to 55 percent through age 8, then decreasing it linearly to 5 percent at age 21, while keeping the other parameters the same, a daily dose of 0.51 µg/kg would be required to produce an urine excretion of 2 µg cadmium /24 hour by age 53. Thus, using child-specific absorption results in a difference of about 2-fold in the daily dose to produce the LOAEL effect. To be public health protective, OEHHA proposes to apply a child-protective factor of 3 to account for childhood absorption differences.

Calculation of the non-cancer chRD for cadmium is based on the following equation:

$$\text{chRD} = \frac{\text{LOAEL}}{\text{UF x CP}} = \frac{1\ \mu\text{g/kg-day}}{30 \times 3} = 0.01\ \mu\text{g/kg-day}$$

Where,

LOAEL = Lowest-observed-adverse-effect-level from Buchet et al., 1990.

UF = Uncertainty factor of 30 (10 for intra-human variability, 3 for LOAEL to NOAEL extrapolation because the LOAEL is based on the minimal effect observed and this is consistent with that applied to calculate the PHG).

CP = Child protective factor of 3 to account for the GI absorption difference between children and adults.

Accordingly, OEHHA is proposing a chRD of 0.01 µg/kg-day for cadmium's non-cancer effect to be used in school-site risk assessment instead of the U.S. EPA's RfD that did not account for a greater GI absorption of cadmium by children.

2.2 Chlordane

Chlordane is a cyclodiene pesticide, one of many organochlorine insecticides. Chlordane was used in large quantities until the U.S. EPA issued a notice of suspension except for use on subterranean structural termite control in 1976 (McConnachie and Zahalsky, 1992). It was banned for all uses in the United States in 1988, but it is still manufactured for export. Like DDT, it persists in the environment, and it is considered a priority persistent, bioaccumulative toxic (PBT) chemical by U.S. EPA (U.S. EPA, 2002).

Chlordane is not a pure chemical pesticide, and all studies investigating its toxicity or mechanism of action have used technical grade chlordane, which is a mixture. Infante et al. (1978) analyzed technical chlordane and reported that it contained 38-48 percent cis- and trans-chlordane, 3-13 percent heptachlor, 5-11 percent nonachlor, 17-25 percent other chlordane isomers, and a small amount of other compounds. Dearth and Hites (1991) identified 147 different compounds in a preparation of technical chlordane that included cis-chlordane (15 percent), trans-chlordane (15 percent), trans-nonachlor (15 percent), and heptachlor (3.8 percent).

OEHHA included chlordane in the "Compilation of Chemicals Potentially Found at School Sites" because it has been targeted by federal and state agencies as a chemical that may present environmental health risks. Chlordane appears on all but one of the chemical compilations that OEHHA has selected to identify chemicals that may be found at school sites. These compilations include:

- Soil contaminants identified at potential school sites in environmental investigations reviewed by the Department of Toxic Substances Control
- Toxic Air Contaminants (TACs) in California identified by OEHHA
- Analytes in the Department of Health Services/Air Resources Board (DHS/ARB) Portable Classroom monitoring study
- Analytes in the U.S. EPA National Health Exposure Assessment Study (NHEXAS)

Chlordane was placed by OEHHA in the compilation of "Candidate Chemicals Based on Critical Health Effects" because 1) it is on the Proposition 65 Developmental and Reproductive Toxin List and 2) a survey of recent scientific literature indicated that it possesses toxicity to organ systems that are developing in children, including the immune system, neuroendocrine and female reproductive systems (Ahmed, 2000; Barone et al., 2000; Barnett et al., 1990; Blyler et al., 1994; Brucker-Davis, 1998; DeRosa et al., 1998; Holladay et al., 2000; Holladay, 1999; Luster et al., 1990; Olea et al., 1998; Reigart, 1995; Spyker-Cranmer et al., 1982; Theus et al., 1992a and 1992b; Voccia et al., 1999). Chlordane exposure has also been associated with childhood cancer (Zahm et al., 1998.)

OEHHA staff prepared a PHG for chlordane in 1997 (OEHHA, 1997). The study on which the PHG is based showed that chlordane acted as an endocrine disruptor and

altered sex steroid-mediated behaviors when exposure occurred during gestation and lactation (Cassidy et al., 1994).

Endocrine disruptors, such as chlordane, are the subject of recent scientific and regulatory concern (U.S. EPA, 1998). They mimic or antagonize estrogens, androgens, and thyroid hormones, as well as their antagonistic analogs, and consequently disrupt the processes or tissues these hormones affect. Organ systems responsive to the sex steroids include the male and female reproductive organs, the central nervous system, and the immune system. The thyroid hormones affect most tissues (Bigsby, 1999). They are of particular concern in regard to children's health because they may disrupt the action of estrogen, androgen and thyroid hormones during critical periods of development and lead to permanent alterations in the reproductive, nervous, and immune systems that are developing during prenatal growth and childhood (Bigsby, 1999).

Existing Health Guidance Values

U.S. EPA Carcinogen Slope Factor: 3.5×10^{-1} per mg/kg-day

Chlordane is classified as B2; probable human carcinogen, using the 1986 Guidelines for Carcinogen Risk Assessment (Integrated Risk Information System (IRIS), 2003, http://www.epa.gov/iris/subst/0142.htm#carc). IRIS also reports that "under the 1996 Proposed Guidelines, it would be characterized as a likely carcinogen by all routes of exposure. These characterizations are based on the following summaries of the evidence available: (1) human epidemiology studies showing non-Hodgkin's lymphoma in farmers exposed to chlordane and case reports of aplastic anemia; chlordane associated with home use are inadequate to demonstrate carcinogenicity; (2) animal studies in which benign and malignant liver tumors were induced in both sexes of four strains of mice and occurred with an elevated, but not statistically significant, incidence in a fifth strain, as well as liver toxicity but no tumors in rats of two strains; and (3) structural similarity to other rodent liver carcinogens." The U.S. EPA oral slope factor is 3.5×10^{-1} per mg/kg-day. This value represents the geometric mean for five data sets with a range from individual data sets of 1.1×10^{-1} to 8.6×10^{-1} using the linearized multistage model (http://www.epa.gov/iris/). The EPA IRIS data base reported that the studies are of good quality and "the confidence is high that chlordane is a mouse liver carcinogen at dietary concentrations above 10 ppm. Although there is indication that the dose-response curve is sublinear in the dose region between 5 and 60 ppm, linearity at low doses cannot be ruled out on theoretical grounds. The tentative evidence is that the hematopoietic system, rather than the liver, is the target organ in humans."

U.S. EPA RfD: 5×10^{-4} mg/kg-day

The oral RfD established by U.S. EPA is 5×10^{-4} mg/kg-day based on a NOAEL of 0.15 mg/kg-day and LOAEL of 0.75 mg/kg-day in a mouse study (Khasawinah and Grutsch, 1989). The critical effect for the LOAEL was liver necrosis, with an uncertainty factor of 300 (10 for interspecies extrapolation, 10 for human variability, and 3 for deficiencies in

the database). The overall confidence given this RfD assessment is medium, both for the quality of the principal study and the sufficiency of the database. The principal study, assigned a confidence of medium, is a rat chronic oral study performed with relatively large group sizes, in which histopathological analyses on the known animal target tissue, the liver, were thoroughly performed. However, the discussion in IRIS stated that "available occupational studies, although limited, give no indication that the liver is a target organ in humans as a consequence of chronic exposure to low levels of chlordane" (http://www.epa.gov/iris/subst/0142.htm#umfinhal).

IRIS also reports that "recent evidence indicates that neurotoxicity, a known human endpoint in acute exposures, may be a relevant endpoint in chronic human exposures, and no chronic animals studies have examined neurotoxicity. Studies on pre-and postnatal animals indicating chlordane mimicry of sex-steroids raise reproductive concerns and no multigenerational reproductive studies, by any route, exist. Thus, there is some concern that the appropriate endpoints have not been examined adequately in the existing database." IRIS further states that "an area of scientific uncertainty in this assessment concerns the role of neurotoxicity, and possibly hematotoxicity, in chronic chlordane toxicity in humans." IRIS also notes that "another area of scientific uncertainty in this assessment concerns the toxicological significance of endocrine mimicry effects of chlordane. Toxicity data for this chemical include a study demonstrating biochemical and behavioral alterations consistent with technical chlordane (or its metabolites) mimicking male sex-steroids (Cassidy et al., 1994). That these effects could include reproductive behaviors is suggested in this study" (http://www.epa.gov/iris/subst/0142.htm#quaoral).

Studies on these endpoints would be of concern for children's health because accidental poisoning studies by chlordane in children have reported neuropsychiatric symptoms, which included learning disabilities, at an incidence four times that found in the general population according to the National Center for Health Statistics (Sherman, 1999). In20 poisoned children, 20 percent had hematological problems and an additional 15 percent had hematological dyscrasias which may be early indicators of leukemia and aplastic anemia (Sherman, 1999).

OEHHA PHG: 0.02 ppb (a safe dose of 1×10^{-5} mg/kg-day)

The PHG developed by OEHHA is 1×10^{-5} mg/kg-day, or 0.02 ppb in drinking water, based on a LOAEL of 0.1 mg/kg-day because of disruption of sex steroid-mediated behaviors in rat identified by Cassidy et al., 1994. The health-protective drinking water concentration for carcinogenic endpoints is calculated to be 0.03 ppb. The U.S. EPA drinking water unit risk is 1×10^{-5} per (µg/L) which translates into risk levels of 10^{-4} to 10^{-6} at concentrations of 3 ppb and 0.03 ppb, respectively (U.S. EPA, 1996).

Current Evaluation Results

Chlordane has been shown to have critical effects on two developing systems due to endocrine disruption. It adversely affects the developing immune system of mice (Spyker-Cranmer et al., 1982; Barnett et al, 1985a; Barnett et al., 1985b; Barnett et al.,

1990; Theus et al., 1992a; Theus et al., 1992b; Blyler et al., 1994), and it alters sex-mediated neurobehavioral endpoints (Cassidy et al., 1994). These effects on the developing endocrine and immune systems show an age-related susceptibility to chlordane. Adult animals exposed to similar or higher doses of chlordane did not show similar effects (Johnson et al., 1986; Barnett et al., 1990; Barnett, 1997).

An endocrine disruptor such as chlordane can act at the level of the hypothalamic-pituitary-adrenal (HPA) axis, disrupting the negative feedback loop between the brain and the immune system. Under normal physiological conditions, activation of the immune system stimulates the release of cytokines, which can then act on the HPA axis to trigger the release of corticosterone. However, if increased levels of corticosterone are released, due to the presence of endocrine disruptors, these high corticosterone levels produce immuno-suppression on virtually all levels of the immune system (Gaillard and Spinedi, 1998; Morale et al., 1995), including depression of the delayed-type hypersensitivity response (Okimura et al., 1986), suppression of granulocyte and macrophage migration (Mizobe et al., 1997), and inhibition of hematopoietic cytokines such as IL-3 and CFU-GM (Gaspar Elsas et al., 2000 and Mucha et al., 2000).

In addition, high levels of glucocorticoids can disrupt all aspects of the hypothalamic-pituitary-gonadal (HPG) axis, including reproductive behavior and the synthesis and release of sex steroids (Viau, 2002) and can interfere with the functioning of the hippocampus, the part of the brain responsible for learning and memory (Kim and Diamond, 2002). It has been shown that the release of adrenocorticotropic hormone (a hormone that stimulates the release of corticosterone) is associated with increased sexual excitation in male rats (Szechtman et al., 1974), and Bowman and colleagues (2001) demonstrated that female rats exposed to stress-induced increases in corticosterone levels showed altered spatial memory performance.

A key finding, suggesting that chlordane disrupts the HPA and HPG axis, was the observation that exposure of the dihybrid mice dams to 0.16 mg/kg-day of analytical reference standard chlordane (which has the same products as technical grade chlordane) from 0-18 days of gestation (Table 2.2.1) produced significantly elevated corticosterone in male and female offspring when they were assayed as adults at 100 and 400 days of age (Cranmer et al., 1984). This indicated a permanent (or long-lasting) effect on the offspring. This dose of technical grade chlordane also reduced metabolism of corticosterone in female BALBc mice and elevated resting plasma corticosterone in male mice at 100 days of age (Spyker-Cranmer et al., 1982). Corticosterone, like cortisol, is synthesized from progesterone by a series of hydroxylations. Testosterone is also synthesized from progesterone, and estradiol is synthesized from testosterone (Stryer, 1981). Chlordane can alter corticosterone levels, and corticosterone is an intermediate in the synthesis of steroids. By this mechanism, chlordane can affect the developing immune system, and it could permanently alter characteristic differences between males and females in non-reproductive and reproductive measures (such as body weight, development of sexual organs, circulating steroid levels, mating behavior, spatial abilities, activity level, or mixed function oxidase levels (Weiss, 2002). The endocrine

disruptive effect of chlordane appears to be corroborated by the study of Cassidy and colleagues (Cassidy et al., 1994) in which a dose of 0.1 mg/kg-day chlordane to the dam and then to the pups until postnatal day 80 caused sex steroid-mediated changes in gender-specific behaviors and functions (Cassidy et al., 1994). There was a dose-responsive decrease in plasma testosterone, which was significant at 5 and 0.5 mg/kg-day, but not significant at 0.1 mg/kg-day.

Table 2.2.1 Summary of Significant Studies on Chlordane

Reference	Protocol	Doses	Critical Effects
Spyker-Cranmer et al., 1982	Pregnant BALB/C mice were dosed until day 18 of gestation and pups nursed on their natural mothers until 21 days of age	0.16 and 8 mg/kg maternal body weight	Delayed Type Hypersensitivity (DTH) was significantly depressed at 8 mg/kg; and depressed but not significantly at 0.16 mg/kg
Cranmer et al., 1984	Pregnant F2 Dihybrid mice were dosed until day 18 of gestation and pups nursed on their natural mothers until 21 days of age.	0.16 and 8 mg/kg maternal body weight	Plasma corticosterone was significantly elevated at 101 days and 400 days in male mice whose mothers were dosed with 0.16 mg/kg-day. It was elevated in female mice at 400 days of age
Barnett et al., 1990	Pregnant BALB/C mice were dosed until day 18 of gestation and pups nursed on their natural mothers until 21 days of age	4 and 8 mg/kg maternal body weight	Hematopoietic stem cells (CFU-GM and CFU-S) in offspring were significantly decreased at 100 and 200 days of age. Adult animals treated with 8 mg/kg chlordane did not have any decrease or differ from controls.
Cassidy et al., 1994	Sprague-Dawley CD rats were dosed from Day 4 of gestation until Day 21 of lactation. Pups were dosed individually from post natal day (PND) 22 until PND 80.	0.1, 0.5, and 5 mg/kg maternal body weight	Females had significant improvements in spatial abilities in the Cincinnati Water Maze test at all doses, males exhibited dose-dependent increases in male-typical mating behavior, and both exhibited maximum response to auditory startle at 0.1 mg/kg when tested at 80 days.

The effects of chlordane on the developing immune system, and their persistence into adulthood, were demonstrated in a series of related studies using prenatal and postnatal exposure to chlordane (Spyker-Cranmer et al., 1982; Barnett et al, 1985a; Barnett et al., 1985b; Barnett et al., 1990; Blyler et al., 1994; Theus et al., 1992a; Theus et al., 1992b Tyrhonas et al, 2003). The experimental protocol common to all the studies was to feed pregnant mice 0.3 mg of peanut butter which was spiked with technical chlordane to provide a maternal dose of 0.16 mg/kg, 4 mg/kg, 8 mg/kg or 16 mg/kg maternal body

weight, although not all doses were used in the assay of each immune system parameter. The pups were allowed to

nurse through day 21. Assays of immune system parameters were performed at various postnatal days, ranging from day 42 to day 200, although not each immune system parameter was assayed at each postnatal time point. Chlordane is fat-soluble, having a log K_{ow} (octanol-water coefficient) of 5.16, so it should be readily transferred from plasma to milk. The total amount of chlordane reaching the pups was determined to be 3.5 mg/kg by analyzing chlordane and its metabolites in the conceptus and in pups at intervals during gestation and through the end of lactation (Theus et al., 1992)

Immune responses, such as delayed type hypersensitivity (DTH), were significantly depressed in offspring at 100 days of age after exposure in utero to a maternal dose of 8 mg/kg-day body weight. A maternal dose of 0.16 mg/kg-day also depressed DTH, although not significantly (Spyker-Cranmer et al., 1982). Pups received chlordane from 0-18 days of gestation, when the mother was dosed, and through 21 days of nursing, when dosing of the mother had ceased. Thus, the pup's exposure dose was actually lower than either 8 mg/kg-day or 0.16 mg/kg-day for its exposure duration.

A decreased DTH response occurs due to functional abnormalities in T lymphocytes, specifically the CD4 T_H1 helper cells. There are three kinds of effector T cells: cytotoxic CD8 T cells, which kill infected cells, and two kinds of CD4 T cells (T_H1, or T helper 1, and T_H2) with different functions (Parham, 2000). It is noteworthy that a decrease in the number of helper/inducer T cells is found in acquired immune deficiency (AIDS) disease, and this decrease is thought to allow infections such as Kaposi's sarcoma, Pneumocystis carinii pneumonia, and cytomegalovirus (CMV) retinitis (Lane and Fauci, 1985).

