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

Child-specific Reference Dose (chRD) for Paraquat

Draft Report
November 2009

Integrated Risk Assessment Branch
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency
Development of Health Criteria for School Site Risk Assessment
Pursuant to Health and Safety Code Section 901(g):

Child-specific Reference Dose (chRD) for Paraquat

Draft Report
November 2009

LIST OF CONTRIBUTORS

Author
David Chan, D.Env.

Reviewers
Jim Carlisle, DVM, Senior Toxicologist, Integrated Risk Assessment Branch
David Siegel, Ph.D., DABT, Chief, Integrated Risk Assessment Branch
Robert Howd, Ph.D., Senior Toxicologist, Pesticide and Environmental Toxicology Branch
Poorni Iyer, Ph.D., Staff Toxicologist, Reproductive and Cancer Hazard Assessment Branch
George Alexeeff, Ph.D., Deputy Director for Scientific Affairs, OEHHA
Jay Schreider, Ph.D., Medical Toxicology Branch, Department of Pesticide Regulation
# Table of Contents

Executive Summary ........................................................................................................................ 1
Introduction..................................................................................................................................... 2
   Developing a chRD ..................................................................................................................... 2
   Challenge ................................................................................................................................ 2
   Process .................................................................................................................................... 4
Paraquat ........................................................................................................................................... 6
   Consideration of Paraquat in School Site Risk Assessment ....................................................... 6
   Existing Health Guidance Values ............................................................................................... 7
   Health Effects of Paraquat .......................................................................................................... 7
   Child-specific Reference Dose for Paraquat ............................................................................. 10
References..................................................................................................................................... 16
   Introduction ............................................................................................................................... 16
   Paraquat ..................................................................................................................................... 19
Executive Summary

The Office of Environmental Health Hazard Assessment (OEHHA) has identified paraquat as a contaminant of concern pursuant to Health and Safety Code (HSC) Section 901(g). HSC Section 901(g) requires OEHHA to establish numerical health guidance values (HGVs) for specific chemicals for use in the assessment of health risks at proposed or existing California school sites. This report summarizes OEHHA’s evaluation of paraquat’s potential health impact in the context of school site risk assessment and discusses the process and basis for developing a child-specific reference dose (chRD) for paraquat. U.S. Environmental Protection Agency’s (U.S. EPA) Integrated Risk Information System and Office of Pesticide Programs reviews of paraquat provided a broad overview on the use, environmental fate, and health effects of paraquat and served as a baseline for OEHHA’s literature search.

OEHHA identified the brain as a sensitive target of paraquat’s toxic effects, particularly in children. The brain is continuously growing and remodeling during fetal life up through adolescence. These changes are normally programmed but can be affected by environmental influences. Unwanted signals or insults from environmental contaminants can adversely affect the brain’s development. There is direct evidence that paraquat can penetrate the central nervous system. Paraquat may affect different systems of the brain including the nigrostriatal dopaminergic system. The developing brain may be particularly sensitive to oxidative insults, a mechanism of action of paraquat.

OEHHA selected two young-animal studies and two adult-animal studies to support development of a chRD as a HGV for paraquat. OEHHA recommends a chRD of $7 \times 10^{-5}$ mg/kg-day for paraquat. This chRD is based on the lowest observed adverse effect level (LOAEL) of 0.07 mg/kg-day from the Fredriksson et al. (1993) neurotoxicity study, divided by a combined uncertainty factor of 1000.

The heart, liver, kidney, and lung are also susceptible to paraquat’s toxic effects. Death of patients within six days of paraquat ingestion was associated with pulmonary, cardiac, renal and/or hepatic failure. In those patients who survived for longer than a week, respiratory failure due to pulmonary fibrosis was the dominant pathological finding. Pulmonary toxicity was also seen in animal studies such as that used by U.S. EPA in establishing the paraquat reference dose (RfD).
Introduction

This introduction serves as a background for the technical evaluation of paraquat. For those that are not familiar with this OEHHA program to develop health guidance values (HGVs) for school site risk assessment pursuant to HSC Section 901(g), it is advisable to review this chapter prior to reviewing the technical analysis.

Developing a child-specific Reference Dose (chRD)

Challenge

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

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 (chRDs) or concentrations should be used. Until the inception of the HSC 901(g) program, these child-specific HGVs were not available. Instead, existing reference doses or concentrations for non-cancer endpoints, which were based on adult human or animal data, were mostly used. The federal Food Quality Protection Act of 1996 was an attempt to address the issue of child sensitivity. In addition to the traditional interspecies and intra-species uncertainty factors, it mandated a safety factor of 10 for developing tolerances for pesticide residues in foods for the protection of children unless data existed to indicate that children were not more sensitive than adults. Thus, a question has been raised that the intra-species uncertainty factor of 10 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 appropriate data is a challenge, OEHHA has strived to do so because children can be more (or less) susceptible to chemical effects due to toxicokinetic and toxicodynamic differences between them and adults, and thus empirical data in the young would be preferable.

Toxicokinetics pertains to the rate of absorption, distribution, metabolism, and elimination of chemical contaminants, and toxicokinetic differences exist between children and adults. 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 to a toxic form or in detoxification of the parent compound. 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 also differ in their capacity to repair damage from chemical insults.