A critical effect on the developing immune system was a significant reduction in the number of granulocyte-monocyte committed stem cells (CFU-GM) and multipotential stem cells (CFU-S) in adult offspring (100 and 200 days of age) of pregnant mice exposed to 4 mg/kg and 8 mg/kg chlordane (Barnett et al., 1990). The bone marrow of offspring exposed to 4 or 8 mg/kg-day chlordane had 63 percent and 75 percent of control CFU-GM at 100 days of age, and at 50 percent and 77 percent at 200 days of age in offspring exposed to 4 mg/kg-day. The multipotential stem cells (CFU-S) were similarly depressed. Female and male offspring exposed prenatally to 8 mg/kg chlordane had 67 percent and 64 percent respectively of control CFU-S, while those exposed to 4 mg/kg-day chlordane had 78 percent and 87 percent of control CFU-S. At 200 days of age the bone marrow CFU-S in female offspring was almost unchanged, and that in males was still significantly reduced. This significant reduction in stem cells that divide and differentiate into mature functional blood cells could produce life-threatening consequences. This decrease, as well a decrease in interleukin-3 (IL-3) stem cells, was confirmed to be present at 42-49 postnatal days when a specific recombinant growth factor was utilized to stimulate differentiation, rather than mouse lung conditioned media containing a mixture of different growth factors (Blyler et al., 1994). IL-3 is a cytokine produced by T helper (T_H1 and T_H2) cells and it is a growth factor for multipotential progenitor hematopoietic cells (Parham, 2000). This toxicity endpoint is significant for humans because blood dyscrasias and bone marrow failures have been reported in people following accidental dermal or inhalation exposure to chlordane at unspecified dose

levels (Infante et al., 1978; Klemmer et al., 1977; Furie and Trubowitz, 1976). Also significant was the gender-specificity when the specific recombinant growth factor was used, as only female offspring had a decrease in myeloid lineage cells. A study on the immune effects of cis-nonachlor, trans-nonachlor and chlordane in adult male and female Sprague-Dawley rats following a 28-day oral gavage treatment also demonstrated gender specific effects on a wide range of immunologic endpoints (Tryphonas et al, 2003).

The studies on the gender specific effects of chlordane on the immune system are also seen in sexually dimorphic behaviors when mice are exposed pre-and postnatally to technical chlordane. Female mice that were exposed to technical chlordane pre- and postnatally acquired male behaviors, and males offspring showed significant increases in male-typical mating behaviors, suggesting that the cyclodienes in technical chlordane mimic sex steroids and/or change their levels (Cassidy et al, 1994).

Clonogenic assays for hematopoietic progenitors have been used in clinical hematology for 30 years (Parent-Massin, 2001) and in research to predict adverse effects of drugs or toxicants, as the rapid rate of cell renewal and differentiation makes the hematopoietic system a susceptible target for xenobiotic toxicity. Xenobiotics that interfere with cell proliferation and differentiation can lead to "bone marrow failure." The two major groups of bone marrow failure are aplastic anemia, where the failure lies in the pluripotent stem cell (colony forming unit – stem cell or CFU-S), and single cytopenia, where the failure lies in the stem cell for one of the committed cell lines, such as the granulocyte/monocyte cell line, the CFU-GM (Parent-Massin, 2001). Most bone marrow failures are characterized by inadequate production of blood cells and, if severe, death of the organism results because existing numbers of stem cells have an inadequate ability to produce mature cells to provide oxygen (anemia), clot blood (thrombocytopenia), or to protect the organism from infection.

Endocrine disruptors such as chlordane can affect neuroendocrine/neurobehavioral endpoints, as well as immune endpoints. The studies of Cassidy and colleagues (Cassidy et al., 1994) confirmed that perinatal chlordane could mimic sex steroids and /or change their levels to masculinize sexually dimorphic functions and behaviors. They dosed pregnant rats with technical chlordane at 0.1 mg/kg, 0.5 mg/kg, and 5 mg/kg during gestation, and they dosed the offspring during 21 days of lactation and from postnatal day 22 to postnatal day 80. Female offspring committed fewer errors than controls in three assays of cognitive and spatial ability in the Cincinnati Water Maze test, appearing to behave more like males, and male offspring exhibited dose-dependent increases in male-typical mating behaviors. The differences in behavior compared to unexposed animals demonstrate that sexual differentiation of the neuroendocrine system has been altered by early life exposure to chlordane. The neuroendocrine-gonadal axis regulates the developmental organization and adult expression of behaviors critical for mammalian survival and reproduction (competitive aggression, exploration, and sexual and parental behaviors), so neurobehavioral alterations induced by endocrine disruptors may impact the survival and fitness of an individual in its environment (Palanza et al., 2002)

Recommendation

Based on studies that describe endocrine disruption and effects on the developing hematopoietic, immune and neuroendocrine systems in young animals, OEHHA recommends that a chRD be developed. The critical effects are alterations in characteristic behavior differences between males and female at doses of 0.1 mg/kg-day maternal body weight and 0.1 mg/kg-day pup weight (Cassidy et al., 1994), and disruption of the hematopoietic and immune systems at a maternal dose as low as 0.16 mg/kg-day maternal body weight (Cranmer et al., 1984).

OEHHA recommends that a non-cancer child-specific RD (chRD) be calculated on the study by Cassidy and colleagues (Cassidy et al., 1994) that showed that a chlordane dosage of 0.1 mg/kg-day (to the pups, as well as the mother) disrupted sex hormone

mediated behaviors. Differences from control were significant at the lowest dose, indicating that 0.1 mg/kg dose is a LOAEL. Because these effects are indicative of endocrine disruption, it is possible that the hematopoietic/immune effects described in the other studies may also occur at this low dose.

Calculation of the non-cancer child-specific RD for chlordane is based on the following equation:

$$\text{chRD} = \frac{\text{LOAEL}}{\text{UF}} = \frac{0.1 \text{ mg/kg-day}}{3000} = 3.33 \times 10^{-5} \text{ mg/kg-day}$$

Where,

LOAEL = Lowest Observed Adverse Effect Level (Cassidy et al, 1994)

UF = Uncertainty factors of 3000 (10 for LOAEL to NOAEL, 10 for interspecies extrapolation, 10 for human variability, and 3 for inadequate database for hematotoxicity, immunotoxicity, neurotoxicity, and the lack of a valid developmental study).

Accordingly, OEHHA is proposing a non-cancer chRD of 3.3×10^{-5} mg/kg-day for chlordane.

Uncertainty and Modifying Factors

OEHHA has applied the additional uncertainty factor 3 for inadequacies in the database for each of three endpoints, hematotoxicity, neurotoxicity, and reproductive toxicity that data suggest may be of concern to human children. This uncertainty factor has been applied in accordance with U.S. EPA (1994) and Renwick et al., (2000), that it is appropriate "if a valid developmental toxicity study was not performed", or "if the study did not examine all developmental endpoints". In the discussion of chlordane on the IRIS database, U.S. EPA noted that "studies on pre-and postnatal animals indicating chlordane mimicry of sex steroids raise reproductive concerns and no multigenerational reproductive studies, by any route, exist. Thus, there is some concern that the appropriate endpoints have not been examined adequately in the existing database" (http://www.epa.gov/iris/subst/0142.htm#umfinhal).

The U.S. EPA RfD was based on hepatic necrosis in mice, even though the discussion on the IRIS database noted that "occupational studies, although limited, give no indication that the liver is a target organ in humans as a consequence of chronic exposure to low levels of chlordane." U.S. EPA reduced confidence in their RfD noting that "an area of scientific uncertainty concerns the role of neurotoxicity and possibly hematotoxicity in chronic chlordane toxicity in humans" (http://www.epa.gov/iris/subst/0142.htm#umfinhal). Neurotoxicity and hematotoxicity

have been reported as principal endpoints of acute chlordane toxicity in both experimentally poisoned animals and accidentally poisoned humans (Grutsch and Khasawinah, 1991; Fleming and Timmeny, 1993). The uncertainty increases when considering exposure of children, rather than adults, because these organ systems are undergoing critical development during childhood.

The reduced numbers of hematopoietic stem cells in offspring, after they had reached maturity, from exposure to a 4 mg/kg-day maternal dose (Barnett et al., 1990a), provides low confidence that a LOAEL which produced minimally significant adverse hematological effects was identified. Immune system toxicity from chlordane is a concern because a report of outcomes from exposure of 20 children to chlordane from pesticide applications noted that 20 percent had hematological problems and an additional 15 percent had hematological dyscrasias (Sherman, 1999). Blood cell dyscrasias are a concern to clinicians because they may later manifest themselves as leukemias and aplastic anemias. The outcome of reduced numbers of stem cells can be bone marrow failure.

The database on chlordane toxicity to children is also considered inadequate because no animal studies have adequately assayed neurotoxicity due to low chlordane exposure concentrations, and there are case reports of human neurotoxicity (Kilburn and Thornton, 1995; Kilburn, 1997). Al-Hachim and Al-Baker (1973) reported that when pregnant mice were exposed to 1 mg/kg-day technical chlordane for only seven consecutive days the pups had poor learning ability or altered motivation in the assay for conditioned avoidance response, raised seizure threshold, and increased exploratory activity. Reports of accidental human exposure to termiticides have resulted in neurobehavioral impairments in adults (Kilburn and Thornton, 1995; Kilburn, 1997) and 70 percent of 20 child patients exposed to chlordane had neuropsychiatric symptoms, which included learning disabilities at an incidence four times that found in the general population, according to the National Center for Health Statistics (Sherman, 1999). School-age children were reported to develop new problems: headaches, visual difficulties, hyperactivity, learning disabilities, frequent ear-nose-throat and chest problems, and gastrointestinal disturbances (Sherman, 1999).

The Food Quality Protection Act required a 10 fold safety factor be applied "for infants and children" for pesticide risk assessments "to take into account…completeness of the data with respect to … toxicity" and OEHHA utilized the 10 fold factor in creating a public health goal (PHG) for chlordane. However, U.S. EPA has been limiting the composite factor to 3,000 when human-equivalent doses are used (U.S. EPA, 1994). As the low dose in the Cassidy et al. (1994) study, which forms the basis for the child-specific RfD, was based on serum levels found in the United States at the 99th percentile (i.e. 1% of the U.S. values are higher), OEHHA decided to utilize only a 3-fold modifying factor to account for the inadequacies in the database.

Additional Comments/Studies:

The experiments from Barnett and colleagues (Spyker-Cranmer et al., 1982; Barnett et al, 1985a; Barnett et al., 1985b; Barnett et al., 1990; Theus et al., 1992a; Theus et al., 1992b; Blyler et al., 1994) support an equivalent or lower dose than the one derived from the Cassidy et al., 1994 study. The experimental protocol of Barnett and colleagues differed from that of Cassidy et al., 1994, in that the pup was not individually dosed. A maternal dose of 8 mg/kg-day was reported to produce a *total* dose of 3.5 mg/kg of chlordane in the pup (Theus et al., 1992). If an equal fraction of 3.5 mg/kg dose were delivered each day of the 18 days of gestation, when the mother was dosed, and the 21 days of lactation, when the mother was not dosed, the pup would receive 0.09 mg/kg-day over the 39 day period. This estimated pup dose is very close to the 0.1 mg/kg-day dose that Cassidy et al. (1994) gave to dams, *and* to pups following weaning and until sacrifice on day 80.

The experiment of Cranmer et al. (1984) suggests that the LOAEL could be lower than 0.1 mg/kg-day. In this study, the dams were dosed with either 0.16 mg/kg-day or 8 mg/kg-day chlordane during gestation. As noted above, the maternal dose of 8/mg/kg-day was shown to produce a total dose of 3.5 mg/kg in the pups when the concentration of chlordane metabolites analyzed on successive days during the 39 days of gestation and lactation were totaled. The average daily dose to the pup was 0.09 mg/kg-day. If the toxicokinetics from the 0.16 mg/kg-day maternal dose is proportional to that from the 8 mg/kg-day maternal dose, then the pups of a dam dosed with 0.16 mg/kg-day (Cranmer et al., 1984) would receive 0.0018 mg/kg-day. Emerging understanding of the hypothalamic-pituitary-adrenal (HPA) axis substantiates the possibility that a low dose may impair the developing immune system.

2.3 Heptachlor/Heptachlor Epoxide

Heptachlor (heptachlorodicyclopentadiene) was used primarily as an agricultural insecticide from 1952 to 1976, as a narcissus bulb and seed treatment and insecticide for fire ant control on pineapple crops until 1976, and as a treatment for subterranean termites until 1987 (Fendick et al., 1990). In 1985, heptachlor alone or in combination with chlordane, accounted for 60-65 percent of the termiticides used in the U.S. (EPA 1987 – see Fendick et al.). In 1987, the EPA and the Agency for Toxic Substances and Disease Registry (ATSDR) classified heptachlor as a priority Group 1 Hazardous Substance, making Superfund money available for cleanup of heptachlor-contaminated sites.

Technical heptachlor contains heptachlor plus related reaction products in approximately a 5:2 ratio (Fendick et al., 1990). Heptachlor is a moderately persistent compound (Ware et al., 1990). In the soil it undergoes multiple transformation and degradation reactions by at least three pathways: epoxidation, hydrolysis, and dechlorination. Epoxidation generates the more persistent and bioaccumulative metabolite, heptachlor epoxide, while hydrolysis is a detoxification reaction (Fendick et al., 1990). U.S. EPA has considered heptachlor and heptachlor epoxide two separate chemicals, and it has established separate RfDs, probably because heptachlor epoxide absorbs strongly to soil and is extremely resistant to biodegradation (Hazardous Substances Databank, http://toxnet nlm.gov), persisting in soils for a long time (Ware, 1988).

OEHHA included heptachlor/heptachlor epoxide in the "Compilation of Chemicals Potentially Found at School Sites" because it has been targeted by federal and state agencies as a chemical that may present environmental health risks. Heptachlor appears on all but two of the chemical compilations that OEHHA has selected to identify chemicals that may be found at school sites. These compilations include:

- Soil contaminants identified at potential school sites in environmental investigations reviewed by the Department of Toxic Substances Control
- Toxic Air Contaminants (TACs) in California identified by OEHHA
- Analytes in the U.S. EPA National Health Exposure Assessment Study (NHEXAS)

OEHHA also included heptachlor/heptachlor epoxide in the compilation of "Candidate Chemicals Based on Critical Health Effects" because heptachlor epoxide is on the Proposition 65 Developmental and Reproductive Toxicant List, and a survey of recent scientific literature indicated that heptachlor and heptachlor epoxide are toxic to organ systems that are developing in children. These organ systems are the immune, nervous, endocrine, and male and female reproductive systems (Brucker-Davis, 1998; DeRosa et al., 1998; Moser et al., 2001; Nicolopoulou-Stamati et al., 2001; Rani et al., 1995; Smialowicz et al., 2001; Voccia et al., 1999). Heptachlor and heptachlor epoxide were also reported to produce cancer (Zahm et al., 1998 and http://toxnet.nlm.nih.gov).

Existing Health Guidance Values

U.S. EPA Carcinogen Slope Factor: 4.5 per mg/kg-day

Heptachlor is classified by EPA as a B2, probable human carcinogen, based on several studies. Davis (1965) fed groups of 100 male and 100 female C3H mice diets with 0 or 10 ppm heptachlor (purity not specified) for 2 years. Survival was low, with 50 percent of the controls and 30 percent of the treated mice surviving until the end of the experiment. A two-fold increase in benign liver lesions over the controls was reported. After a histologic reevaluation, Reuber (as cited in Epstein, 1976), as well as four other pathologists, remarked a statistically significant increase in liver carcinomas in the treated male (64/87) and female (57/78) groups by comparison to controls (22/73 and 2/53 for males and females, respectively). The NCI (1977) reported a significant dose-related increase of hepatocellular carcinomas in male and female B6C3F1 mice.

U.S. EPA RfD: 5×10^{-4} mg/kg-day

The current oral RfD for heptachlor given by U.S. EPA in 1991 is 5×10^{-4} mg/kg-day (http://toxnet.nlm.nih.gov). This value was derived from a three ppm dietary NOAEL in a two-year rat feeding study where the critical effect was liver weight increase (Velsicol Chemical, 1955, cited by U.S. EPA http://toxnet.nlm.nih.gov/). The LOAEL in this study was 5 ppm or 0.25 mg/kg-day and an uncertainty factor of 300 was employed. EPA reports that there is low confidence that this RfD is accurate because the principal study is of low quality; the database on chronic toxicity is incomplete. There are no teratology, reproductive, or studies in young animals.

An RfD for heptachlor epoxide was based on a study in which adolescent dogs were fed heptachlor epoxide for 60 weeks (Dow Chemical Company, 1954). The LOAEL of 0.5 ppm (0.0125 mg/kg-day) was based on an increased liver-to-body weight ratio in both males and females as a critical effect (Dow Chemical Co., 1958, cited in U.S. EPA IRIS online file, http://toxnet.nlm.nih.gov/). An uncertainty factor of 1000 was employed. EPA indicates there is low confidence that the RfD is accurate because the principal study is of low quality, and the chronic toxicity studies are of low quality. There were no rat or rabbit teratology studies.

OEHHA PHG: 8 ppt (a safe dose of 1×10^{-4} mg/kg-day)

OEHHA staff prepared a Public Health Goal (PHG) for heptachlor of 8×10^{-6} mg/L drinking water, based on a cancer slope factor of 4.1 mg/kg-day $^{-1}$ and a 1×10^{-6} cancer risk (OEHHA, 1999b). Heptachlor exposure produced a dose-related increase in the incidence of hepatocellular carcinoma in male and female B6C3F1 mice (NCI, 1977) and hepatocellular carcinoma in male and female C3H mice (Davis, 1965).

In developing a PHG for heptachlor, OEHHA (1999) considered two studies when non-cancer effects were reviewed. The first was the two-year rat feeding study where the critical effect was liver weight increase that was used by EPA when it last revised the heptachlor RfD (Velsicol Chemical, 1955, cited by U.S. EPA, http://toxnet.nlm.nih.gov). Uncertainty factors of 10 for interspecies variability and 10 for interindividual variability were used. The second study (Cassidy et al., 1994) reflected the recent concern about the endocrine disruption effects of chlorinated cyclodiene and other chlorinated pesticides. The critical effect in this study was the alteration of sex steroid-mediated behaviors by prenatal and early-in-life exposure to 0.1mg/kg/day technical chlordane which contains 10 percent heptachlor. In this calculation, the uncertainty factors are: LOAEL to NOAEL extrapolation (10), interspecies variability (10), and interindividual variability (10), resulting in a "safe" non-cancer human dose of 1×10^{-4} mg/kg/day

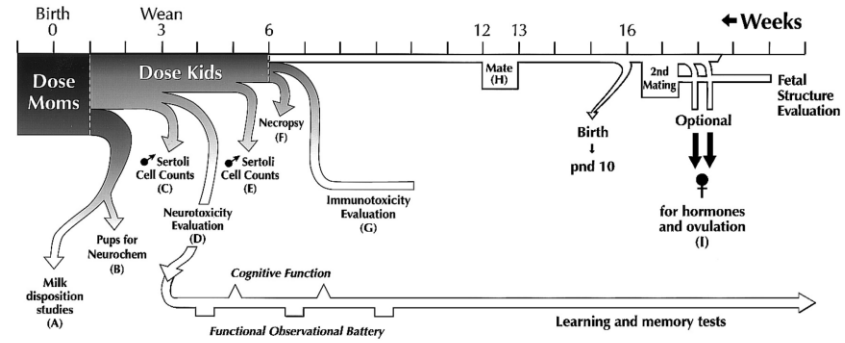
Current Evaluation Results

The effects of heptachlor that are specific for children are its disturbance of the development of the endocrine system and of the organs that respond to endocrine signals when exposure occurs during prenatal and/or early postnatal life (Colborn, 1993). These effects are permanent.

Contamination of the commercial milk supply of Oahu, Hawaii, with heptachlor for 15 months, from 1981 to 1982, and the subsequent finding of heptachlor epoxide in human milk, prompted new studies on rats to look for possible effects of heptachlor and its persistent primary metabolite at the concentrations to which children were exposed. The Hawaii Heptachlor Research and Education Foundation (HHREF) cosponsored these studies with the U.S. EPA and NIEHS in order to evaluate many aspects of the impact of heptachlor exposure during the perinatal/juvenile period of development, using a broad battery of tests of immune and reproductive system function.

The doses (0, 0.3, 3, or 30 mg/kg-day of 99% pure heptachlor) employed were adjusted so that the low dose gave milk values of heptachlor epoxide that approximated the 95^{th} percentile of human milk heptachlor and heptachlor epoxide values in Oahu, Hawaii in 1981 (Baker et al., 1991; Siegel, 1988 in Smialowicz et al., 2001). The period of exposure was designed to approximate the last trimester of pregnancy through 18 years of age in humans. The experimental design for the studies of endocrine disrupting effects on immune, neurobehavioral, and reproductive is given in Figure 2.3.1.

Figure 2.3.1 Experimental Design



	A	B	C	D	E	F	G	H	I
♀	8 (F_0)	10		10		~10	12	15	20
♂		10	8	10	8	~10	12	15	

Figure taken from Moser et al., 2001 and Smialowicz et al., 2001)

The results of one subset of this study (Smialowicz et al., 2001) indicated that exposure of rats to heptachlor during the last trimester of gestation through puberty adversely affects adult functioning of the immune system by suppressing the primary IgM and secondary IgG antibody response in male offspring. Rats were exposed to 0, 0.03, 0.3, or 3.0 mg/kg-day from gestation day 12 to postnatal day 7, followed by direct dosing through postnatal day 42. The LOAEL was identified as 0.03 mg/kg-day because the primary IgM antibody response to Sheep Red Blood Cells (SRBCs), as measured by enzyme linked immunosorbent assay (ELISA) was suppressed at 8 weeks of age and at 21 weeks of age. At the same dose, the secondary IgG response was also suppressed at 25 weeks of age. These responses require three major immune cell types: macrophages, the CD4+ T-helper cells, and B cells. Alterations in or dysfunction of any of these cells and cell interactions can result in aberrant antibody production (Luster et al., 1988 in Smialowicz, 2001). The suppression of these T cell-dependent antibody responses persisted through the first six months of life at all doses employed, including the lowest dose, 0.03 mg/kg-day, which was administered through 6 weeks of age.

The response to SRBC is one of the most sensitive functional parameters in animals exposed to immunosuppressants (Luster et al., 1992). Consequently, it is included in the battery of tests required by the Federal Insecticide, Fungicide, and Rodenticide (FIFRA) guidelines for detection of immunosuppressants (Smialowicz, 2001). In both animals and humans, T-cell dependent responses are involved in protection against viral, bacterial, and parasitic infections (Blanden, 1974 in Smialowicz, 2001). Consequently, the suppression of the primary IgM and secondary IgG antibody responses suggests potential increased susceptibility to infectious diseases.

In another subset of rats from the same large study, heptachlor produced significant differences in tests for cognitive abilities that are associated with the development of neuroendocrine pathways (Moser et al., 2001). Rats were evaluated for neurological and behavioral alterations using a functional observational battery (FOB), an automated measure of motor activity, passive avoidance, and a Morris water maze test (Moser et al., 2001). Rats dosed prenatally and postnatally until day 21 had changes in activity measures, but those in which dosing continued until day 42 had alterations in autonomic, neuromuscular, and excitability measures. The most pronounced effects of heptachlor occurred in rats treated until day 42 and tested with the Morris water maze test. The Morris water maze test (Morris, 1984) was devised to resolve theoretical controversies about the basis of spatial and working memory. Normal rats learn very quickly to swim directly towards a platform from any starting position at the circumference of a pool. The accurate directionality of their escape behavior provide evidence that the rats escape by learning the position of the platform relative to distal cues. Thus, their performance can be compared to those of animals exposed to potential neurotoxins to assay spatial learning and memory (Morris, 1984).

Heptachlor exposure slowed acquisition of the spatial task and impaired recall during probe trials: the treated male rats at all dose levels did random searching for the platform, rather than developing an efficient search strategy. Working memory, which was assayed by requiring the rats to learn a new position for the platform each day, was significantly decreased in the low dose (0.03 mg/kg-day) male rats which had been dosed with heptachlor prenatally and postnatally until Day 21. The escape latency (mean time to find the new location) was 27.9 seconds compared to 20.5 seconds in control (Smialowicz, et al., 2001).

Cyclodiene pesticides bind to the chloride channel portion of the receptor for the neurotransmitter gamma aminobutyric acid $(GABA)_A$, block the inhibitory actions of and thus affect a variety of neurological functions in both adult and young animals (Abalis et al., 1986; Cole and Casida, 1986; Gant et al., 1987 in Moser, 2001). Acute actions of cyclodiene pesticides include excitation, hyperstimulation, and convulsions (Cole and Casida, 1986; Fendick et al., 1990). In young mammals, the development of the nervous system is quite protracted, and specific processes of migration, proliferation, and differentiation occur from gestation throughout childhood and into adolescence. These processes occur in sequence, so disturbance of earlier processes can disrupt later developmental events. Cyclodiene insecticides alter expression of the $GABA_A$ receptor. Since the neurotransmitter, GABA, influences development of serotonergic, dopaminergic, and cholinergic neurotransmitter systems, cyclodiene pesticides may produce long-lasting alterations in brain function (Lauder et al., 1998 in Smialowicz et al., 2001).

Recommendation

Heptachlor:

The experiments of Smialowicz et al. (2001) and Moser et al. (2001) describe several critical effects in young male rats at a LOAEL of 0.03 mg/kg-day heptachlor during the last half of gestation and the first 21 or 42 postnatal days. The most significant effects include suppression of the primary IgM and secondary IgG antibody response (Smialowicz et al., 2001), and decreased performance on measures of cognitive function, such as impaired recall (Moser et al., 2001).

Technical grade heptachlor and chlordane are mixtures of pure compound plus related reaction products (Ware, 1990). The PHG developed by OEHHA in 1999 utilized a study (Cassidy et al., 1994) that described a disruption of sex-steroid mediated behaviors in female mice at a dose of 0.01 mg/kg-day of technical grade chlordane, which contains 10 percent heptachlor. More recent studies (Smialowicz et al., 2001, Moser et al, 2001), which used heptachlor of 99 percent purity, have allowed OEHHA staff to develop the chRD for heptachlor without the ambiguity associated with testing a mixture.

Calculation of the non-cancer chRD is based on the following equation:

$$\text{chRD} = \frac{\text{LOAEL}}{\text{UF}} = \frac{0.03 \text{ mg/kg-day}}{1000} = 3.0 \times 10^{-5} \text{ mg/kg-day}$$

Where,

LOAEL = Lowest Observed Adverse Effect Level from Smialowicz et al., 2001 and Moser et al., 2001

UF = Uncertainty factor of 1000 (10 for LOAEL to NOAEL, 10 for inter-species extrapolation, 10 for human variability)

Accordingly, OEHHA is proposing a non-cancer chRD of 3.0×10^{-5} mg/kg-day for heptachlor.

Heptachlor Epoxide**:**

Heptachlor has not been used since 1987 when its use was restricted. Heptachlor in the soil undergoes multiple transformation and degradation reactions, and epoxidation generates the more persistent and bioaccumulative metabolite, heptachlor epoxide (Fendick et al., 1990), so children at school sites may be exposed to heptachlor epoxide.

The principal study used by U.S. EPA to calculate an RfD (http://toxnet.nlm.nih.gov) was a 60-week dog feeding study (Dow Chemical Co., 1958) in which the LOAEL was an increased liver-to-body weight ratio. Although liver-to-body weight ratio is not a child-

specific endpoint, the exposure period began in adolescence and continued into young adulthood.

Since adolescent animals were exposed, OEHHA has decided to utilize the same study and the same uncertainty factors to calculate a non-cancer child-specific RD as the U.S. EPA RfD:

$$\text{chRD} = \frac{\text{LOAEL}}{\text{UF}} = \frac{0.0125 \text{ mg/kg-day}}{1000} = 1.3 \times 10^{-5} \text{ mg/kg-day}$$

Where,

LOAEL = Lowest Observed Adverse Effect Level from Dow Chemical Co, 1958

UF = Uncertainty factor of 1000 (10 for LOAEL to NOAEL, 10 for inter-species extrapolation, 10 for human variability)

Accordingly, OEHHA is proposing a non-cancer chRD of 1.3×10^{-5} mg/kg-day for heptachlor epoxide.

2.4 Methoxychlor

Methoxychlor, 2,2-bis(p-methoxyphenyl)-1,1,1-trichloroethane, is structurally related to DDT. Because of its lower toxicity and bioaccumulation potential, methoxychlor became an attractive replacement of DDT (ATSDR, 1994). It was registered as an insecticide against a wide range of pests, including houseflies, mosquitoes, cockroaches, and various arthropods commonly found on fields crops, vegetables, fruits, stored grain, livestock, and domestic pets.

While DTSC's school site review efforts continue, methoxychlor has already been detected at a school site. Recent studies show that demethylated metabolites of methoxychlor are an estrogenic agent and an endocrine disruptor. Accordingly, OEHHA believes it is important to further review methoxychlor pursuant to Health and Safety Code Section 901(g). As endocrine disruptors, methoxychlor metabolites may have adverse effects on different developing organ systems. These chemicals may disrupt the development and functioning of the female reproductive system, and the brain, and the male reproductive system (vom Saal et al., 1983, 1997; Nonneman et al., 1992; Hess et al., 1997). The endocrine disruption effect of methoxychlor has also been demonstrated in a non-human primate model (Golub et al., 2003). As such, the effect of methoxychlor or its metabolites on school children is a concern.

Earlier studies may have inadequately characterized the dose-response relationship of methoxychlor (NTP, 2001). In reviewing environmental estrogens, the NTP Peer Review Subpanel found that overall the classic estrogenic activity of methoxychlor was limited to doses greater than 5 mg/kg-day because testing at lower doses had not been incorporated into the experimental design. An updated review of the pertinent literature is necessary to ensure that the appropriate LOAEL or NOAEL will be considered in setting a child-specific guidance value.

Pertinent Guidance Values

U.S. EPA RfD: 0.005 mg/kg-day

U.S. EPA used the 1986 Kincaid Enterprises, Inc. study to establish its RfD. Young adult female New Zealand White rabbits were assigned into 3 dose groups of 17 animals each, 5.01, 35.5, and 251.0 mg/kg-day, and a control group (a total of 68 animals). The females were artificially inseminated and the day of insemination considered as gestation day 0. All animals were dosed from days 7 through 19 of gestation. All surviving dams were sacrificed on gestation day 29.

Maternal toxicity was observed as excessive loss of litters (abortions) and statistically significant decreases in body weight in the mid- and high-dose groups, and the deaths in the high dose group. No specific toxicity was noted in the low dose (5.01 mg/kg-day), which was deemed to be the NOAEL.

An uncertainty factor of 1000 was applied to the NOAEL in developing the RfD; of which 100 was used to account for the inter-and intra-species differences and an additional 10 was used to account for the poor quality of the critical study and for the incomplete database on chronic toxicity.

OEHHA PHG: 0.03 mg/L (a safe dose of 0.005 mg/kg-day)

In reviewing literature for the purpose of establishing a Public Health Goal (PHG) for methoxychlor in drinking water (OEHHA, 1999c), OEHHA found the chemical to be negative in several mutagenicity tests. However, a positive test was reported for the induction of forward mutations in the mouse lymphoma assay. Large doses of methoxychlor decreased locomotor activity and caused tremors. Reproductive effects have been caused by the estrogenic activity of the o-demethylated metabolites of methoxychlor. These metabolites also bind estrogen receptors in animal and human tissues.

OEHHA identified the investigation by Chapin et al. (1997) as the most relevant study for use in developing a PHG for methoxychlor in drinking water. The investigation focused on effects of perinatal methoxychlor exposure on adult rats' nervous, immune, and reproductive system function. Dams were dosed orally at gestation day 14 through postnatal day (pnd) 7 and then pups were directly dosed at pnd 7 through pnd 42 at dosages of 0, 5, 50, and 150 mg/kg-day. Critical effects included a reduction in serum FSH, ovary weight and uterine weight at all dosages. The findings suggested that methoxychlor, as an exogenous estrogenic agent, had interfered with the normal programming of the ovarian-pituitary axis.

Applying the LOAEL of 5 mg/kg/day and an uncertainty factor of 1000 (10 each for inter-species extrapolation, intra-human variability, and LOAEL to NOAEL extrapolation), OEHHA calculated a safe dose of 0.005 mg/kg-day. This in turn was used to derive a PHG of 0.03 mg/L.

Current Evaluation Results

Stoker et al. (1999) investigated the effect of perinatal exposure of methoxychlor on the prostate of adult rat. The study showed that a perinatal dose of 50 mg/kg methoxychlor to the dam only from gestation day 18 to postnatal day 5 resulted in offspring with increased lateral prostate weight and inflammation at 90 days of age.

Welshons et al. (1999) reported increases in adult prostate size in mice from fetal exposure to methoxychlor. Females were dosed from day 11 to day 17 of pregnancy at 20 or 2000 μg/kg maternal body weight per day. Pups were weaned on postnatal day 23. When males reached 8.5 months old (adult), a randomly selected male from each litter was individually housed for 4 weeks to eliminate any effects of having been housed with other males before the selected male was sacrificed for various examinations. Prostatic weights were significantly increased in the 20 and 2000 μg/kg groups; however, the

methoxychlor effect is greater in the lower dose group. This observation is corroborated by a recent study (Golub et al., 2003), supporting the view that methoxychlor could produce a non-linear (non-monotonic) dose response.

Table 2.4.1 Effects of Methoxychlor on Prostate Weight

	Prostate (mg)
Control	40.0±3.0
Methoxychlor (20 μg/kg)	64.5±3.7
Methoxychlor (2000 μg/kg)	60.3±4.1

The finding of prostate enlargement is not surprising as the prostate contains both androgen and estrogen receptors (Kumar et al., 1995) and it has been observed that estrogen can stimulate the growth of the stromal compartment of the prostate (Ekman, 2000). In Marker et al.'s review, the authors discuss that prostatic development is very sensitive to levels of estrogenic compounds (Marker et al., 2003). Male rodent embryos that have been exposed to relatively higher serum estradiol and lower testosterone have increased expression of androgen receptor in the prostate and larger prostate in adulthood. Overall, the Welshons study demonstrates that exposure of methoxychlor during a critical developmental window has resulted in an irreversible effect on the prostate.

vom Saal et al. (1995) discussed evidence that during fetal life, hormones have marked effects on subsequent behaviors. Male mice are particularly active in urine-marking behavior to indicate their social status. Urine marking was used as the end point to measure the effect of methoxychlor. Females received 0, 1, 10, 100, 1000, or 5000 μg/d from day 11 to day 17 of pregnancy. Two males from each litter were randomly selected when they were 60 days old and housed individually for four weeks to eliminate any effects of having been housed with other males. Urine-marking tests were conducted for one hour in clean cages with the floor lined by a sheet of Whatman No. 2 filter paper. The filter paper was then removed and discrete urine marks (which fluoresce under UV light) deposited on it were counted. The lowest dose (1 μg/day or 20 μg/kg-day based on 0.05 kg maternal weight) of methoxychlor significantly increased urine-marking behavior in male offspring.