Toxicodynamics, on the other hand, deal with the effects of chemicals on tissues and organ systems. To clarify toxicodynamic differences between adults and children, 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.

In evaluating various chemicals, OEHHA has become increasingly aware that toxicodynamic differences between adult and early-in-life exposure may have different manifestations of toxicity. While higher-dose chemical exposure during adulthood may produce overt pathological alterations, lower-dose exposure during critical periods in gestation or childhood may alter early biochemical events or “upstream” factors that result in “re-programming” of the signal transduction pathways. This in turn may produce “silent dysfunctions” of gene expressions. The dysfunctions only manifest themselves when the genes are called to action later in life. These outcomes are difficult to recognize or detect by traditional toxicological measures of pathology and clinical chemistry. Furthermore, in some investigational studies, exposure needs to occur during the critical window and assays need to be done at the right time to detect early-in-life
Endocrine disrupting chemicals (EDCs) and neurotoxicants are examples of chemicals that can produce irreversible biochemical changes that may not be recognized as toxicity until the dysfunction is manifested in adulthood. The brain 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). Even functional tests, such as neurobehavioral assays, may not detect deficits in behavior or cognition at the time of childhood exposure; deficits may only appear in adulthood when the function is required.

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 can they directly impact the maturation and proper functioning of the endocrine system, they can 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 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, the use of a study in children or young animals as the basis for a child-specific HGV is preferred. In cases when epidemiological studies involving an adult population, or studies involving adult animals, are used, the challenge is to determine whether it is possible to integrate other experimental studies that suggest a greater sensitivity in the young with adult studies 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 78 chemicals (OEHHA, 2002). 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-effects 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.

OEHHA has revised its guidelines for establishing Reference Exposure Levels (RELs) under the Air Toxics Hot Spots Program (OEHHA, 2008). Procedures for accounting for toxicokinetic and toxicodynamic differences in children have been incorporated into the revised guidelines. OEHHA scientists working on health guidance values for children as mandated by Health & Safety Code 901(g) have observed the Air Toxics Hot Spots guidelines in evaluating and developing chRDs or child-specific reference concentrations (chRCs). Several evaluation considerations, which are consistent with the Hot Spots guidelines, are discussed as follows. 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 of studies that are of equivalent quality or validity. When evaluating various studies that use different test methods to measure effects on the same organ system, the lowest LOAEL (lowest observed adverse effect level) or NOAEL (no observed adverse effect level) 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 when there is sufficient information to strongly suggest child-specific sensitivity but insufficient quantitative data from young animal studies to permit the use of these data. Fourth, quantifying differences in susceptibility between a developing organ system and a mature one are hampered by the availability of studies that compare an effect in young animals with one in adult animals. Available data are mainly from developmental toxicity studies that limit dosing to the mother during pregnancy. OEHHA staff finds 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 when the development of the critical organ system continues to occur during childhood.
Paraquat

Paraquat dichloride (commonly known as paraquat) is currently registered for the control of weeds and grasses in agricultural and non-agricultural areas (USEPA, 1997). It is used as a preplant or preemergence herbicide on vegetables, grains, cotton, grasses, sugarcane, peanuts, potatoes, and on areas for tree plantation establishment. Paraquat is applied as a directed spray postemergence herbicide around fruit crops, vegetables, trees, vines, grains, soybeans, and sugarcane. It is used for dormant season applications on clover and other legumes, and for chemical fallow. It is also used as a desiccant or harvest aid on cotton, dry beans, soybeans, potatoes, sunflowers, sugarcane and as a post-harvest desiccant on tomatoes. Finally, it is applied to pine trees to induce turpentine production. Paraquat dichloride is also used on non-crop areas such as public airports, electric transformer stations and around commercial buildings to control weeds. More recently, the registrant proposed new use of paraquat on ginger and okra, and changes to the use patterns on soybeans, wheat, cotton, cucurbits, onions, and tanier (USEPA, 2006).

Table 1 provides a summary of paraquat use in California (CDPR, 2009). The 10-year data do not indicate an increasing or a decreasing use trend, but rather, suggest a sustained use of paraquat.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>PARAQUAT DICHLORIDE</td>
<td>1,046,375</td>
<td>879,847</td>
<td>976,158</td>
<td>752,604</td>
<td>869,243</td>
<td>990,382</td>
<td>952,964</td>
<td>1,019,690</td>
<td>1,144,220</td>
<td>966,583</td>
</tr>
</tbody>
</table>

Consideration of Paraquat in School Site Risk Assessment

California’s Department of Toxic Substances Control (DTSC), in reviewing school site risk assessment documents submitted by school districts, has found paraquat at some of those sites (Chan, 2004). Accordingly, paraquat sampling and analysis is required at proposed school sites that have a history of its use at the property (DTSC, 2002). The environmental fate of paraquat has been reviewed (USEPA, 1997) and other studies have also shown that this chemical adsorbs relatively strongly in soil (Knight and Tomlinson, 1967; Staiff et al., 1980). Because of this adsorptivity, a question could be raised regarding the bioavailability of paraquat. This issue needs to be addressed on a case-by-case basis because different soil types may affect paraquat bioavailability to different degrees. Paraquat bioavailability should be determined during site-specific soil sampling and analysis. OEHHA’s current focus is to evaluate toxicological data in developing a chRD necessary for site-specific risk assessment. A chRD for paraquat would be used to assess the potential health risk of school children only if site-specific sampling and analysis indicate the occurrence and bioavailability of this chemical.
Existing Health Guidance Values

The Agency for Toxic Substances and Disease Registry has not developed Minimal Risk Levels for this chemical. The Integrated Risk Information System (IRIS) of U.S. EPA has developed an RfD for paraquat (USEPA, 1991). The RfD of 0.0045 mg/kg-day is based on a 1-year dog study (Kalinowski et al., 1983). Alderly Park beagle dogs, grouped in six per sex per dose, were fed diets for 52 weeks containing paraquat dichloride. Treatment groups received 0, 0.45, 0.93, or 1.51 mg/kg-day of paraquat. Clearly defined chronic toxicity of the lungs was reported for the 0.93 and 1.51 mg/kg-day treatment groups. This included fibrosis and inflammation, which is consistent with a diagnosis of pneumonitis. Therefore the no-observed-adverse-effect-level (NOAEL) and lowest-observed-adverse-effect level (LOAEL) for the pneumonitis endpoint are 0.45 and 0.93 mg/kg-day, respectively. U.S. EPA applied an uncertainty factor of 100 (10 for interspecies extrapolation and 10 for human variability) to the NOAEL in deriving the RfD (USEPA, 1991). That RfD was also used by U.S. EPA’s Office of Pesticide Programs in evaluating the risk of paraquat in the re-registration and tolerance setting processes (USEPA, 1997; USEPA, 2006).

In its risk assessment prioritization report, the California Department of Pesticide Regulation (CDPR) indicated that paraquat has received a high priority designation in the risk characterization process (CDPR, 2007). Health guidance values for paraquat will also be developed as a part of that risk characterization process.

Health Effects of Paraquat

The following is a focused review of the health effects of paraquat in context of the school site risk assessment program. A broader review is contained in U.S. EPA’s Reregistration Eligibility Document on Paraquat (USEPA, 1997).

Grant et al. (1980) observed that the heart, liver, kidney, and lung are the major target organs of paraquat in acute human poisoning. The amount of 20 percent paraquat solution ingested ranged from 20 to 800 ml (dose range = 57 – 2,286 mg/kg). Patients who died within six days of paraquat ingestion exhibited pulmonary, cardiac, renal and/or hepatic failure. In those patients who survived for longer than a week, respiratory failure due to pulmonary fibrosis was the dominant pathological finding. Animal studies such as that used by U.S. EPA in establishing the paraquat RfD corroborate that the lungs are susceptible to paraquat. That susceptibility may be due to a sodium-independent uptake mechanism that leads to the accumulation of paraquat in the lungs (Rose and Smith, 1977).

OEHHA, in reviewing literature, finds that the brain is also a target organ of paraquat. While Koller (1986) speculated that paraquat, a divalent cation, does not cross the blood-brain barrier (BBB) readily, human brain damage due to paraquat poisoning was observed (Grant et al., 1980; Hughes, 1988). Dey et al.(1990), who studied the tissue distribution of paraquat in Sprague-Dawley rats, provided direct evidence that paraquat can penetrate the central nervous system. $^{14}\text{C}$-labeled paraquat at 72 µmol/kg (13.4 mg/kg) was dissolved in sterile water and injected subcutaneously in the thigh of adult male rats. The total radioactivity from various tissues was counted at specific intervals. Table 2 summarizes tissue concentration data from Dey et al. The
data clearly show the presence of paraquat in the brain after that single subcutaneous injection. The data further reaffirm the distribution of paraquat in the heart, liver, lung, and kidney.