The increased in urine marking behavior observed by vom Saal et al. could reflect an increased reactivity to novel environment or could be an index of heightened territoriality (aggression). However, a followup study showed that methoxychlor did not induced male territorial aggression by measuring residents' attack on intruders and the intensity of attacks (Palanza et al., 1999). Another study, on the other hand, illustrated that methoxychlor had no effects on males in terms of open field exploration, locomotor activity, and rearing, which were indices of exploratory activity and novelty seeking (Palanza et al., 2002).

Recommendation

OEHHA recommends that a chRD for methoxychlor be developed based on the data from Welshons et al. (1999). While the exposure period used to demonstrate the significant effect on the prostate was in the fetal period, OEHHA feels that the prostate data are applicable for school age children. The human prostate development is biphasic, with much of the growth occurring at puberty. It is small (weighs about 2 g) in childhood and undergoes exponential growth to about 20 g at puberty (Hayward et al., 2000). This system remains vulnerable during the K-12 schooling period. Exposure to methoxychlor during the postnatal period may result in the abnormal development and maturation of the prostate. Therefore, the Welshons data are an appropriate basis for evaluating hazards at schools.

OEHHA has also considered the appropriateness of using the maternal dose to calculate the chRD. OEHHA finds that methoxychlor crosses the placenta and partitions into the lipids of milk (OEHHA, 1999). It is likely that the corresponding pup dose is higher on a per kilogram body weight basis. However, the demethylated (phenolic) metabolites rather than methoxychlor were shown to be the active species that displayed the endocrine disruption potential. The polar metabolites would not cross the placenta effectively. Additionally, methoxychlor that crosses the placenta would not be metabolized effectively by the pup whose P-450 enzymes are not fully developed. Thus, it would not be too conservative to use the maternal dose in this case to calculate the chRD.

Calculation of the non-cancer chRD for methoxychlor is based on the following equation:

$$\text{chRD} = \frac{\text{LOAEL}}{\text{UF}} = \frac{20\ \mu\text{g/kg-day}}{1000} = 0.02\ \mu\text{g/kg-day}$$

Where,

LOAEL= Lowest-observed-adverse-effect-level based on Welshons et al. (1999)

UF= Uncertainty factor of 1000 (10 for inter-species extrapolation, 10 for intra-human variability, and 10 for LOAEL to NOAEL extrapolation)

Accordingly, OEHHA is proposing a non-cancer chRD of 0.02 μg/kg-day for methoxychlor to be used in school-site risk assessment.

2.5 Nickel

Nickel, an important industrial metal, comprises 0.008 percent of the earth's crust (Duke, 1980 as cited in ATSDR, 1997). The production, use, and disposal of nickel have led to its mobilization in the environment and human exposure. Nickel is used in aircraft frames, jet engines, gas turbines, and turbosuperchargers, boats, hulls, propellers, and pumps (OEHHA, 2001). Nickel alloys are used in pumps and pipes to resist corrosion in petro-chemical industries. In addition, nickel is used in making coins and jewelry; as catalysts; and in magnets, batteries, and color pigment. Nationwide in 2000, 651,000 pounds of nickel were emitted into the air (with nickel plating operations as a major source of emission), 30,000 pounds were discharged into surface water, 17,000 pounds were injected underground, 2,032,000 pounds were disposed of onsite, and 8,700,000 pounds were disposed of offsite (U.S. EPA, TRI2000).

Nickel was selected for further evaluation pursuant to Health and Safety Code Section 901(g) because it meets both criteria for selection identified in OEHHA's 2002 report (OEHHA, 2002):

- DTSC reported the presence of nickel at two percent of the potential school sites evaluated to date. ARB reported its occurrence in California air (OEHHA, 2002). In addition, U.S. EPA and ARB/DHS have deemed nickel as a chemical of interest in their NHEXAS and Portable Classroom Study, respectively.

- OEHHA (2001) found a number of studies concerning the reproductive effects of nickel compounds. Nickel also adversely affected the immune functions in animals. The administration of nickel to rats increased the concentration of the metal in the hypothalamus and pituitary and inhibited prolactin secretion.

Pertinent Guidance Values

U.S. EPA RfD: 0.02 mg/kg-day

U.S. EPA's RfD is based primarily on the results of a two-year feeding study using rats given 0, 100, 1000 or 2500 ppm nickel (estimated as 0, 5, 50 and 125 mg Ni/kg bw) in the diet (Ambrose et al. 1976). In the 1000 and 2500 ppm groups (50 and 125 mg Ni/kg bw, respectively) body weights were significantly decreased compared with controls and the females had significantly higher heart-to-body weight ratios and lower liver-to-body weight ratios than controls. Since no significant effects were reported at 100 ppm (5 mg Ni/kg bw), this dose was a NOAEL. In this study, two-year survival was poor, particularly in control rats of both sexes (44 of 50 died), raising some concern about the interpretation of the results of this study. A subchronic study conducted by American Biogenics Corp. (ABC, 1986) also found 5 mg/kg-day to be a NOAEL, which supported the Ambrose et al. (1976) chronic NOAEL of 5 mg/kg-day.

An uncertainty factor (UF) of 300 (10 for interspecies extrapolation, 10 to protect sensitive populations, and 3 to account for inadequacies in the reproductive studies) was applied to the NOAEL of 5 mg/kg-day to compute an RfD of 0.02 mg/kg-day.

OEHHA PHG: 11.8 µg/L (a safe dose of 1.1×10^{-3} mg/kg-day)

OEHHA (2001) established a Public Health Goal (PHG) for nickel in drinking water that is based on three reproductive studies in rats (Smith et al., 1993; Springborn Laboratories, 2000a, b). In the Smith study 61-64 day old female rats (at puberty) were dosed at 0, 1.3, 6.8, or 31.6 mg/kg-day for 11 weeks prior to mating and then continuously during two sequential gestation and lactation periods. Breeder males were unexposed. The proportion of dead pups per litter was significantly increased in the 31.6 mg/kg-day group in both breedings and also in the 1.3 mg/kg-day group in the second breeding. Thus, 1.3 mg/kg-day was considered the LOAEL for this study.

The first Springborn report (Springborn Laboratories, 2000a) summarized a one-generation reproduction range-finding study in rats. 102 day-old animals (at sexual maturity) were dosed at 0, 10, 20, 30, 50, or 75 mg nickel sulfate hexahydrate/kg-day for two weeks prior to mating. OEHHA observed significant pup mortality at the lowest dose (10 mg nickel sulfate hexahydrate/kg-day or equivalent to 2.2 mg nickel/kg-day) and deemed it as the LOAEL for this study.

Following the range-finding study, Springborn Laboratories (2000b) conducted a two-generation reproduction study. Nickel sulfate hexahydrate was administered at 0, 1, 2.5, 5, or 10 mg/kg-day. Dosing of the F_0 animals began at 10 weeks prior to mating and dosing of the F_1 rats began on postpartum day 22 (just after weaning, at a young age). For both generations, daily dosing of the dams was continued until lactation day 21. In this two-generation study, no adverse effects were observed even at the highest dose, 10 mg/kg-day (2.2 mg nickel/kg-day).

In reviewing these three studies in totality, OEHHA concluded that the 1.1 mg nickel/kg-day (5 mg nickel sulfate hexahydrate/kg-day) dose in the two-generation study was the appropriate NOAEL for use in calculating the PHG. It represents the highest NOAEL that is lower than the LOAEL from either the Smith, or Springborn range-finding, study.

OEHHA applied this NOAEL in conjunction with an uncertainty factor of 1000 (10 for inter-species extrapolation, 10 to account for human variability, and 10 for database deficiencies for carcinogenic effect via oral route) for calculating a safe dose of 1.1 µg/kg-day. The safe dose was in turn used to derive the PHG.

Current Evaluation Results

Nickel has been cited in the PHG report as having adverse effects on several sensitive organ systems that are undergoing development in school children (OEHHA, 2001). For

example, it affected the hypothalamus-pituitary axis and inhibited prolactin secretion; it reduced a variety of T-lymphocytes and natural killer cell-mediated immune functions; and it impacted the reproductive system and viability of offspring. Against this background, OEHHA targeted the literature search using the criteria outlined in the Introduction Section. We came up with a list of 18 references; all of which were qualitative studies and thus not usable in the context of the current task.

OEHHA modified its strategy, which stipulated a broad-based literature search. A total of 18,410 references were compiled. These references and their abstracts were put into a Procite database. The database, in turn, was queried in an attempt to identify quantitative studies with nickel doses in the range, or below that, of 1.1 mg/kg-day. The purpose is to run another check that we have identified the "lowest" LOAEL or NOAEL during the PHG review. The results support that conclusion.

Recommendation

The current broad-based literature search has not identified data to suggest that the NOAEL should be changed from that used as the basis for the PHG. The PHG NOAEL addresses the reproductive end point that is one of the targeted organ systems for this review, and the exposure time and duration of rats stipulated in the Smith and Springborn studies covers the critical windows for exposure of pre-school and school children. As such, the PHG NOAEL should be used to develop a child-specific RD for use in school-site risk assessment.

OEHHA has considered the appropriateness of using an oral absorption factor for calculating the chRD for nickel. Child-specific GI absorption and the matrix effect on absorption (bioavailability) are the factors that have been reviewed. OEHHA noted that human absorption of nickel depends on the dietary matrix (OEHHA 2001). The absorption of nickel from water is about 10 times greater than that from food. Assuming the matrix effect of soil and food are equivalent in retarding absorption, the soil matrix effect could reduce the absorption by 10 times. This suggests the need for a correction for absorption because the chRD is based on studies involving the administration of soluble nickel in drinking water or by gavage; whereas, in the school setting nickel would be in a soil matrix. With respect to GI absorption, Alexander et al. (1974) estimated a 40 percent absorption for healthy children on a balanced diet that consisted of milk, cereal, and other food. The child absorption value of 40 percent is calculated by taking the difference between intake and fecal excretion. The adult absorption value of 1.6 percent calculated by Diamond et al. (1998) using McNeeley et al. (1972) data is based on urine excretion. Applying Diamond's method to Alexander's urine excretion data for re-calculation would yield a child absorption value of 19 percent. Thus, children are likely to have a higher GI absorption of nickel by 11.8 times (19/1.6). Considering the retardation of absorption by the soil matrix and the higher GI absorption in children in totality, OEHHA determines that an absorption factor is not required. Risk assessors should note that OEHHA in this case has considered the bioavailability of nickel in developing the chRD. Thus, further

correction for oral bioavailability would not be required when conducting the exposure assessment.

Because a PHG can be based on a cancer or non-cancer endpoint, OEHHA applied a factor of 10 to account for database deficiencies for carcinogenic effect via oral route in deriving a PHG safe dose for nickel. Since a non-cancer chRD by definition addresses the non-cancer endpoint only, OEHHA in this situation has not applied that database deficiency factor in calculating the chRD for nickel.

Calculation of the chRD for nickel is based on the following equation:

$$\text{chRD} = \frac{\text{NOAEL}}{\text{UF}} = \frac{1.1\ \text{mg/kg-day}}{100} = 11\ \mu\text{g/kg-day}$$

Where,

NOAEL = No-observed-adverse-effect-level from Smith et al., 1993; Springborn Laboratories, 2000a, b.

UF = Uncertainty factor of 100 (10 for inter-species extrapolation, 10 for human variability).

Accordingly, OEHHA is proposing a non-cancer chRD of 11µg/kg-day for nickel to be used in school-site risk assessment.

3. Conclusion

This report summarizes OEHHA's evaluation of cadmium, chlordane, heptachlor (and its metabolite heptachlor epoxide), methoxychlor, and nickel. Based on the evaluation, OEHHA proposes to establish a chRD for each of these chemicals pursuant to the second part of Health and Safety Code Section 901(g). They are listed in Table 3.1 along with other pertinent numerical health criteria.

Table 3.1 Numerical Non-cancer Health Criteria

	OEHHA's Proposed chRD (mg/kg-day)	OEHHA's PHG Safe Dose (mg/kg-day)	U.S. EPA's RfD (mg/kg-day)
Cadmium	1×10^{-5}	1×10^{-5}	5×10^{-4}
Chlordane	3.3×10^{-5}	1×10^{-5}	5×10^{-4}
Heptachlor	3×10^{-5}	1×10^{-4}	5×10^{-4}
Heptachlor epoxide	1.3×10^{-5}	1.3×10^{-5}	1.3×10^{-5}
Methoxychlor	2×10^{-5}	5×10^{-3}	5×10^{-3}
Nickel	11×10^{-3}	1.1×10^{-3}	2×10^{-2}

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APPENDIX A

Keywords Used in Literature Search

gestation*
infant
neonatal
neonate*
newborn
perinate
perinatal
perinatally
lactation
puberty
adolescent
adolescence
kids
young
youth
children
child
juvenile
school
pediatric
prepubertal
peripubertal
age
sacrificed
lactation
pup
pups
postnatal*
preweanling
weanling*
early postnatal exposure
offspring
immature
childhood
developmental
growth
developing
development
rotarod
utero
early postnatal exposure
synaptogenesis
cell physiology
myelination
myelin sheath
apoptosis
locomotor skill
motor activity
learning
Psychological
Phenomena and
Processes
memory
pseudoglandular
canalicular
saccular
morphogenesis
Respiratory Tract
Diseases
Splenic diseases
spleen
hematopoeisis
extramedullary
thymus gland
autoimmunity
endocrine glands
brain
gonads
ovary
testis
urogenital system
kidney
ureters
bladder
urethra
ovaries
uterus
fallopian tubes
vagina
clitoris
testes
seminal vesicles
prostate seminal ducts
penis
breast/gd
mammae
udder
sperm count
sperm motility
sex maturation
vaginal opening
preputial separation
litter size
Estrogens
androgens
Leydig Cell Tumor
Leydig Cells
Sertoli Leydig Cell
Tumor
Sertoli Cell Tumor
Sertoli Cells
maze learning
sex hormones
steroid
receptors
GABA
body weight
cincinnati maze
navigation times
escape reaction
Startle reaction
startle
spatial behavior
crowding
personal space
territoriality
mating behavior
sex behavior
motor activity
chloride channels
gaba receptors
auditory startle
Neuropsychological
Tests
Reaction Time
Psychomotor
Performance
Battery
Physiology
Nervous System
Psychological
Phenomena and
Processes
Behavior and Behavior
Mechanisms

Psychological Tests
Behavioral Disciplines and Activities
Ovarian Function Tests
Pain Measurement
placental Function Tests
Pulmonary Ventilation
Respiratory Function Tests
Speech Articulation Tests
Speech Discrimination Tests
Thyroid Function Tests
Pancreatic Function Tests
Ethology
hearing tests
vision
visual perception
ethological
photic stimulation
uterotrophic
Immune system
immunity
immunotox*
Nervous system
nervous system diseases
Neurologic Manifestations
neurotox*
Respiration system
Respiratory Tract Diseases
respirat*
lung
lungs
nasal
airway
Neurosecretory Systems
neuroendocrin*
Psychomotor Agitation
Neurobehavioral Manifestations
Psychomotor Performance
Psychophysiology
behavior
neurobehav*
reproduction

APPENDIX B

Public Comments

EVALUATION OF THE PROPOSED CHILD-SPECIFIC REFERENCE DOSES (CHRDS) FOR SCHOOL SITE RISK ASSESSMENT

Comments of the Nickel Producers Environmental Research Association

August 18, 2003

NIPERA

Table of Contents

Page

NiPERA

1. INTRODUCTION

These are Comments of the Nickel Producers Environmental Research Association (NiPERA) on the June 2003 draft "*Proposed Child-Specific Reference Doses (chRDs) for School Site Risk Assessment – Cadmium, Chlordane, Heptachlor/Heptachlor Epoxide, Methoxychlor, and Nickel*" issued by the California Environmental Protection Agency's Office of Environmental Health Hazard Assessment (OEHHA) during June 2003. OEHHA recommends a chRD value for nickel of 3.7 µg Ni/kg body weight based on three reproductive studies of nickel in rats (Smith *et al.*, 1993; Springborn Laboratories, 2000a, b). The critical study in this assessment is the report by Smith *et al.*, 1993 where a purported LOAEL of 1.3 mg/kg is used to justify the selection of the mid-dose (1.1 mg Ni/kg) from the Springborn Laboratories, 2000b study as the starting point for establishing the chRD.

As previously commented upon in discussions of the derivation of the Public Health Goals (PHG) for nickel, NiPERA questions the use of the Smith study for any risk characterization purposes. In addition, NiPERA disagrees with the application of a 3-fold safety factor for differences between adults and children in nickel absorption from the GI tract as discussed below. NiPERA believes that there is no evidence to demonstrate or suggest that school age children are any more susceptible to the systemic effects nickel than adults and that the chRD should be established at 22 µg Ni/kg (rounded to 20 µg Ni/kg) based upon the highest NOAEL from the Springborn Laboratories, 2000b study.