Table 2

| Tissue Paraquat Concentration (nmol/g ± SD) (N ≥ 3/Time Point) |
|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| Tissues          | Time             | Brain            | Heart            | Liver            | Blood            | Lung             | Kidneys          | Spleen           | Carcass          |
|                  | 10 min           | 10.4 ± 0.8       | 25.5 ± 4.5       | 31.8 ± 2.7       | 36.6 ± 6.5       | 55.8 ± 9.9       | 267.7 ± 40.0     | 25.3 ± 3.5       | 18.7 ± 2.2       |
|                  | 20 min           | 11.3 ± 3.4       | 35.1 ± 8.9       | 34.6 ± 5.6       | 58.1 ± 7.0       | 55.6 ± 9.0       | 319.1 ± 49.2     | 40.6 ± 8.0       | 24.3 ± 5.3       |
|                  | 30 min           | 10.4 ± 1.9       | 29.9 ± 4.9       | 31.6 ± 7.9       | 56.0 ± 2.8       | 54.0 ± 8.2       | 328.7 ± 71.7     | 39.1 ± 11.8      | 25.2 ± 3.8       |
|                  | 40 min           | 8.3 ± 2.0        | 34.1 ± 4.1       | 31.6 ± 5.0       | 46.8 ± 2.8       | 64.2 ± 8.4       | 358.9 ± 41.8     | 59.7 ± 7.7       | 27.3 ± 1.9       |
|                  | 50 min           | 6.5 ± 2.0        | 24.3 ± 4.6       | 27.8 ± 2.1       | 36.3 ± 4.9       | 62.9 ± 9.7       | 315.9 ± 60.8     | 44.6 ± 18.5      | 24.4 ± 3.3       |
|                  | 60 min           | 5.2 ± 0.5        | 19.8 ± 1.0       | 19.7 ± 3.5       | 25.2 ± 3.9       | 39.1 ± 5.5       | 205.8 ± 14.6     | 39.5 ± 9.5       | 24.7 ± 1.9       |
|                  | 4 hr             | 2.2 ± 0.2        | 7.7 ± 0.7        | 3.9 ± 0.4        | 0.96 ± 0.1       | 32.3 ± 7.4       | 28.0 ± 3.5       | 13.6 ± 1.6       | 5.1 ± 0.1        |
|                  | 24 hr            | 1.2 ± 0.3        | 4.7 ± 1.1        | 2.2 ± 0.5        | 0.36 ± 0.2       | 15.7 ± 2.2       | 11.2 ± 4.8       | 3.1 ± 0.6        | 2.7 ± 0.4        |
|                  | 3 days           | 1.0 ± 0.1        | 1.7 ± 0.1        | 1.0 ± 0.1        | 0.12 ± 0.01      | 3.6 ± 0.3        | 2.8 ± 0.6        | 1.3 ± 0.1        | 1.2 ± 0.04       |
|                  | 7 days           | 1.0 ± 0.1        | 0.8 ± 0.3        | 0.6 ± 0.1        | 0.04 ± 0.01      | 1.3 ± 0.2        | 1.6 ± 0.5        | 0.9 ± 0.1        | 0.9 ± 0.3        |

Source: Dey et al. (1990)

Mechanistic studies suggested that paraquat enters the brain via an active uptake system, the BBB neutral amino acid transporter (McCormack and Di Monte, 2003; Shimizu et al., 2001). Brain accumulation and neurotoxicity of paraquat in mice was completely prevented by co-administration of amino acids such as valine and phenylalanine. These amino acids served as competitive substrates for the same BBB transporter.

The developing brain in children is a sensitive target organ (Rice and Barone, 2000; Weiss, 2000). From gestation through adolescence, the nervous system continues to remodel and change in response to epigenetically programmed events and environmental influences (Monk et al., 2001; Webb et al., 2001). Unwanted signals or insults from environmental contaminants could adversely impact the developmental course. While paraquat exposure in higher doses during adulthood may produce pathological alterations such as pneumonitis, exposure (in lower doses) during critical periods in childhood may alter biochemical factors that result in “re-programming” of the signal transduction pathways. Such re-programming may adversely affect the development of brain functions. In addition, infants and young children, having immature BBB, may be more vulnerable. Corasaniti et al. (1991) showed a higher concentration of paraquat in the brain of 2-week old rats compared to 3-month old rats given the same dose. The developing brain may be particularly sensitive to oxidative insults, a mechanism of action of paraquat (discussed below). In a review, Bayir et al. (2006) provided animal data to show that the antioxidant system, which helps alleviate oxidative stress, is not fully developed in the immature brain. The authors further demonstrated that infants and children are more susceptible by evaluating cases of oxidative stress induced by TBI (traumatic brain injury). Moreover, Fredriksson et al. (1993) showed that low-dose exposure of mice to paraquat produced irreversible changes in the brain that were not recognized as toxicity until the behavioral dysfunction was manifested in adulthood. This low-dose “silent effect” is of concern, even though the mechanism of action has not been elucidated.
Paraquat may also be a risk factor for Parkinson’s disease (PD). The hallmark of PD is progressive and selective dopaminergic neuron loss in the substantia nigra. After more than 50 percent of neuronal loss in the substantia nigra and 75 percent depletion of striatal dopamine content, patients start to exhibit the clinical symptoms, including resting tremor, bradykinesia, rigidity, and postural instability (Steece-Collier et al., 2002). Exposure of children to paraquat may initiate the neurodegenerative process in a “silent state” until clinical symptoms are manifested later in life. Dinis-Oliveira et al. (2006) reviewed paraquat as an etiological factor of PD. Paraquat is structurally similar to 1-methyl-4-phenylpyridinium (MPP+), an active metabolite of N-methyl-4-phenyltetrahydropyridine (MPTP) that is known to cause the clinical, biochemical, and pathological features of PD (Calne and Langston, 1983). A case-control study that included 120 PD patients in Taiwan demonstrated a strong association between paraquat exposure and PD risk (Odds Ratio, 3.22; 95% Confidence Interval, 2.41 to 4.31) (Liou et al., 1997). In another case-control study in 1988 in a rural area of British Columbia, Hertzman et al. (1990) also showed an association between paraquat exposure and PD.