1. 2. HEALTH EFFECTS

2. 2.1 The Use of a 3-fold Uncertainty Factor To Account for Greater Childhood Absorption of Nickel from the GI Tract is Scientifically Unjustified.

The assignment of a 3-fold uncertainty factor for increased GI tract absorption of nickel by children as compared to adults is not supported by a careful review of the available data. In particular, the derivation of the safety factor used to account for child to adult absorption of nickel from the GI tract is flawed in several critical ways.

The first error in the calculation of the proposed chRD is a comparison of absorption values generated under different assumptions. Specifically, the human child data quoted in the derivation of the chRD are derived from a quasi-Bioavailability Study while the available animal data and the adult human data used for comparison are obtained from Balance studies (Table 1).

Balance studies measure metal intake and excretion from which, the difference between intake and excretion can provide an estimate of absorption (Diamond *et al.*, 1998). Balance studies are widely used to estimate absorption in humans because the approach is noninvasive.

An alternative approach determines the Bioavailability of a compound. Specifically, the relationship between metal intake and tissue metal concentrations, or body burden, can be used to estimate gastrointestinal absorption. Such approaches are collectively referred to as Bioavailability Assessments. Bioavailability can be defined as the fraction of the oral dose that enters the systemic circulation (Diamond *et al.*, 1998).

The 40% absorption value (Alexander *et al.*, 1974) quoted in the draft chRD is based upon a comparison of a calculated absorption factor where the fecal nickel concentration is subtracted from the total nickel consumed in the children's diet. The remaining nickel out of the total consumed is assumed by the authors to be the absorbed fraction or systemic load. While the parameters measured by the authors are those of a Balance Study, they then attempted to calculate Bioavailability (*i.e.*, the systemic burden of nickel). Unfortunately, there are problems with using this calculated value for risk characterization. Specifically, Alexander and co-workers did not exactly match the ingested nickel quantity to the quantity excreted in feces. The authors note that they marked the beginning and end of the 72-hour evaluation period with oral Carmine and Edicol Blue markers, respectively. While the Carmine marker was apparently used as the starting point to correlate with the diet collection, the authors note that the feces were not collected until the Edicol Bule marker appeared. This could account for the aberrant (~20%) calculated systemic "retention" of nickel reported by Alexander and co-workers.

It has been clearly documented in humans and animals that nickel excretion follows 1st-order kinetics and occurs via the kidneys. It has also been demonstrated that nickel ion is not retained in the body. Specifically, Sunderman *et al.*, (1989) compared the absorption of nickel in humans in food and water. This study found that 26% of a dose of soluble nickel given in water was excreted in the urine, while fecal elimination of nickel averaged 76 ±19% of the dose ingested in water accounting for 100% of the administered dose of nickel. A similar pattern of nickel excretion has been observed in animals. In non-fasting rats receiving $^{63}NiCl_2$ by oral gavage, 3-6% of the initial dose was excreted in the urine while fecal excretion accounted for 94-97% of the oral dose (Ho and Furst, 1973). In another study, male rats administered unlabeled soluble nickel chloride, nickel nitrate, or nickel sulfate by gavage in a starch-saline solution, urinary excretion within 24 hours of dosing accounted for 94-96% of the 10% absorbed dose (Ishimatsu *et al.*, 1995).

Given the documentation for excretion of absorbed nickel in urine, the actual absorption value for the healthy children in the Alexander *et al.* (1974) study can be calculated. **By dividing the amount of nickel in urine (1.63 µg) by the amount of nickel ingested (8.73 µg) the absorption of nickel from food over a 72-hour period in the study by Alexander *et al.* (1974) can be calculated as 19%.** This approach to calculating nickel absorption is the same as the approach followed in research by Ishimatsu *et al.*, 1995; Sunderman *et al.*, 1989 ; Cronin *et al.*, 1980; Christensen and Lagassoni, 1984; Spruit and Bongaarts, 1977 and Nielsen *et al.* 1999 where the absorption value was calculated by dividing the urine concentration by the amount consumed orally.

In conducting such a comparison, acute versus chronic exposures must be taken into account. Unfortunately, not only does the draft chRD fail to take these temporal patterns of nickel absorption in adults into account (discussed below), but it cites an absorption value (1.6%) from a study that has nothing to do with calculating absorption (*i.e.*, McNeely *et al*., 1972). The study compares the nickel excretion rates of citizens in Hartford, CT versus those in Sudbury, ON Canada. McNeely and co-workers note that the drinking water and air concentrations of nickel in Sudbury are orders of magnitude higher than Hartford and that the urinary levels are also higher in the Sudbury citizens that were examined. **However, no effort was made to quantify the total oral nickel exposure of the human volunteers in the study by McNeely *et al*. (1972) and consequently, it is impossible to calculate an absorption value and the study by McNeely *et al*. (1972) makes no claim whatsoever regarding nickel absorption.**

As noted, it is known that temporal patterns of meal consumption have a profound effect on the calculation of absorption in Balance Studies (Diamond *et al.*, 1998). A reasonable comparison of the Alexander *et al.* (1974) data to adult Balance Study data would require the averaging of various temporal measurements of nickel absorption from acute exposure adult studies to simulate the 72-hour measurements used in the Alexander study. A recent Balance Study evaluation of the temporal impact of nickel exposure before, during, and after meals, reported by Nielsen *et al.* in 1999, makes this measurement possible. The results from this study demonstrate that an average Balance Study absorption value for adult humans is approximately 12%. However, it should be noted that the variance of the data reported by Alexander *et al.* (1974) was approximately 50% indicating that the 19% childhood absorption value calculated from Balance data in the Alexander study is not biologically different from the 12% absorption value calculated from the Balance data reported by Nielsen *et al.* (1999).

While important from the point of view of scientific accuracy, the comparison of human childhood Balance absorption values to adult Balance absorption values is irrelevant for the derivation of a chRD. Specifically, the toxic endpoint that is being extrapolated in this risk characterization is not being measured in human adults, consequently it is irrelevant to include an adjustment for human adult to human child GI absorption of nickel in the derivation of a chRD. Instead, the toxic effect is being extrapolated from adult rat studies. The correct comparison for adjusting the risk characterization is to compare the temporally adjusted gastrointestinal absorption of rats to the gastrointestinal absorption of human children to calculate an appropriate safety factor. GI tract absorption data for rats were reported by Ishimatsu *et al.* (1995). In their study non-fasted male Wistar rats were administered a single dose of 10 mg nickel (nickel sulfate hexahydrate in a 5% starch saline solution) by gavage. Subsequent urinary nickel level determination demonstrated a Balance absorption of 10%.

The absorption value calculated by Ishimatsu *et al.* (1995) represents the low point of the temporal nickel absorption curve in rats since it is based on an acute exposure at a time of minimal absorption of nickel from the GI tract (*i.e.*, nickel administration when food is present in the G.I. tract since rats are nocturnal feeders). When the 10% (low end) acute absorption value for rats reported by Ishimatsu *et al.* (1995) is compared to the 19% childhood absorption value calculated from Balance data in the Alexander study, it is clear that they are not biologically different from each other (considering both the 50% variance of the data reported by Alexander *et al.* (1974) and the acute non-fasted derivation of the data reported by Ishimatsu *et al.* (1995)).

From these data it is clear that a 3-fold correction factor for the gastrointestinal absorption of nickel is not scientifically justified in calculating a chRD.

Table 1: Balance Studies of Nickel Absorption from the GI Tract of Rats and Humans

Study	N	Vehicle or Exposure Media	Duration	Fasting Status	Absorption (% of Dose)
Ishimatsu *et al.*, 1995	8♂ Rats	gavage in starch solution	acute	not fasted	10
Alexander *et al.*, 1974	8 children	as a component of diet	chronic	not applicable	19
Nielsen *et al.* 1999	8	food 4hr prior to nickel in water	acute	12hr fast	23.2
Nielsen *et al.* 1999	8	food 1.5hr prior to nickel in water	acute	12hr fast	7.1

Nielsen *et al.* 1999	8	food and nickel in water together	acute	12hr fast	3.4
Nielsen *et al.* 1999	8	food 1.5hr prior to nickel in water	acute	12hr fast	7.1
Nielsen *et al.* 1999	8	food 0.5hr after nickel in water	acute	12hr fast	12.8
Nielsen *et al.* 1999	8	food 1hr after nickel in water	acute	12hr fast	16.7

Nickel absorption (Balance Study method) was estimated from reported measurements of urinary nickel excretion Acute doses were administered as nickel sulfate

2.2 The NOAEL for Nickel Should be 2.2 mg Ni/kg/day

The 1-generation reproductive study conducted by Smith *et al.* (1993) was used by OEHHA as a deciding factor in selecting an animal study NOAEL from which to extrapolate a chRD It is clear that the reproductive toxicity LOAEL for nickel reported by Smith *et al.* (1993) influenced the decision to use the Springborn (2000b) NOAEL of 1.1 µg Ni/kg as the reference level in the calculation of the chRD. NiPERA has previously argued the merits of the study by Smith *et al.* (1993) in comments titled, "*Comments of the Nickel Producers Environmental Research Association, Inc. on the Draft Public Health Goal for Nickel in Drinking Water*", November 5, 1999. In those comments NiPERA noted the lack of a dose response for the observed effect at the low exposure levels and the lack of replication of the response pattern between matings as evidence that the 1.3 µg Ni/kg exposure was not in fact an effect level. Subsequently, a Freedom of Information Act request was submitted for the individual animal data and final report for the Smith *et al.* (1993) study which was conducted at the U.S. EPA's Cincinnati Laboratory. However, no evidence of the study, its report, or the individual animal data was found, even when the request eventually was forwarded to Dr. Smith. Consequently, it is not possible to shed further light on the response patterns seen in the study by Smith *et al.* (1993). If the Smith study is not considered, it becomes clear from an analysis of the remaining generational studies of nickel exposure to rats that that the NOAEL derived in the Springborn 2000b study of 2.2 mg Ni/kg should be used for risk characterization in setting regulatory standards.

3. CONCLUSION

As previously commented upon in discussions of the derivation of the PHG for nickel, NiPERA questions the use of the Smith *et al*. (1993) study for any risk characterization purposes. In addition, and as discussed, NiPERA disagrees with the application of a 3-fold safety factor for differences in nickel absorption from the GI tract. There is no credible scientific evidence to demonstrate or suggest that school age children are any more susceptible to the systemic effects of nickel than adults (particularly given the reproductive nature of the toxicity endpoint). In addition, there is also no credible scientific evidence to demonstrate that children absorb nickel at higher rates than the rats in the study from which the chRD was derived (Springborn 2000b) or even than human adults. Consequently, the chRD should be established at 22 µg Ni/kg (rounded to 20 µg Ni/kg) based upon the highest NOAEL from the Springborn Laboratories, 2000b study as shown below.

$$\frac{NOAEL}{UF} = chRD$$

$$\frac{2.2mg/kg/day}{10X10} = 0.022mg/kg/day \quad \text{or 22 μg/kg/day}$$

Where:
NOAEL = No Observable Adverse Effect Level
UF = Uncertainty Factor:
10 for animals to humans
10 for individual variations
chRD = child-specific Reference Dose

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Before the
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

Comments of the Nickel Development Institute

on the

PROPOSED CHILD-SPECIFIC REFERENCE DOSES (chRDS) FOR SCHOOL SITE RISK ASSESSMENT

DRAFT REPORT JUNE 2003

Communications regarding these Comments should be addressed to:
Neil J. King
Wilmer, Cutler & Pickering 2445
M Street, N.W.
Washington, D.C. 20037-1420
Phone: (202) 663-6061
Fax: (202) 772-6061
e-mail: Neil.King@Wilmer.com

August 18, 2003

Introduction

These are Comments of the Nickel Development Institute ("NiDI") on the June 2003 Draft Report entitled "Proposed Child-Specific Reference Doses (chRDs) for School Site Risk Assessment - Cadmium, Chlordane, Heptachlor/Heptachlor Epoxide, Methoxychlor, and Nickel." The Draft Report was issued by the California Environmental Protection Agency's Office of Environmental Health Hazard Assessment ("OEHHA") pursuant to Health and Safety Code Section 901(g). In it, OEHHA recommends a chRD value for nickel of 3.7 µg Ni/kg body weight based on three reproductive studies of nickel in rats (Smith *et al.,* 1993; Springborn Laboratories, 2000a, 2000b).[1]

As a trade association of the western world's major nickel producers, NiDI has an interest in scientific evaluations and regulatory actions affecting nickel, NiDI is puzzled as to why nickel was selected to be among the first five chemicals for development *of a chRD,* and *we* believe *the* recommended chRD *is* unwarranted. These points are discussed briefly below.

I. OEHHA's Selection of Nickel as One of the First Five Chemicals for Development of a chRD Is Puzzling.

OEHHA's inclusion *of nickel in the first group of five* chemicals *for* which *a chRD* is being developed implies that nickel is among those chemicals that are commonly found at school sites in California and determined to be of greatest concern to children because of child-specific physiological sensitivities to the chemical.[2] We see no basis for reaching that conclusion in the case of nickel. OEHHA itself admits it does not have

[1] These references are as cited in the Draft Report.

[2] *See* Draft Report at 1, 4.

information "sufficient to conclude that the compiled chemicals are found in most schools or that children have a greater sensitivity compared to adults."[3] OEHHA's case regarding the presence of nickel at school sites is particularly flimsy.

The Department of Toxic Substances Control reports the presence of nickel at only 2% of potential school sites evaluated to date.[4] That hardly seems a basis for concluding that nickel is "commonly found at school sites." Furthermore, what does the "presence" of nickel at these 2% of school sites mean? That nickel is detectable in soil? So what? Nickel is the 24th most abundant element and occurs naturally in the earth's crust-at an average concentration of 86 ppm.[5] Naturally one would expect to find *some* nickel in *any* soil, whether at school sites or anywhere else, The relevant question is not whether nickel can be detected in soil at a school site, but whether it is present at a level indicating significant contamination. OEHHA does not suggest that it is.

OEHHA also says the Air Resources Board ("ARB") reports the occurrence of nickel in California air. So it does. Here's what the ARB reports: a mean concentration of 4.5 *nanograms of* nickel per cubic meter and a median concentration of just 3 *nanograms per* cubic meter.[6] Assuming a child inhales as much as 20 cubic meters of air per day, this amounts to inhalation of *less than 0.1 microgram of nickel per day,* two

[3]Id at 5-6.

[4]Id. at 36.

[5]*See Agency for Toxic Substances and Disease Registry,* Toxicological *Profile for* Nickel Update (September 1997) at 2, 192.

[6]*See Annual Statewide* Nickel *Summary at the following* ARB *website page:* http://www.arb.ca.govlaqd/toxicslstatepageslnistate.html.

orders of magnitude less than a child's normal dietary intake of nickel.[7] Thus, while nickel will be found at very low levels in ambient air at school sites (as it is elsewhere), this clearly should not be of concern.

OEHHA goes on to observe that EPA and ARB have looked at nickel in the NHEXAS *pilot project and the Portable Classroom Study,* respectively.[8] That *may* be so, but it does not show nickel to be of any special concern at school sites. The real question-which OEHHA does not address-is what, if anything, did EPA and ARB conclude about nickel in the NHEXAS pilot project and Portable Classroom Study.

In *sum, the fact* that *nickel is found at low nanogram/m^3 concentrations in* California's ambient air and is present (presumably in soil) at 2% of tested school sites does not justify placing nickel among the top five chemicals for the development of a chRD.

II. OEHHA's Recommended chRD Value of 3.7 μg Ni/kg-Day Is Unjustified and Should Be Increased to at Least 20 Ng Ni/kg-Day.

Assuming a chRD is to be established for nickel at all, it should be considerably higher than the value of 3.7 μg Ni/kg body weight recommended by OEHHA. The specific problems with OEHHA's calculation of the chRD involve: (1) its use of the lower NOAEL of 1.1 mg Ni/kg-day from the two-generation Springborn Laboratories, 2000b study as the starting point for the calculation, rather than using the higher NOAEL of 2.2 mg Ni/kg-day from that same study; and (2) its application of a Child Protective Factor of 3 to account for a supposed difference in GI absorption between children and adults. As explained more fully in the Comments being filed by the Nickel Producers

7 ***See*** pp. *4-5, infra.*

8 *See* Draft Report at 36.

Environmental Research Association ("NiPERA"), neither OEHHA's choice of a NOAEL nor its application of a factor of 3 to account for supposed differences in GI absorption is justified. When the calculation is made on the basis of an appropriate NOAEL without an unjustified GI absorption adjustment, the resulting chRD is 22 μg Ni/kg-day, which NiPERA has conservatively rounded down to 20 μg Ni/kg-day.[9]

Additional conservatism is built in to NiPERA's proposed chRD of 20 μg Ni/kg-day, because the animal studies from which the chRD is derived involved the administration of soluble nickel to rats in drinking water or by gavage. By contrast, the presumed risk, if any, associated with the presence of nickel at school sites would involve ingestion of nickel in soil-because (as noted above) levels of nickel in the ambient air are negligible, and drinking water at schools will come from public drinking water systems as opposed to school site-specific sources. As OEHHA recognizes, the absorption rate of nickel from water is about ten times as great as the absorption rate from food.[10] Accordingly, a chRD derived from animal studies where nickel was administered in water will reflect greater absorption than would be expected when comparable amounts of nickel are ingested in soil. in that respect, the chRD for nickel reflects an implicit, though unstated, protective factor.