Further, basic features of the human disease were reproducible in paraquat-treated animal studies. Using tyrosine hydroxylase (TH)-immunoreactive and Nissl techniques, McCormack et al. (2002) found that paraquat induced dopaminergic neuron cell death in the substantia nigra (SN) of mice. Brooks et al. (1999) showed that paraquat - like MPTP - elicited in mice a dose-dependent decrease in SN dopaminergic neurons assessed by fluoro-gold labeling, a decline in striatal dopamine nerve terminal density assessed by the measurement of TH-immunoreactivity, and a reduction of ambulatory activities. Exposure of mice to 10 mg/kg of paraquat weekly via i.p. (intraperitoneal) injection for three consecutive weeks also led to the formation of intraneuronal aggregates having characteristics of Lewy bodies, a distinct pathological feature of PD (Manning-Bog et al., 2002). The effect was most pronounced at two days after the last paraquat administration.

Paraquat’s toxicity stems from its redox reactions in the cell (Dinis-Oliveira et al., 2006). Based on that mechanism, paraquat could impact other systems of the brain, and not just the SN dopaminergic system. Paraquat can be reduced by nicotinamide adenine dinucleotide phosphate (NADPH)-cytochrome P-450 reductase, NADPH-cytochrome c reductase, or the mitochondrial complex I (nicotinamide adenine dinucleotide (NADH)-ubiquinone oxidoreductase) to form a paraquat monocation free radical. The free radical is re-oxidized in the presence of oxygen, generating superoxide radical in that process. This redox cycling of paraquat has been further demonstrated in microglial cultures (Bonneh-Barkay et al., 2005). The continued regeneration of paraquat via redox cycling could amplify the accumulation of superoxide radicals. In turn, this would set off the well known cascade of reactions producing other reactive oxygen species (ROS)—hydrogen peroxide and hydroxyl radical. Hydroxyl free radicals, being highly reactive, have especially been implicated in cellular dysfunctions and tissue damage through their interaction with lipids, proteins, DNA and RNA. For example, lipid peroxidation of the inner mitochondrial membrane could cause the release of cytochrome c into the cytosol, setting the stage for apoptosis (Ott et al., 2002). The redox reaction between paraquat and the mitochondrial complex I could also lead to the inhibition of electron transport and poisoning of the energy production system, which is critical for brain functions (Dinis-Oliveira et al., 2006). In contrast, MPTP’s mode of action begins with the conversion of MPTP into MPP+ by monoamine oxidase B (MAO-B) in astrocytes (Singer and Ramsay, 1990). MPP+ then enters dopaminergic nerve
terminals and is concentrated in mitochondria, where it inhibits Complex I of the oxidative phosphorylation cascade. This action is associated with reduced adenosine-5'-triphosphate (ATP) formation and the formation of free radicals. In the end, the mitochondrial permeability transition pore's electrochemical gradient is abolished and apoptosis is induced.

Data from various studies suggest that paraquat could impact different systems of the brain including the SN dopaminergic system. Microinfusion of paraquat into non-dopaminergic areas of the rat brain, such as the locus coeruleus, raphe nuclei, and hippocampus, produced dose-dependent neural degeneration similar to that observed in dopaminergic neurons (Bagetta et al., 1992; Calo et al., 1990; Iannone et al., 1988). These data, however, do not necessarily indicate that these brain systems are equally sensitive. Recent studies seem to suggest that the dopamine system may be more vulnerable to oxidative stress. It is well established that iron catalyzes hydroxyl radical formation (Graf et al., 1984). Zucca et al. (2006) investigated the iron content in human locus coeruleus and substantia nigra, and found that iron deposits were abundant in the substantia nigra, but very scarce in the locus coeruleus. Peng et al. (2007) further demonstrated that iron exacerbated paraquat-induced neurotoxicity in vitro and showed that iron administration exacerbated paraquat-induced dopaminergic neuronal degeneration in mice.

**Child-specific Reference Dose for Paraquat**

Paraquat is neurotoxic and it is likely to adversely affect the developing brain. Paraquat can penetrate the central nervous system, and infants and young children having an immature BBB are especially at risk. Data further suggest that the immature brain is highly susceptible to oxidative stress caused by paraquat. Thus, it is appropriate to develop a chRD for paraquat.

Much of the literature in peer-reviewed journals deals with paraquat and the dopaminergic system because of the interest in investigating the causal relationship between paraquat and PD. OEHHA also notes that the Paraquat Information Center (2007) provides a link to the German Federal Institute for Risk Assessment’s report, which gives an expert opinion that there is no definitive causal relationship between paraquat and PD. However, this report was not published in a peer-reviewed journal and thus it is unknown if the document has gone through an independent scientific peer review process. It appears that the issue of paraquat and PD will continue to be scrutinized and debated. While the endpoints of the following studies also relate to the dopaminergic system, OEHHA is considering them from the viewpoint of paraquat’s effects on the brain and brain functions. OEHHA is not drawing any conclusion that those effects will necessarily lead to PD as a disease outcome.

Two young-animal studies and two adult studies have been selected in considering a chRD for paraquat. Given that the development of the dopaminergic system in the striatum occurs during the brain growth spurt period (Giorgi et al., 1987), Fredriksson et al. (1993) designed a study to investigate whether paraquat would affect the dopaminergic system and the behavior of the adult mouse in a manner similar to MPTP, when it is administered to mice during this critical window of development. Five treatment groups, each consisting of at least 12 C57 black male mice from three different litters, were used. Using either the egg lecithin and peanut oil emulsion vehicle (as a control), 0.3 mg/kg-day of MPTP, 20 mg/kg-day of MPTP, 0.07 mg/kg-day of paraquat, or 0.36 mg/kg-day of paraquat was administered orally at postnatal days (PND) 10 and 11. Twelve mice from each group were used in behavioral testing and of these eight were taken for
neurochemical analysis. Three indicators of spontaneous behavior were measured at PND 18, 60, and 120: locomotion (low-level grid of infrared beams to measure horizontal movement), rearing (high-level infrared beams to measure vertical movement), and total activity (detection of vibration motion such as from grooming). On PND 125, mice were sacrificed and neo-striata were dissected for neurochemical analysis. Dopamine (DA), DA metabolites--3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), serotonin (5-HT), and 5-HT metabolite--5-hydroxyindoleacetic acid (5-HIAA), were measured.

Fredriksson et al. observed no changes in body weight gain or overt toxicity as a result of exposure to paraquat or MPTP. When spontaneous activities were measured at PND 18, no significant differences between the control and 0.36 mg/kg-day paraquat group were seen (low-dose paraquat and MPTP treatment groups were not tested). At PND 60, all paraquat and MPTP treatment groups demonstrated hypoactivity as measured by locomotion and total activity parameters for the first and second of the three 20-minute time periods. At PND 120, all paraquat and MPTP treatment groups demonstrated significant hypoactivity in all three testing parameters for two of the three time periods. The results from neurochemical analyses indicated that exposure to the 20 mg/kg-day MPTP or to 0.36 mg/kg-day paraquat significantly reduced DA, DOPAC, and HVA levels. Exposure to 0.3 mg/kg-day MPTP significantly reduced only the DA level and the 0.07 mg/kg-day paraquat exposure significantly reduced only the HVA level. Neither MPTP nor paraquat affected the 5-HT or 5-HIAA levels. The significance of the low-dose paraquat neurochemical results is debatable. The reduction of HVA alone does not strongly suggest damages to the dopaminergic neurons. Thus, the LOAEL derived for paraquat in this study is 0.07 mg/kg-day based on hypoactivity and not the reduced HVA level.

Thiruchelvam et al. (2002) tested the hypothesis that developmental exposure to paraquat, maneb, or a combination of both would result in permanent nigrostriatal DA system neurotoxicity. In context of the OEHHA evaluation, only the part of the study that pertains to early life exposure to paraquat is summarized. C57BL/6 male mice in groups of at least 14 were i.p. (intraperitoneally) injected with either vehicle (saline as the control) or 0.3 mg/kg-day of paraquat between PND 5-19. Chambers equipped with infrared photobeams were used to quantify locomotor activities at six weeks, six months, and eight months. Photobeam breaks were recorded each minute for 45 minutes for horizontal, vertical, and ambulatory movements. After the last locomotor activity measurement, the mice were sacrificed. The striatal block from 10 mice in each group was dissected out for neurochemical analysis. Levels of DA, DOPAC, HVA and 5-HT were measured. The brains of four mice per group were also fixed for immunolabeling to identify tyrosine hydroxylase (TH)-positive dopaminergic neurons. Peripheral organs, including the lung, heart, kidney, and liver, were fixed for histopathological examination.

Thiruchelvam et al. observed no treatment-related changes in body weights. No pathological changes were noted in the peripheral organs. The paraquat-treated group showed a statistically significant 14 percent decrease in locomotor activities at the 6-week interval. However, decreases in locomotor activities at 6-month and 8-month intervals were not statistically significant. While no significant changes in 5-HT were observed in the paraquat-treated group, DA and DOPAC levels were significantly reduced. Stereological analysis of the TH-positive cells indicated a significant decrease in DA neurons in mice treated with paraquat. Thus, the i.p.
LOAEL is 0.3 mg/kg-day based on dopamine decrease and dopaminergic neuronal reduction endpoints.

Because gastrointestinal (GI) absorption data in mice were not available, OEHHA employed rat data for i.p.-to-oral route conversion. Chui et al. (1988) and Daniel et al. (1966) estimated that about 6 percent of paraquat dichloride was orally absorbed in rats. Based on an assumed oral absorption of 6 percent, OEHHA converted the i.p. LOAEL from the Thiruchelvam study into an oral equivalent LOAEL of 5 mg/kg-day (0.3/0.06). OEHHA believes that this i.p.-to-oral conversion is valid because paraquat does not appear to be metabolized in the liver (USEPA, 1997). After oral administration (gastric intubation) of single doses of paraquat dichloride to Wistar male and female rats, most of the administered radioactivity (69-96%) was excreted in feces as unchanged paraquat. After subcutaneous injection of these compounds, 73-96% of the administered radioactivity appeared in the urine as unchanged paraquat.