In sum, OEHHA's recommended chRD of 3.7 μg Ni/kg-day is not well founded, and it does not withstand a reality check. For a 20 kg child, this amounts to a total acceptable daily intake of 74 μg Ni/day. OEHHA says the average daily dietary intake

[9] *See* NiPERA Comments at 6.

[10] *See* OEHHA, Public Health Goals for Chemicals in Drinking Water: Nickel (August 2001) at 54, Table *26.*

of nickel in food is 200 μg /day.[11] That value presumably is for adults. Assuming it is reduced by 50% for children, a child's average dietary intake of nickel in food would be 100 μg Ni/day-or about 50 percent greater than the total acceptable daily intake of 74 μg Ni/day implied by a chRD value of 3.7 μg Ni/kg-day. Obviously, something does not compute. The problem is the unsupported and unrealistic chRD value of 3.7 μg Ni/kg-day recommended by OEHHA. If a chRD value for nickel is to be established at all, it should be no lower than 20 . μg Ni/kg-day, as shown in the Comments filed by NiPERA.

[11] *See id.* at 56, n.1.

APPENDIX C

External Peer Review Panel Comments

A Review of the Document entitled, "Development of Health Criteria for School Site Risk Assessment Pursuant to Health and Safety Code Section 901(g): Proposed Child-specific Reference Doses (chRDs) for School Site Risk Assessment – Cadmium, Chlordane, Heptachlor/Heptachlor Epoxide, Methoxychlor and Nickel":

by

David A. Eastmond, Ph.D.
Environmental Toxicology Graduate Program
University of California, Riverside

April 19, 2004

General Comments

The establishment of child-specific reference doses (chRDs) represents a new and important regulatory initiative that is being undertaken by OEHHA. As described in the draft report, the approach that OEHHA has selected to develop chRDs represents a logical extension of its current approach for setting reference doses (and public health goals) for non-carcinogenic agents. For the chRDs, OEHHA is focusing primarily on organ systems that are actively developing in children and uses a supplemental uncertainty factor to provide additional protection for children who might be exposed to the agent at a school site. In general, the approach seems reasonable and appropriate. However, two additional items should be noted. In addition to protecting school-aged children, it appears that OEHHA has elected to extend its mandate to cover children from the time of conception through age 18, including infants and toddlers. While I can see some rationale for doing this, I am not certain that this extended mandate is warranted. It expands the focus to cover developmental toxicants that act exclusively during embryonic and fetal development. Secondly, OEHHA has selected to consider the most sensitive species and endpoints in its evaluations, meaning that the lowest LOAEL or NOAEL from the available literature has been selected. While in general I agree with this approach, these studies need to be critically evaluated and used only if they have been determined to be of good quality with results that are likely to be reproducible and relevant to humans. This is particularly important when evaluating agents that are reported to disrupt normal endocrine function. Methods to evaluate many of these effects have not been standardized and for many of the endpoints, it is not certain at this time that the reported effects will be reproducible, particularly those which have been reported to occur at very low doses. There are also significant questions about the likely relevance of these low dose effects in humans (Witorsch, 2002). In these cases, I would recommend that a more cautious approach be taken with the chRds being based upon the results of more established or definitive studies, with perhaps a larger uncertainty factor applied to provide added protection.

With regards to the specific questions that OEHHA would like addressed, 1) The information presented appears to be accurate. 2) The approaches used seem to be appropriate. I have a few concerns about the some of the studies selected as basis for the chRDs, especially for methoxychlor. In some cases it would be useful to have additional supporting information. These concerns are described in more detail below. 3) A number of the key studies reviewed and used for the derivation of the chRds were developmental studies in which dosing began when the test animals were in utero and continued for a portion of their lives. While these are clearly relevant for assessing overall health effects, I am not certain that effects that occur primarily from exposure during gestation are relevant for children's exposures at school sites. 4) The individual uncertainty factors used for the chRDs are fairly standard. However, given the low NOAELs or LOAELs identified and the large overall uncertainty factors applied, I would consider the derived chRDs to be very, and likely overly, health protective. 5) Any errors or omissions that were identified are presented below under specific comments.

Specific Comments on Methoxychlor and Nickel

Methoxychlor

As indicated above, the information for this section appears to be accurate. I do, however, have concerns about the studies selected for derivation of the chRD. In my opinion, these represent exploratory studies that, while interesting and potentially significant, are not sufficiently definitive to justify their use for setting a chRD at this time. My opinion is similar to that of the ATSDR (2002) which decided not to use these studies in establishing their MRL for methoxychlor. In their report, they identified a number significant concerns and weaknesses with the Welshons et al. (1999) study and "concluded that without additional data to confirm the causal relationship between exposure to extremely low doses of methoxychlor and increased prostate weight in adult male offspring (and other estrogen-related effects), derivation of an MRL in the nanogram range is not justified". Extended excerpts from their critique found in Appendix A of their recent profile is reproduced below for your reference. They had fewer comments on the vom Saal (1995) report but I believe that the overall conclusion should be the same: The use of this study to derive a chRD in the nanogram range is currently not justified.

It should be noted that in a series of additional studies, Palanza, vom Saal and collaborators have report that methoxychlor administration at very low doses to pregnant mice affects the neurobehavioral development of in utero-exposed offspring later in life (Palazana et al., 1999, 2001, 2002). I was only able to examine one of these articles. In this study, the effects were seen were quite variable. According to the authors, significant effects were seen at the lowest doses tested. Given the variable results and non-standardized nature of the studies, I would also not recommend that these be used to establish a chRD at this time. However, I believe that OEHHA should be aware of these studies and critically review them.

In addition to the other concerns, Witorsch raises some important questions about the relevance of the results from these low dose studies to humans (Witorsch, 2002). These relate primarily to differences in endocrine physiology during pregnancy, and particularly the differences in the circulating levels of estrogenic hormones in rodents and humans. Consequently, the low-level

effects seen in mice may not be expected to have the same effects in similarly exposed humans. This would appear to be relevant to methoxychlor as well as the other compounds to be evaluated by OEHHA that have estrogenic effects. I believe that these potential concerns should be reviewed, discussed, and, if warranted, incorporated in the document.

Additionally, if the reported low dose effects are real, they may occur uniquely during fetal development. For example, the neurodevelopmental and prostate enlarging effects would appear to be due to exposure during fetal development. As a result, the rationale for their usage for establishing a chRD for a school setting strikes me as questionable.

Nickel

As with many metals, the toxic effects of Nickel can vary depending upon its form. IARC in its monograph uses the descriptor "Nickel and Nickel Compounds" whereas the EPA in IRIS has different entries for metallic nickel and different nickel forms. It would appear that the results presented pertain primarily to the soluble forms of nickel. This should be mentioned in the document. For example, the EPA's RfD that is cited and described is for the "Nickel, soluble salts" entry.

In describing the results of the Smith et al. 1993 study, I think it is important to note that in the second breeding the number of dead pups at postnatal day one was significantly increased at all tested doses. In addition, the proportion of dead pups per litter was also significantly increased at the lowest 1.3 mg Ni/kg dose.

Additional information should be provided as to why a child-specific factor of three is adequate to correct for the difference in Ni absorption between children and adults.

It should be noted that the proposed Ni chRD of 3.7 µg/kg-day is less than the average intake of Ni by children from food [as estimated by the Canadian government (Canadian Government, 1994)]. The Canadian estimates range from 22 µg/kg-day for infants (0-0.5 yrs of age) to 5.7 µg/kg for children aged 12 to 19 years of age.

Moreover, the average concentration of Ni in soil samples from the western United States is reported to average 19 µg/g (arithmetic mean with a range from <5 – 700 µg/g; ATSDR, 1992). As a result, soil consumption by pica children at many schools could easily exceed the chRD, even in non-contaminated areas.

Additional Comments

There are a number of minor errors in the Executive Summary: These should be "pertinent scientific" and "factor of 90".

I don't believe that SHBG was defined in the text.

Page 13. According to the document, the Alexander et al (1974) indicates that the absorption of cadmium by children averages 55%. This seems quite high. OEHHA should try to verify this number from another source.

Page 17. The incidence of hematological effects in children poisoned with chlordane reported by Sherman (1999) seem very high. The accuracy of these numbers should be confirmed.

Page 21. Palanza et al., 2002 is not in the references.

Page 25 and later. http://toxnet.nlm.nih.gov is an indirect URL to access the IRIS database. A more direct URL such as http://www.epa.gov/iris/ should be used.

Page 27 – 30. The text indicates that the doses of heptachlor administered were 0, 0.3, 3 or 30. Yet, later it identifies the LOAEL as 0.03 mg/kg-day. This is either incorrect or confusing and should be corrected.

As an inconsistency, the US EPA Carcinogen Slope Factor and the basis for the RfD have not been presented for heptachlor epoxide as they have for the other compounds.

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vom Saal FS, Nagel SC, Palanza P, Boechler M, Parmigiani S, Welshons WV. (1995) Estrogenic

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Witorsch RJ. (2002) Low-dose in utero effects of xenoestrogens in mice and their relevance to humans: an analytical review of the literature. Food Chem Toxicol. 40(7):905-12.

ATSDR (2002) Toxicological Profile for Methoxychlor (excerpts from Appendix A, pages A-3 – A-7)

A variety of candidate MRL studies were considered for derivation of an acute-duration oral MRL for methoxychlor. There were several hypothesis-generating studies at extremely low doses that were not definitive enough to use for MRL derivation. A synopsis of each candidate MRL study and the reasons for not using it follow; these two candidate studies probably bracket the upper and lower bounds of where the true MRL should lie.

Upper Bound
Reference: Gray LE, Otsby J, Ferrell J, et al. 1989. A dose-response analysis of methoxychlor-induced alterations of the reproductive development and function in the rat. Fund Appl Toxicol 12:92-109.

Experimental design: In block 2 of this study, groups of eight immature Long-Evans hooded rats of each sex were exposed to either 0, 25, or 50 mg/kg/day technical grade methoxychlor for 59–104 days beginning at 21 days of age by gavage in corn oil. Females were monitored for onset of vaginal opening, onset of estrus, estrus cyclicity, fertility, litter size, number of implantation sites, organ weights, and ovarian and uterine histology. Males were monitored for preputial separation, testis weight, and sperm count.

Effects noted in study and corresponding doses: Female rats exposed to 25 mg/kg/day or more exhibited younger age at vaginal estrus and vaginal opening after 1 week of exposure. Vaginal opening occurred at an average age of 26 days in rats exposed to 25 mg/kg/day, compared with an average vaginal-opening age of 32–33 days in control rats. Atypical vaginal smears (decreased leukocytes, increased cornification) were noted in females exposed to 50 mg/kg/day. This study identifies an acute oral LOAEL of 25 mg/kg/day for reproductive/developmental effects in female rats.

Lower Bound
Reference: Welshons WV, Nagel SC, Thayer KA, et al. 1999. Low-dose bioactivity of xenoestrogens in animals: fetal exposure to low doses of methoxychlor and other xenoestrogens increases adult prostate size in mice. Toxicol Ind Health 15:12-25.

Experimental design: Adult female pregnant CF-1 mice were administered 0 (n=9), 0.02 (n=6), or 2.0 (n=5) mg methoxychlor/kg/day in corn oil on gestation days 11–17. Pups were weaned at postpartum day 23 and males were housed together until 8.5 months of age. One male from each litter was housed individually for 4 weeks, then killed, and the prostate, seminal vesicles, preputial glands, liver, and adrenals were removed and weighed (seminal vesicles and preputial glands were blotted to remove fluid before weighing).

Effects noted in study and corresponding doses: Prostate weight was statistically significantly increased by 61 and 51% in the 0.02 and 2.0 mg/kg/day groups, respectively; seminal vesicle weight was increased by 20% in the 2.0 mg/kg/day group; and liver weight was decreased by 5% in both treatment groups. Although no positive control was done in this study, the methoxychlor-induced prostate enlargement was greater than the enlargement observed in previous studies by the same investigators examining effects from gestational exposure to other estrogen-receptor ligands (estradiol or DES). There were no statistically significant exposure-related changes in body weight or weights of preputial glands, testes, or adrenals. No histological examinations were performed.

Problems with using this study for risk assessment calculations: This is a hypothesis generating study, not a definitive one. There are several areas in which its design is less than ideal. EPA 1998 Health Effects Test Guidelines OPPTS 870.3700 Prenatal Developmental Toxicity Study (EPA 1998) recommends that for a definitive study, each treatment group has 20 pregnant dams yielding offspring; Welshons et al.(1999) only used 5–6 pregnant dams in the treatment groups. These guidelines do not specify how many offspring from each litter should be measured. In good developmental studies, the litter is considered the unit of measurement for statistical calculations (Tyl 2000), but the measurement of the litter response still needs to be accurate. In many thorough developmental studies, such as the Chapin et al. (1997) study used for intermediate MRL derivation, every animal in the litter is assessed and for sex specific end points, each animal of a given sex is measured. The use of only one male from each litter raises questions about the representativeness of the measurement of the litter response; some type of selection bias might have occurred. In an analysis of a similar study in which only one or two animals out of a litter were measured for effects on prostate size, it was found that only measuring one animal per litter resulted in incorrect conclusions 50% of the time, when compared to a study in which all male offspring in each litter were measured (Elwsick et al. 2000a, 2000b; Janszen et al. 2000).

Measuring only one male out of each litter for a characteristic known to vary between litter members may not be a good experimental design strategy. Prostate size in untreated rodents normally varies between males in a litter depending on how they are positioned relative to the females in the litter; males positioned between two females are exposed to more estrogen and consequently have larger prostates than males positioned between two other males (Timms et al. 1999). It is unknown whether treatment with an exogenous estrogen-like compound would decrease the variability of prostate weight within a litter.

As mentioned above, *in utero* exposure to estrogens is one factor that influences prostate weight and some exposure to estrogen may result from the intrauterine position of male fetuses in relation to females. This is a natural consequence of the physiology of multiparous animals. The magnitude of the prostate weight differences resulting from intrauterine position has not been precisely measured; data on this

topic would facilitate comparisons with the magnitude of effects produced by methoxychlor. It would be interesting to have data on prostate weights in adult males whose intrauterine position was known via observation after caesarian section delivery. Timms et al. (1999) did measure cross-sectional areas of prostate histology sections, and lengths of prostate buds and estimated prostate volume in rats with a computer model. Data on differences between cross sectional *area* in prostates from males positioned between two other males versus males positioned between two females is presented in Figure 2 of the publication, but no direct comparisons are made of *volume* or weight. It appears from the figure that budding areas of certain parts of the prostate can vary by a factor of about 2-fold as a function of intrauterine position.

Another problem with the Welshons et al. (1999) study is its lack of appropriate positive controls; ideally, one of these would have included various doses of estradiol. Vom Saal et al. (1997) includes data on the prostate weight effects of *in utero* exposure to estradiol continuously delivered from implanted silastic capsules, but this method differs from the once a day dosing of methoxychlor in the Welshons et al. (1999) paper. The prostate effects of the extremely potent synthetic estrogen DES administered by the same methods as Welshons et al. (1999) have been reported from experiments done at a different time (vom Saal et al. 1997). Although lower doses of DES produced the same increased prostate weight effect in these experiments as methoxychlor did in the Welshons et al. (1999) study, big differences in the magnitude of the response and less than expected differences in the effectiveness of DES and methoxychlor in producing this response raise some questions about how exactly reproducible the results of this experimental protocol are. As can be seen in the table below, the maximum percent increase in prostate weight produced by methoxychlor is 61.5% while that produced by DES is only 29%. Also, there is only a 100-fold difference between the doses of methoxychlor and DES producing the maximal prostate weight increase; a greater fold difference would have been expected based on the fact that DES is an extremely potent estrogen receptor agonist while methoxychlor is a weak one (Dodge et al. 1996; Kuiper et al. 1998; Ousterhout et al. 1981).

Welshons et al. 1999		vom Saal et al. 1997	
Methoxychlor µg/kg/day	mg prostate weight adjusted by ANCOVA for body weight (percent increase from controls)	DES µg/kg/day	mg prostate weight thought to be adjusted by ANCOVA for body weight (percent increase from controls)
0	40.0 (-)	0	41.5 (-)

20	64.5 (61%)	0.002	40.0 (-4%)
2000	60.3 (51%)	0.02	48.0 (20%)
		0.2	55.0 (38%)
		2.0	49.0 (21%)
		20	47.0 (19%)
		200	32.0 (-20%)

It has been shown that low levels of estradiol (0.32 pg/mL serum, a 50% increase in free-serum estradiol over the endogenous level, released continuously from a silastic implant) and DES (0.02, 0.2, and 2.0 µg/kg/day) administered to pregnant mice during gestation produces increased prostate weight in adult male offspring, while higher and lower exposure levels of estradiol (0.21 and 0.56 pg/mL and above) and DES (0.002 and 200 µg/kg/day) resulted in a decrease in prostate weight (vom Saal et al. 1997). This results in an inverted U-shaped dose-response curve. Gestational exposure to low levels of methoxychlor (0.02–2.0 mg/kg/day) have also been shown to result in increased prostate weight (Welshons et al. 1999), while exposure of weanling to adult rats to high levels (100–1,400 mg/kg/day) of methoxychlor have been shown to result in decreased prostate weight (Shain et al. 1977; Tullner and Edgcomb 1962), as well as testicular atrophy (Bal 1984; Hodge et al. 1950; Shain et al. 1977; Tullner and Edgcomb 1962). Adult mice exposed to 60 mg/kg/day methoxychlor developed testicular degeneration (Wenda-Rozewicka 1983).

Although the Welshons et al. (1999) study is not definitive enough to be the basis of an MRL, the suggestion that gestational exposure to methoxychlor, and other estrogenic compounds, can increase prostate weight at some dose is worthy of further investigation. This issue has been featured prominently in Section 3.12.2, Identification of Data Needs, of this Toxicological Profile.

A peer review panel was organized by the National Institute of Environmental Health Sciences (NIEHS), National Institute of Health (NIH), National Toxicology Program (NTP) to examine low-dose effects of endocrine disruptors (NTP 2001). The panel examined a number of studies involving estrogen and several estrogenic chemicals (including methoxychlor), androgens, and antiandrogens, as well as biological factors and study design, statistics, and dose-response modeling. The panel's overall conclusions included:

(1) While low-dose effects have been observed in some laboratory animals with certain endocrine disruptors, they are compound- and end point-specific, and in some cases they have not been replicated in studies by other investigators. Additionally, the toxicological significance of some of the end points is not known.
(2) The shape of the dose-response curve may be low-dose linear, threshold appearing, or non-monotonic. The curve shape varies with the end point and dosing regimen.
(3) Previously reported key low-dose findings need to be replicated, and studies are needed to characterize target tissue dosimetry, identify sensitive

molecular markers, and determine the long-term health consequences of low-dose effects.

(4) The current testing paradigm for reproductive and developmental toxicity should be revisited to determine if changes need to be made regarding dose selection, animal model selection, age of animals when evaluated, and end points measured.

Conclusions regarding methoxychlor studies included:

(1) Methoxychlor is a weakly estrogenic chemical that can induce uterotrophism in immature rodents.
(2) There is a wide range of changes in estrogen sensitive organs at doses of 5 mg/kg/day and higher.
(3) Some immune effects, which need to be further evaluated to determine their toxicological significance, were seen following exposure to 1 mg/kg/day.
(4) More data are needed on the differences in toxicology of technical grade and pure methoxychlor.

Therefore, ATSDR has concluded that without additional data to confirm the causal relationship between exposure to extremely low doses of methoxychlor and increased prostate weight in adult male offspring (and other estrogen-related effects), derivation of an MRL in the nanogram range is not justified. However, the current data do suggest that low-dose effects of methoxychlor may be real, and more definitive studies are necessary to examine them further. An intermediate oral MRL of 0.005 mg/kg/day has been derived based on data that are well supported by the database and based on an end point that is well-established for methoxychlor (accelerated onset of puberty).

A potential acute-duration oral MRL of 0.00002 mg/kg/day can be derived from the LOAEL of 0.02 mg/kg/day in the Welshons et al. (1999) study dividing by an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).

Other Studies Considered for Use as the Basis for an Acute Oral MRL:

(1) A LOAEL of 0.02 mg/kg/day for increased urine-marking behavior in mice when placed in a new territory (vom Saal et al. 1995). Only two male pups per litter were tested and apparently, each was only tested one time; the accuracy of the assessment of the test was somewhat questionable; it is unknown how reproducible the results are, and there was no indication of what, if any, statistical methods were used to evaluate the results.

(2) A LOAEL of 1.8 mg/kg/day for aggressive behavior (infanticide) in mice toward an unrelated pup (Parmigiani et al. 1998). The strain of mouse used was the “house mouse,” and no information was provided on the specifics

of the strain or whether they were inbred; only two male pups per litter were tested; the effect was only seen at one middle dose (no dose-response); and no effect was seen with DES, a positive estrogenic control.

(3) A LOAEL of 0.02 mg/kg/day for decreased aggression of young male mice toward male siblings at postpartum day 39, but not at postpartum 54 (Palanza et al. 1999). The effect was transient and there was no dose-response (no effect was seen at 200 or 2,000 mg/kg/day).

(4) A LOAEL of 16.7 mg/kg/day methoxychlor for a 2-fold increase in uterine weight in ovariectomized mice (Tullner 1961). There was little information about the condition of the animals used in the experiments; the estrogenic activity of methoxychlor was discovered serendipitously following the dusting of the mice (being used for an experiment not related to methoxychlor) for parasite control.

Jim Carlisle, Senior Toxicologist
Office of Environmental Health Hazard Assessment
Integrated Risk Assessment Section
1001 I Street
P.O. Box 401
Sacramento, CA 95812-4010

Dr. Carlisle,

This is my review of the draft *Report on Development of Health Criteria for School Site Risk Assessment Pursuant to Health and Safety Code Section 901(g): Proposed chRfDs for School Site Risk Assessment.* I have reviewed the draft for all five compounds but include here my evaluation for chlordane, heptachlor/heptachlor epoxide, and methoxychlor as I have experience and expertise in the targets and mechanism(s) of toxic action for these compounds. As I indicated earlier I do not feel I am qualified to evaluate the draft for nickel and cadmium and have not done so in this report. I can send my comments on nickel and cadmium if you request but feel there are many other with superior knowledge.

Overall, I find the information relating to the published literature and conclusions drawn from them to be well-organized, accurate and logical. The approach taken to find relevant information was appropriate and the interpretations of the literature reviewed were scientifically sound. There will always be more recent literature than can be cited and I have indicated a two recent reports that I think are particularly relevant. Still, the current literature review is entirely adequate and would not be challenged. The calculations for chRfds are mathematically correct and the assumptions made in each case are both logical and defensible.

I have just a few comments on each of the three specific halogenated hydocarbon compounds listed below:

The literature review of chlordane is adequate and the interpretation valid. However, he identification of gender specific effects for a large number of halogenated hydrocarbons (HHCs), and specifically for chlordane, was implied but indicated. This quality of chlordane and other HHCs should be clearly indicated now as future regulations may require gender-specific standards. The recent report of Tyrhonas et al. (2003) underscores the potential targeted of chlordane on the immune system using the rat as an in vivo animal model. Also, on page 18, last paragraph, the discussion of the relationship of corticosterone to male sexual behavior and its position in the steroidogenic pathway is not well written. As it is now presented one could

interpret these statements to indicate that the glucocorticoids have sex-behavior properties and corticosterone is a precursor to sex steroid… and of these interpretations would be true.

The section on heptachlor/heptachlor epoxide is well written except for the need to explain why studies with chordane/heptachlor mixtures were used as evidence for heptachlor toxicity. Even I don't quite understand how the adverse effects of chlordane were excluded in the interpretation of these studies. There is a growing literature on the targeting of heptachlor on signal transduction in a wide range of cells including human platelets and murine hepatocytes. These reports underscore the likelihood that heptachlor can reprogram cells and lead to hyperplastic diseases.

The review of methoxychlor is appropriate. The gender differences in several experimental studies were note and this seems pertinent to the establishment of exposure standards. None of the sections presented experimental data using the nonhuman primate animal model in in-vivo experiments and this is understandable if such studies do not exist. There is, however, one such recent study on methoxychlor (Golub et al., 2004). I have included this reference below as it deals directly with pre-adolescent exposures and sexual development which is pertinent to this report.

Bill L. Lasley, Ph.D.
Department of Population Health and Reproduction
University of California, Davis

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October 6, 2004
Review of OEHHA chRD Report on Cadmium

Reviewer:
Isaac N. Pessah, Ph.D.
Professor of Toxicology
University of California, Davis

I. Scientific Accuracy

A small number of longitudinal studies suggest that environmental exposure to cadmium is associated with irreversible and possibly progressive renal failure or uraemia (1-4). However studies on mortality due to renal disease in populations exposed to cadmium have provided contradictory results (5-7). A possible explanation for inconsistencies may be that the results of some studies have been distorted by biases and confounding factors, which may have caused an overestimation of risk of severe kidney damage induced by environmental cadmium exposure.

The results Cadmibel study reported by Buchet et al in 1990 (8), a cross-sectional study of a Belgian population of 1699 subjects aged 20-80 years, assessed if environmental exposure to cadmium is associated with renal dysfunction. Five variables (urinary excretion of retinol-binding protein, N-acetyl-beta-glucosaminidase, beta 2-microglobulin, aminoacids, and calcium) were found significantly associated with the urinary excretion of cadmium (a marker of cadmium body burden), suggesting the presence of tubular dysfunction. The probability of values of these variables being abnormal when cadmium excretion exceeded 2-4 micrograms/24 h was reported to be 10%.

A follow up investigation by the same group reported in 1999 (9) was to follow the course of the cadmium-induced renal effects ascertained in the most exposed subgroup from the Cadmibel study. This study also examined the relation between indicators of renal tubular effects and cadmium body burden and the possible development of glomerular dysfunction. The association between cadmium body burden and renal factors was examined by multivariate logistic and linear regression. The main finding of this study is that no sign of glomerular dysfunction or progression of cadmium induced renal damage was found in this population with environmental exposure to cadmium and followed up over 5 years. Thus the renal effects due to a cadmium body burden as found in this environmentally exposed population are weak, stable, or even reversible after the introduction of measures to reduce exposure, and that tubular effects are not necessarily associated with a subsequent deterioration in glomerular function.

The basis of the calculation of chRD for cadmium is based on the results of the original Belgian study published in 1990 (8) that indicated a LOAEL of $1X10^{-3}$mg/kg-day. In this

regard, ‘Buchet 1999’ is not cited correctly on page 1, since this citation represents the results of a 5-year follow up study.?? Considering the current data indicating that persistent renal effects attributable to a cadmium body burden are somewhat weak, even at the higher exposure levels (9), the uncertainty factor of 30 (10 for intra-human variability and 3 for LOAEL to NOEL extrapolation) seem adequate. The difference in absorption of cadmium by children ≤8years of age averages 55% according to Alexander et al (10) compared to the 5% applied for the adult subjects studied by Buchet et al 1990 (8). The model assumptions used to derive the child-protective factor of 3 are based on enhanced absorption of 55% children through age 8 and decreasing linearly through age 21. The model predicts a 2-fold lower daily dose to produce the LOAEL effect (8) when childhood absorption is included, and the protective factor of 3 seems reasonable to derive the chRD.

II. Completeness

The chRD for cadmium is exclusively based on studies of renal toxicity. Although somewhat controversial, there is no clear evidence that cadmium produces progressive or irreversible renal damage during adulthood exposure (9). Although the evaluation of non-renal effects of cadmium is briefly summarized on page 13, the possible influence of genetic factors such as polymorphisms in accentuating cadmium toxicity in certain individuals are not presented. For example, recent evidence for significantly enhanced toxicity of cadmium in mice whose expression of metallothioneins was disrupted directly or indirectly by interfering with metal responsive transcriptional factors such as MTF-1 (which regulate transcription of metallothioneins). The latter have been recently are important not only for liver development but also for cadmium detoxification in the adult liver (11-13).

III. Appropriate Public Health Protection

The review seems appropriate for public health protection if one does not consider genetic susceptibility and target organs other than the renal system.

IV. Specific Suggestions

Correct the reference to ‘Buchet 1999’ to Buchet et al 1990 on page 1.
An updated review of the literature seems warranted. This is especially important in the areas of known polymorphisms than confer heightened sensitivity to the toxicity of cadmium. A more detailed analysis of non-renal targets seems timely.

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October 6, 2004
Review of OEHHA chRD Report on Nickel

Reviewer:
Isaac N. Pessah, Ph.D.
Professor of Toxicology
University of California, Davis

I. Scientific Accuracy

Overall the chRD for the non-carcinogenic hazards associated with exposure to nickel is based solely on rodent studies. The guidance value of 20µg/kg-day (the U.S. EPA RfD) used by OEHHA is based on a questionable 2-year study in rats in which the control animals exhibited 88% mortality (Ambrose et al 1976). A subsequent subchronic study in rats (ABC, 1986) reported a NOAEL of 5mg/kg-day. This NOAEL was corrected by an uncertainty factor of 300 (10 for interspecies extrapolation, 10 for sensitive populations, and 3 for the inadequacies of reproductive studies) to arrive at the RfD 0f 0.02 mg/kg-day.

In 2001, OEHHA further considered the reproductive studies of Smith et al (1993) and Springborn Laboratories (2000a,b) that indicated negative impact on reproductive outcome in nickel treated females commencing 11 weeks prior to mating. A LOAEL of 1.3-2.2 mg/kg-day was obtained from these studies. OEHHA used a NOAEL of 1.1 mg/kg-day and an uncertainty factor of 1000 to derive the safe dose. However the chRD is calculated based on an uncertainty factor of 100 and a child protective factor of 3 (total safety factor of 300). Thus the chRD of 3.7 µg/kg-day is arrived by incorporating a child protective factor of 3 that is based on a reported difference in dietary absorption of nickel of 40 % in healthy children (Alexander et al, 1974) *vs.* 1.6 % in healthy adults (NcNeely et al, 1972). It is unclear why a 25-fold higher efficiency in nickel absorption in children translates to a protective factor of only 3.

II. Completeness

The chRD for nickel is based predominantly on qualitative rodent studies since no human epidemiological studies were considered. Some recent studies on occupational exposure to nickel should be considered in future analysis of the chRD (e.g., Sorahan 2004). Although this study examines cancer rates in individuals with occupational exposures, the results may influence factor of 10 applied by OEHHA for database deficiencies in the literature about carcinogenic effects by the oral route.

III. Appropriate Public Health Protection

The review seems appropriate if one considers the paucity of information concerning nickel toxicosis in humans. The chRD appears to account for this lack of information.

IV. Specific Suggestions

None

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APPENDIX D

OEHHA Response to Comments

Response to General Comments

David A. Eastmond, Environmental Toxicology Graduate Program,University of California, Riverside

Comment1: In addition to protecting school-age children, it seems the program has extended the mandate to also cover the period of conception in a school setting.

Response1: California schools provides diverse services to students, which may include special facilities for pregnant students and daycare centers for infants and toddlers in addition to typical services to K-12 students. This information prompted OEHHA to consider conception through age 18 as the appropriate program coverage. Since there is another program within OEHHA that deals with reproductive and developmental toxicants, and will contribute prenatal analysis to the school site program, the school site program has decided to focus on analyzing chemicals that could impact children postnatally. Response3 will further explain that prenatal data, as appropriate, could be used in developing child-specific reference doses (chRDs) for school children in school site risk assessment. A change has been made in the text to reflect the focus of the school site program is on infants through age 18.

Comment2: OEHHA has elected to consider the most sensitive species and endpoint in its evaluation. While accepting this in concept, OEHHA should be very careful to ensure quality studies are selected. It is particularly important when evaluating endocrine disruptors. Methods for evaluating many of these effects have not been standardized. A more cautious approach would be to use definitive studies, with perhaps a larger uncertainty factor applied to provide added protection.

Response2: OEHHA shares the view that studies need to be evaluated carefully to ensure appropriate ones are selected. To the extent that definitive studies are available, they will be considered. A judgment will be made on which scenario will provide less uncertainty—a definitive study with a larger uncertainty factor applied or an otherwise good-quality study that does not meet all the U.S. EPA test guidelines. As indicated in the response to Dr. Eastmond's comment1 on methoxychlor, the peer review panel convened by the National Toxicology Program pointed out that the current testing paradigm for reproductive and developmental toxicity should be revisited to address the requirements for measuring low-dose effects of endocrine disruptors. Until the test guidelines have been revised and updated, appropriate definitive studies are unlikely to be available. From the public health policy perspective, it seems to make sense to use appropriate non-definitive studies in the interim to establish chRDs.

Comment3: A number of studies used for deriving the chRDs were prenatal studies. While they are relevant for assessing overall health effects, Dr. Eastmond is not certain that effects occurred primarily in utero are relevant for children's exposure at school sites.

Response3: 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 to use these studies for development of a child-specific health guidance value (chRD or chRC) if the studies are reasonable to assume that the effect of the chemical on the target organ in the offspring animal would likely occur on the same target organ undergoing development after birth in humans. From a public health protection standpoint, the OEHHA scientists working on health guidance values for children as mandated by Health & Safety Code 901(g) have included this perspective as a part of the procedures in evaluating and developing chRDs. The entire set of procedures is presented in the Introduction under Process.

Response to Comments on Cadmium

Isaac Pessah, Professor of Toxicology,University of California, Davis

Comment1: The citation of Buchet 1999 in the executive summary is incorrect. It should have been Buchet 1990.

Response1: Error is corrected.

Comment2: The possible influence of genetic factors such as polymorphism in accentuating cadmium toxicity is not presented. Specifically, genetic variability of metallothioneins in individuals may cause different susceptibilities.

Response2: A paragraph pertaining to this has been added.

Comment3: This review seems appropriate for public health protection if one does not consider genetic susceptibility and target organs other than the renal system.

Response3: For public health protection, OEHHA has applied a 10X factor to consider human variability including genetic variation, and selected the most sensitive organ, the kidney, as the endpoint for the chRD.

David A. Eastmond, Environmental Toxicology Graduate Program,University of California, Riverside

Comment1: OEHHA cited Alexander et al. (1974) who indicate that the absorption of cadmium by children averages 55%. This seems quite high and OEHHA should try to verify this value from another source.

Response1: OEHHA was not able to locate another source regarding cadmium absorption in children. The data suggest that children's absorption is 11 times higher than that of adults. However, the dose-response is not a linear function of absorption. Through modeling, OEHHA has demonstrated that the 11-fold difference in absorption has only resulted in a 2-fold difference in dose that produces the same response. The modeling illustrates that even if OEHHA erred in using a higher absorption value, the validity of the chRD has not been greatly impacted.