Brooks et al. (1999) examined whether systemic administration of paraquat to C57bl/6 adult male mice would produce a neurobehavioral syndrome and dopaminergic neurotoxicity. The investigators demonstrated that paraquat, like the established dopaminergic neurotoxicant MPTP, caused a dose-dependent reduction in TH-labeled cell bodies and diminished ambulatory activities, a behavioral change correlated with damage to the nigrostriatal circuitry. In this experiment, 30 mice were randomly distributed into five groups and each group received one of the following i.p. treatments: saline (as control), 5 mg/kg of paraquat, 10 mg/kg of paraquat, 10 mg/kg of MPTP, or 30 mg/kg of MPTP. Paraquat was reconstituted in saline and administered in a total of three doses, with the doses separated by one week. OEHHA averaged the weekly paraquat doses to derive equivalent daily doses of 0.7 mg/kg-day and 1.4 mg/kg-day, respectively. OEHHA recognizes that this averaging method has its limitation and does not produce a highly accurate estimate of the LOAEL because rapid clearance of paraquat was observed (Daniel and Gage, 1966). From the estimated daily i.p. doses, OEHHA calculated equivalent oral doses of 11.6 mg/kg-day and 23.3 mg/kg-day based on a 6 percent oral absorption. Behavioral testing was carried out one week after the final injection. Horizontal, vertical, and ambulatory locomotor activities were measured by infrared beam breaks at five minute intervals over the course of a 60 minute session. Upon completion of the behavioral assessment, the animals were sacrificed and the brain was sectioned for immuno-labeling to identify TH-positive cells. Fluoro-gold was also introduced into the striatum of mice to retrogradely label the substantia nigra projecting neurons before dosing. Fluoro-gold labeled cells, which could be visualized in the brain sections, were cross-matched with TH positive cells to confirm the identity of dopaminergic neurons.

Brooks et al. found that both the high and low doses of paraquat and MPTP caused a dose-dependent reduction of nigrostriatal neurons. Analyses further revealed that the low and high dosages of paraquat reduced the density of striatal dopaminergic terminals by 87 percent and >94 percent, respectively. Similar dose-dependent decrements were observed in the MPTP-treated groups. The results from neurobehavioral testing indicated that paraquat and MPTP produced similar locomotor effects. Both high and low doses caused pronounced decreases in ambulatory activities in the final 5-minintute intervals of the assessment. Thus, in this study, the i.p LOAEL for paraquat is 0.7 mg/kg-day and the estimated oral LOAEL is 11.6 mg/kg-day, based on the dopaminergic neuronal reduction and hypoactivity endpoints.
Since a number of paraquat investigations were relatively short-term studies, Ossowska et al. (2005) decided to examine whether longer-term (up to 24 weeks) paraquat administration would produce a slowly progressing and selective degeneration of nigrostriatal neurons. Wistar male rats, 7-8 animals per group, were i.p. injected with saline (control) or 10 mg/kg of paraquat once a week for four, eight, 12 or 24 weeks. This yields an average daily i.p. dose of 1.4 mg/kg-day and an estimated oral dose of 23.3 mg/kg-day based on a 6 percent oral absorption. The dose used in the Ossowska et al. study is equivalent to the high dose used in the Brooks et al. investigation. Upon completion of dosing, animals were sacrificed accordingly at four, eight, 12, or 24 weeks for histological evaluation of TH-positive neurons in the substantia nigra and TH positive pre-synaptic terminals in the striatum. Levels of DA and its metabolites DOPAC, 3-methoxytyramine (3-MT), HVA; 5-HT and its metabolite 5-HIAA; and noradrenaline (NA) were also measured.

Ossowska et al. performed histological analyses on the 4-week, 8-week and 24-week groups. They found that paraquat administration for four weeks caused a 17 percent reduction of TH-positive neurons in the substantia nigra; for eight weeks, a 28.5 percent reduction; and for 24 weeks, a 37 percent reduction. TH-immunoreactive pre-synaptic terminals in the striatum were not altered after four or eight weeks of paraquat treatment but decreased significantly after 24 weeks. Neurochemical analyses indicated that long-term paraquat administration induced a biphasic dopaminergic response in the striatum. Levels of 3-MT and HVA were significantly elevated after 4 weeks of treatment, followed by an increase in the levels of DA and its metabolites after eight weeks. After 12 weeks, DA and its metabolites returned to their control values. After 24 weeks, DA and DOPAC concentrations dropped by 26-31 percent and 27-36 percent, respectively. The authors, in interpreting these results, suggested that during the early phases of paraquat-induced degeneration, surviving nigrostriatal neurons became hyperactive in dopamine releases to compensate for the losses of neurons. However, this compensatory mechanism either could not keep up or just broke down as neuron-degeneration continued, leading to the observed decrease in DA levels after 24 weeks. Based on the histological and neurochemical endpoints, the estimated oral chronic LOAEL is 23.3 mg/kg-day.

With respect to paraquat effects on the serotonin and noradrenaline systems, Ossowska et al. found that there were certain increases in 5-HT, 5-HIAA, and NA during 4-12 weeks of treatment. However, no significant changes in 5-HT and NA systems were observed after 24 weeks. The authors concluded that their present study did not provide proof of whether paraquat would adversely affect non-dopaminergic neurons.

The above well-planned scientific studies collectively paint a cohesive picture that paraquat is a neurotoxicant and impacts brain functions. OEHHA used the Fredriksson et al. study as the basis for developing a chRD for paraquat for the following reasons:

- First, in accordance with OEHHA’s adopted procedures stated in the Introduction, young animals in their critical window of brain development (brain growth spurt) were used in the experiment. Early life exposure resulted in irreversible motor deficits that were manifested later in life, long after the withdrawal of paraquat treatment.
Second, it has more treatment groups than the Ossowska et al. and Thiruchelvam et al. studies to facilitate dose-response assessment.

Third, as indicated in Table 3, it provides the lowest LOAEL among the four studies.

Fourth, a smaller uncertainty factor would need to be applied. Because Fredriksson et al. dosed the animals during the critical period of brain development (Giorgi et al., 1987; Rice and Barone, 2000; Weiss, 2000) and during the time when the BBB was not completely matured (Corasaniti et al., 1991), a child safety factor would not be required. Producing an effect with only “two hits” (two-day exposure) suggests that the target at that time was very sensitive and thus a subchronic-to-chronic factor would not be necessary. In the case of the Brooks et al. study, a subchronic-to-chronic factor would be necessary to account for the relative short exposure duration. Moreover, a child safety factor would be required if either the Brooks et al. or Ossowska et al. study were used because they employed adult animals.

Fifth, because oral dosing was the route of administration in the Fredriksson et al. study, there is no need to apply an absorption factor to estimate an oral LOAEL, mitigating the uncertainty associated with this type of estimation.

In all, as shown in Table 3, a health-protective chRD having the least uncertainty would result with the use of the Fredriksson et al. study.

**Table 3**

<table>
<thead>
<tr>
<th>NOAEL or LOAEL* mg/kg-day</th>
<th>LOAEL-to-NOAEL</th>
<th>Subchronic-to-Chronic</th>
<th>Inter-species</th>
<th>Intra-species</th>
<th>Child Safety</th>
<th>Health Criterion mg/kg-day</th>
<th>Endpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td>IRIS RfD</td>
<td>0.45</td>
<td>1</td>
<td>1</td>
<td>10</td>
<td>10</td>
<td>1</td>
<td>4.50E-03</td>
</tr>
<tr>
<td>Fredriksson</td>
<td>0.07</td>
<td>10</td>
<td>1</td>
<td>10</td>
<td>10</td>
<td>1</td>
<td>7.00E-05</td>
</tr>
<tr>
<td>Brooks</td>
<td>11.6</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>1.16E-04</td>
</tr>
<tr>
<td>Thiruchelvam</td>
<td>5</td>
<td>10</td>
<td>1</td>
<td>10</td>
<td>10</td>
<td>1</td>
<td>5.0E-03</td>
</tr>
<tr>
<td>Ossowska</td>
<td>23.3</td>
<td>10</td>
<td>1</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>2.33E-03</td>
</tr>
</tbody>
</table>

*Oral dose or estimated oral equivalent dose if dosing was not by the oral route.

The Brooks, Thiruchelvam, and Ossowska investigations also strengthen the findings of Fredriksson et al. Neurochemical but not histological analyses were performed to assess paraquat’s effect on the dopaminergic system in the Fredriksson et al. study. Exposure to the 0.36 mg/kg-day paraquat significantly reduced DA, DOPAC, and HVA levels, whereas the 0.07
mg/kg-day paraquat reduced only the HVA level. A question could be raised regarding the significance of the hypoactivity observed at 0.07 mg/kg-day when the neurochemical parameters did not provide a clear indication of dopaminergic effects. Ossowska’s data suggested that during the early phases of paraquat-induced degeneration, surviving nigrostriatal neurons could become hyperactive in dopamine releases to compensate for the reduction due to the loss of neurons. Thus, the compensatory mechanism may have counter-balanced the dopamine reduction that resulted from neural degeneration so that a significant change in the total dopamine levels was not observed by Fredriksson et al.

While the specific contribution of nigrostriatal damage to hypoactivity is not clearly understood, Brooks et al. in their study showed a strong association between these two parameters. Brooks et al. also reproduced hypoactivity results that were similar to that of Fredriksson et al. Brooks’ study strengthens Fredriksson’s observations that the hypoactivity is not an artifact.

Like the Fredriksson study, the Thiruchelvam study provided data to show developmental exposure to paraquat resulted in permanent neurotoxicity. The replication of this observation further increases the confidence in Fredriksson’s study.

In conclusion, OEHHA is recommending a chRD of 7.00 x 10^{-5} mg/kg-day for paraquat. An uncertainty factor of 1000 (10 for interspecies extrapolation, 10 for human variability, and 10 for LOAEL-to-NOAEL conversion) is applied to the LOAEL of 0.07 mg/kg-day from the Fredriksson et al. study in deriving this chRD.
References

Introduction


http://www.oehha.ca.gov/public_info/public/kids/schools1205.html


vom Saal FS, Timms B G, Montano M M, Palanza P, Thayer K A, Nagel S C, Dhar M D,


Paraquat


Chan D. (2004) Personal communication with Sharon Fair, Department of Toxic Substances Control.