Response to Comments on Chlordane

Bill L. Lasley, Department of Population Health and Reproduction, University of California, Davis

Comment 1. The identification of gender-specific effects of chlordane were implied but not indicated.

Response: The discussion on p. 22 has been amended to provide a broader discussion of the observation of gender-specific effects.

Comment 2. The recent report of Tyrphonas et al, 2003 underscores the immune system as a target of chlordane using the rat as an in vivo animal model.

Response: A good point. The discussion on p.22 has been amended to add that report and give more breath to the discussion

Comment 3. The discussion of the relationship of corticosterone to male sexual behavior and its position in the steroidogenic pathway is not well written to discern the precise pathway.

Response:

David A. Eastmond, Environmental Toxicology Graduate Program, University of California, Riverside

Comment 1 The incidence of hematological effects in children poisoned with chlordane reported by Sherman (1999) seems very high.

Response: The author created a compendium of health problems suffered by 70 persons following exposure to chlordane/heptachlor. In 46 adults, 37% had hematological problems and 19% of 48 adults had hematological problems that were classified as hematological dyscrasias. Out of 20 child patients 1-18 years, 20% had hematological problems and 15% were serious enough to be classified as hematological dyscrasias. The experimental studies in rats and mice that are cited in this report corroborate the observation from human exposure that the hematopoietic system is a target for chlordane/heptachlor.

Comment 2. A more direct URL for IRIS such as http://www.epa.gov/iris should be cited.

Response: The URL of http://toxnet.nlm.nih.gov leads one to the site in which one can access several other databases of useful toxicological information, in addition to IRIS. The more direct URL to IRIS is appreciated.

Response to Comments on Heptachlor

David A. Eastmond, Environmental Toxicology Graduate Program,University of California, Riverside

Comment 1. The text indicates that the doses of heptachlor administered were 0.0.3, 3.0 or 30 and yet the LOAEL is identified as 0.03 mg/kg-day.

Response:

Comment 2. The US EPA Carcinogen Slope Factor and the basis for the RfD have not been presented for heptachlor epoxide.

Response:

Bill L. Lasley, Department of Population Health and Reproduction, University of California, Davis

Comment 1. Why did OEHHA (1999) use a study with a mixture of chlordane/heptachlor as evidence of heptachlor toxicity?

Response. The Public Health Goal published in 1999 used a study with a mixture of chlordane/heptachlor as evidence of heptachlor toxicity. The chRD was based on a newer study, that of Smialowicz et al, 2001 and Moser et al, 2001, which used 99% pure heptachlor. The Pesticide and Environmental Toxicology Branch of OEHHA surveys the literature for new studies revises the PHGs when better studies are published. We have made them aware of the study we used.

Response to Comments on Methoxychlor

Bill L. Lasley, Department of Population Health and Reproduction, University of California, Davis

Comment1: The review of methoxychlor is appropriate. However, a recent study on methoxychlor (Golub et al., 2004) using the nonhuman primate animal model should also be

included in the review.

Response1: The Golub study pertains to the effect of methoxychlor on pubertal growth and reproductive system maturation in female rehesus monkeys. While relatively higher doses were used in the study, it illustrates the endocrine disruption effects in a non-human primate. It corroborates the results of the Welshons et al. (1999) study on rodents in which the latter shows a non-linear (non-monotonic) dose-response of methoxychlor. This information has been added to the OEHHA report.

David A. Eastmond, Environmental Toxicology Graduate Program,University of California, Riverside

Comment1: The studies selected for deriving the chRD should not be used. ATSDR's analysis regarding these studies is used to support that view:

Welshons et al. (1999)

- The study is not a definitive study because it does not meet the U.S. EPA health effect test guidelines. The peer review panel organized by the National Toxicology Program (2001) to examine low-dose effects of endocrine disruptors has also not embraced this study as a definitive study for methoxychlor.
- The study lacks positive control.
- In comparing the magnitude of DES and methoxychlor induced prostate responses, DES, a more potent synthetic estrogen, produces a weaker response in a separate study using the same experimental procedure.

vom Saal et al. (1995)

- Small sample size.
- Unknown if results are reproducible.
- Unclear if results were subject to statistical analysis.

Response1: The overarching theme is that because these studies are not definitive studies, they should not be used as a basis for the chRD. OEHHA also feels that an appropriate definitive study should be used if it is available. However, as the peer review panel convened by the National Toxicology Program pointed out, the current testing paradigm for reproductive and developmental toxicity should be revisited to address the requirements for measuring low-dose effects of endocrine disruptors. Until the test guidelines have been revised and updated, appropriate definitive studies are unlikely available. From the public health policy perspective, it seems to make sense to use appropriate non-definitive studies in the interim to establish health criteria. OEHHA has seen ATSDR and other public agencies followed this perspective in developing health criteria. ATSDR's methoxychlor review appears to be an exception. Consistent with this public health philosophy, OEHHA scientists, working on the school site risk assessment program, have adopted a number of procedural guidelines to facilitate the development of chRDs, which include the following:

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 because 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 would likely 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 pups (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 to occur 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.

With respect to the Welshons study lacking a positive control, OEHHA agrees that it would be better to have a positive control; however, lacking one in itself does not invalidate the use of this study. Moreover, the comparison of DES and methoxychlor responses using two different studies is not scientifically sound and should not be used to cast a doubt on the results of the Welshons study.

With respect to the comments on the vom Saal study, a larger sample size and the results of a study that has been replicated are clearly better; however, OEHHA has yet to see a consensus statement from the scientific community that any peer-reviewed study not meeting the U.S. EPA test guideline on sample size, or not been replicated, should not be used. Regarding the question on statistical analysis, with the error bars indicated in figure 1 of the paper, it is apparent that statistical analysis has been performed.

Comment2: Since the vom Saal et al. (1995) study, a series of additional studies (Palazana et al. 1999, 2001, 2002) has been published to examine the neurobehavioral effects of low-doses of methoxychlor. The effects seen in these studies were quite variable, casting doubt on the validity of the vom Saal study.

Response2: The increased in urine marking behavior observed by vom Saal et al. could reflect an increased reactivity to a novel environment or could be an index of heightened territoriality (aggression). However, the 1999 Palazana study showed that methoxychlor did not induced male territorial aggression by measuring residents' attack on intruders and the intensity of attacks. The 2002 Palazana et al. study, on the other hand, illustrated that methoxychlor had no effects on males in terms of open field exploration, locomotor activity, and rearing, which were indices of exploratory activity and novelty seeking. OEHHA agrees with the view that the 1995 vom Saal study should not be used as a basis for the chRD until this neurobehavioral endpoint has received a thorough investigation.

Comment3: A reference was made to Witorsch (2002) who suggested that the low-dose in utero effects of xenoestrogen seen in mice will unlikely be observed in human. Witorsch indicated that a human fetus is subject to a much higher level of estrogen throughout pregnancy. Additional exposure to a weaker xenoestrogen at low doses would pose little incremental impact. Thus, the mouse may not be a good model for human.

Response3: The proposition that the mouse may be more sensitive and produce potential false positive indication of human effect is based on the traditional concept of dose-response. It fails to take into an account of the fundamental principle of hormone-receptor biology. Just comparing the hormone levels and certain physiological characteristics between the two species are insufficient to make this conclusion. Witorsch did not provide additional data pertaining to receptor regulation in these two species during the course of pregnancy, or the relationship between receptor occupancy and response. One of the classic and still most revealing examples of endocrine disruption effects is the high incidence of adenocarcinoma of the vagina in young women born to mother who had taken DES. These clinical findings prompted a series of animal studies. A similar cancer of the vagina was produced in mice exposed to DES in utero. Contrary to the suggestion of Witorsch, the mouse proved to be an appropriate model in studying endocrine disruption effects[1].

Comment4: If the reported low dose effects are real, they may occur uniquely during fetal development. The rationale for the use of these prenatal studies for establishing a chRD for a school setting is questionable.

Response4: The endpoints from which the proposed chRD is based are the prostate and nervous system. Their development and maturation are not complete at birth.[2, 3] These organ systems are still vulnerable to perturbations by methoxychlor in the postnatal period prior to their maturation. Thus, it is very restrictive to think that the low-dose effects would only occur during fetal development.

Response to Comments on Nickel

Nickel Development Institute

Comment1: OEHHA's selection of nickel as one of the first five chemicals for development of a chRD is puzzling because there is insufficient information to indicate that nickel is a contaminant of concern at school sites.

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[1] Vandenbergh, J.G. (2004), Animal Models and Studies of in Utero Endocrine Disruptor Effects. ILAR Journal, Vol. 45, #4, 438-442.

[2] Rice, D et al. (2000). Critical Periods of Vulnerability for the Developing Nervous Systme: Evidence from Humans and Animal Models. Environmental Health Perspectives, Vol 108 Suppl. 3, 511-533.

[3] Cunha, G.R et al. (1987). The Endocrinology and Developmental Biology of the Prostate. Endocrine Reviews, Vol.8, #3, 338-362.

Aumuller, G. (1991). Postnatal development of the Prostate. Bulletin de l'Association des Anatomistes, vol. 75, #229, 39-42

Response1: In the final report, *Development of Health Criteria for School Site Risk Assessment Pursuant to Health and Safety Code 901(g): Identification of Potential Chemical Contaminants of Concern at California School Sites*, OEHHA indicates that from the public health protection perspective, the office should not wait for definitive information regarding the occurrence of a chemical before proceeding to developing a chRD. OEHHA also believes this to be a reasonable approach in the context of the school site risk assessment process. A given chRD will be applied in the site-specific risk assessment only if the corresponding chemical has been identified as a contaminant of concern for that site. Accordingly, the chRD for nickel will not be applied unless it is definitively identified as a site-specific contaminant of concern.

Comment2: Neither OEHHA's choice of a NOAEL nor its application of a factor of three to account for GI absorption difference between children and adults is justified. The higher NOAEL of 2.2 mg/kg-d discussed by the Nickel Producers Environmental Research Association should be used.

Response2: See OEHHA's response to comment 3 of NiPERA (Nickel Producers Environmental Research Association) regarding the NOAEL, and response to comments 1 and 2 of NiPERA regarding the safety factor for GI absorption.

Comment3: The chRD, which is based on studies involving the administration of soluble nickel in drinking water or by gavage, reflects an unstated conservatism because exposure at school sites would involve the ingestion of nickel in soil. According to the OEHHA's PHG document, the absorption of nickel from water is about 10 times greater than that from food.

Response3: This statement assumes that soil exerts the identical matrix effect as food on the absorption of nickel. While OEHHA thinks that less nickel would be absorbed when it is in a soil matrix compared to when it is in a water solution, NDI has not provided data to show that absorption of nickel from water is 10 times greater than that from soil. It appears that the degree of nickel absorption will depend on the soil type and pH.

Comment4: OEHHA's proposed chRD is unrealistic. For a 20 kg child, exposed to a dose of 3.7 ug Ni/kg-d (proposed chRD), this would result in an acceptable daily intake of 74 ug Ni/day. OEHHA's PHG document indicates the average daily dietary intake of nickel is 200 µg/day for adults. Assuming it is reduced by 50 percent for children, a child's dietary intake of nickel would be 100 µg/day—about 50 percent greater than the total acceptable daily intake of 74 µg/ day computed from the chRD value.

Response4: It should be noted that the PHG document cited a North Dakota study that estimated the average daily dietary intake of 168$\pm$11 ug, and not 200 ug. Even if 200µg/day is correct and children's daily intake is indeed 50 % of adult intake, the 100µg/day dietary intake for children is less than the allowable intake of 220 µg/day computed from the revised chRD of 11 µg/kg-day.

Nickel Producers Environmental Research Association

Comment1: The use of a three-fold uncertainty factor to account for greater childhood

absorption of nickel is scientifically unjustified. The factor is based on the comparison of child and adult absorption values that are generated under different assumptions. The child absorption value of 40 percent provided by Alexander et al. (1974) is calculated from taking the difference between intake and fecal excretion. The adult absorption value of 1.6 percent calculated by Diamond et al. (1998) using McNeeley et al. (1972) data is based on urine excretion. Alexander's urine excretion data should be used instead, which would yield a child absorption value of 19 percent. The absorption difference between children and adults is deemed statistically insignificant when comparing the 19 percent value with the 12 percent adult absorption value derived from averaging measurements reported by Nielsen et al. (1999).

Response1: The Alexander study involves the measurement of nickel intake from diet. The Nielsen study, on the other hand, involves several different scenarios to examine the influence of fasting and food intake at various time intervals on the absorption of nickel from drinking water. To appropriately compare with the dietary condition of the Alexander study, one should not use the average result of all Nielsen's study scenarios. Instead, only those scenarios that simulate dietary intake (i.e., the intake of water and eggs together, or if they are mixed prior to consumption) should be used. Nielsen shows that adults absorb 2.3-3.4 percent of the nickel under those scenarios. These values are in agreement with the 1.6 percent value estimated by Diamond et al. (1998). Even using a child absorption value of 19 percent proposed by NiPERA for comparison, OEHHA still finds a significant difference in child and adult absorption of nickel.

Comment2: Since the chRD is based on animal studies, rat absorption data should be used to compare with children absorption values to determine an appropriate safety factor. Accordingly, the 10 percent rat absorption value reported by Ishimatsu et al. (1995) should be used to compare with the 19 percent child absorption value calculated from Alexander's urine excretion data. This comparison also shows that the absorption difference is statistically insignificant.

Response2: OEHHA does not necessarily agree with the concept presented here. However, the Nickel Development Institute pointed out that the chRD, which is based on studies involving the administration of soluble nickel in drinking water or by gavage, reflects an unstated conservatism because exposure at school sites would involve the ingestion of nickel in soil. Taking the issue in totality—children are likely to have a higher GI absorption of nickel by 11.8 times (19/1.6) but the soil matrix effect could reduce the absorption by 10 times (if matrix effect of soil and food is equivalent), OEHHA has decided to withdraw the three-fold safety factor for absorption.

Comment3: The Smith et al. (1993) study was used by OEHHA as a deciding factor in selecting the NOAEL from which to extrapolate a chRD. NiPERA has previously questioned the merits of the Smith's study when commenting on the *Draft Public Health Goal for Nickel in Drinking Water*. NiPERA expresses a continuing concern for the use of the study. NiPERA suggests that the chRD should be based on the highest NOAEL (2.2 mg Ni/kg-d) from the Springborn Laboratories (2000b) study.

Response3: The Public Health Goal is based on three reproduction toxicity studies in rat (Smith et al., 1993; Springborn Laboratory, 2000a, 2000b). OEHHA identified the oral dose of 1.12 mg

Ni/kg-d as the appropriate NOAEL value from the Springborn Laboratories (2000b) study. This NOAEL is compared with the LOAEL of 2.2 mg/kg-d identified in the Springborn Laboratories (2000a) study and the LOAEL of 1.3 mg/kg-d identified in the study reported by Smith et al. (1993). OEHHA maintains that all relevant studies should be considered and that this NOAEL established by the PHG process should be applied in developing the chRD.

David A. Eastmond, Environmental Toxicology Graduate Program,University of California, Riverside

Comment1: Only soluble forms of nickel could pose toxicity and this should be clarified in the text.

Response1: OEHHA has noted this suggestion.

Comment2: Additional information should be provided to justify the factor of three to account for absorption difference.

Response2: OEHHA will not apply this factor. See Response2 to Nickel Producers Environmental Research Association.

Comment3: The chRD of 3.7 µg/kg-day is less than the Canadian's intake estimates from food for infants (22 µg/kg-day) and children aged 12-19 (5.7 µg/kg-day).

Response3: The Canadian's estimates seem to be higher than what OEHHA has observed in developing the PHG document. The PHG document contains only adult intake data and thus OEHHA can only compare that to the corresponding adult data in the Canadian document. The Canadian document indicates an adult intake of 4.4 µg/kg-day or 308 µg/day (assume adult weight of 70 kg); whereas, the North Dakota study cited in the PHG document provides an estimated dietary intake of 168$\pm$11 µg/day. Morover, in Response4 to the Nickel Development Institute, OEHHA elaborates that the dietary intake for children would be lower than the allowable intake computed from the revised chRD of 11 µg/kg-day.

Comment4: Average concentration of nickel in uncontaminated soil is about 19 µg/g. As a result of soil consumption by pica children, the intake could exceed the chRD in non-contaminated areas.

Response4: U.S. EPA's risk assessment guidelines acknowledge this type of situations and provide for the subtraction of documented background concentration in calculating the exposure from contaminated soil.

Isaac Pessah, Professor of Toxicology,University of California, Davis

Comment1: The guidance value of 20 µg/kg-day (U.S. EPA RfD) is based on a questionable study in which the controls exhibited 88% mortality.

Response1: OEHHA basically summarized U.S. EPA's work and has not recommended the RfD for use.

Comment2: It is unclear why a 25 fold higher efficiency in nickel absorption in children translates to a protective factor of only 3.

Response2: OEHHA has decided not to apply this factor after reviewing additional data. See Response2 to Nickel Producers Environmental Research Association.

Comment3: Sorahan 2004, which examines cancer rates in individuals with occupational exposures, could be used to re-evaluate the factor of 10 applied for database deficiencies for carcinogenic effects in developing the PHG.

Response4: OEHHA will review this document in the next PHG update.