# 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

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**Integrated Risk Assessment Branch**
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# 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

exposure effects. 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 1**

| Paraquat Use in California | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | POUNDS APPLIED | | | | | | | | | |
| | 1998 | 1999 | 2000 | 2001 | 2002 | 2003 | 2004 | 2005 | 2006 | 2007 |
| PARAQUAT DICHLORIDE | 1,046,375 | 879,847 | 976,158 | 752,604 | 869,243 | 990,382 | 952,964 | 1,019,690 | 1,144,220 | 966,583 |

**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. $^{14}CH_3$-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) ($N \geq 3$/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 | 9.2 ± 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-mininute 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**

| | NOAEL or LOAEL* mg/kg-day | LOAEL -to- NOAEL | Subchronic-to-Chronic | Inter-species | Intra-species | Child Safety | Health Criterion mg/kg-day | Endpoint |
|---|---|---|---|---|---|---|---|---|
| IRIS RfD | 0.45 | 1 | 1 | 10 | 10 | 1 | 4.50E-03 | pneumonitis |
| Fredriksson | 0.07 | 10 | 1 | 10 | 10 | 1 | 7.00E-05 | hypoactivity |
| Brooks | 11.6 | 10 | 10 | 10 | 10 | 10 | 1.16E-04 | hypoactivity, SN neuron reduction |
| Thiruchelvam | 5 | 10 | 1 | 10 | 10 | 1 | 5.0E-03 | SN neuron and dopamine reduction |
| Ossowska | 23.3 | 10 | 1 | 10 | 10 | 10 | 2.33E-03 | SN neuron reduction and biphasic dopamine level changes |

*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 $7.00 \times 10^{-5}$ 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.

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# APPENDIX 1: OEHHA Response to Public Comments

**Response to comments submitted by Timothy Pastoor, Ph.D., D.A.B.T., Andy Cook, M.A., Jonathan Akins, PhD., D.A.B.T., Montague Dixon,M.S., Syngenta Crop Protection, Inc., Greensboro, NC**

GENERAL COMMENTS

Comment: Paraquat does not meet any of the OEHHA evaluation criteria, obviating the need for a paraquat child-specific reference dose (chRD).

Response: These OEHHA criteria need to be discussed in context of the purpose of Health and Safety Code (HSC), Section 901(g). This law provides a mechanism to ensure that any contaminant present in the school environment will not pose a health risk to school children. It prescribes a school site risk assessment process and requires the development of chRDs for use as a risk assessment tool. A chRD will be applied in a site-specific risk assessment only if the corresponding chemical has been identified as a contaminant of concern for that site. Accordingly, the chRD for paraquat will not be applied unless it is definitively identified as a site-specific contaminant of concern.

In this context, the purpose of the criteria is to facilitate the prioritization of chemicals for review rather than to accept or reject chemicals for consideration. In our 2009 document cited by Syngenta, OEHHA has specifically indicated that while prioritization is usually made on the basis of exposure and health effect potential, the availability of health-effects data is often the overriding consideration in the selection of chemicals. The OEHHA model is similar to that of U.S. Environmental Protection Agency (U.S. EPA) in its development of reference doses (RfDs), and of Agency for Toxic Substances and Disease Registry (ATSDR) in its establishment of minimal risk levels (MRLs). OEHHA strives to develop as many chRDs as appropriate to provide the necessary tools for risk assessors who will likely encounter different contaminants at different school sites. OEHHA's evaluation has led to the development a draft chRD for paraquat, which, when finalized, will become a risk assessment tool in the event paraquat is encountered at any future school site.

EXPOSURE POTENTIAL

Comment 1: Paraquat adsorbed strongly to soil and thus will not be bioavailable.

Response 1: Syngenta provided a paper by Ospenson and Pack in Appendix 1 to further show that it is difficult to extract paraquat from soil. Based on this line of reasoning, Syngenta suggests that paraquat would not be bioavailable. It should be underscored that chemical extraction experiments do not adequately simulate conditions and actions of the gastrointestinal system. With hydrochloric acid, other ion-exchange species, and churning motion simultaneously occurring in the stomach, it is likely that a certain amount of paraquat will become bioavailable. The extent of paraquat bioavailability and exposure would be determined as a part of site specific risk assessment. The chRD for paraquat would be applied to evaluate the risk only if exposures occur.

Comment 2: Negligible paraquat residues were found on school sites and DTSC has revised their guidelines to indicate that routine analysis for paraquat is not required for field areas.

Response 2: Syngenta cited that DTSC, in reviewing nearly two dozen sites for presence of paraquat, has detected paraquat at low levels at only one site. While routine analysis is not required, the DTSC guidelines also indicate that paraquat analysis may be required in storage and mixing/loading areas. OEHHA does not dispute that the detection of paraquat will be an infrequent event; however, we cannot say with certainty that there will be no detection in the future, nor can we predict the concentrations of paraquat that will likely be encountered. Consistent with HSC Section 901(g), it is incumbent upon OEHHA to consider a chRD for paraquat so that there will be an appropriate risk assessment tool available when paraquat is detected at future school sites.

Comment 3: Paraquat use around schools is restricted, no paraquat was detected in ambient air and groundwater monitoring, and only one sample out of 399 was positive for paraquat in surface water monitoring.

Response 3: This should be discussed in context of the conceptual school-site model. While paraquat use around existing schools is restricted, new schools could be sited in former agricultural areas where paraquat had been applied, stored, or mixed/loaded. Ambient air and water exposures are not a concern. Rather, potential ingestion of contaminated soil especially by young school children and inhalation of suspended soil particles are the exposure scenarios.

HEALTH EFFECT CONSIDERATIONS

Comment 1: No other U.S./California authoritative body has identified paraquat as a health concern.

Response 1: OEHHA disagrees with this statement. Based on health concerns, U.S. EPA has developed an RfD for paraquat. Syngenta pointed out that Australia, Brazil, the European Union, World Health Organization, and Canada also have similar Acceptable Daily Intake criteria for paraquat. Moreover, it should be the available health-effects information and not the precedent set by an authoritative body that drives the consideration of a chRD.

Comment 2: OEHHA placed significant emphasis on the developmental neurotoxicity of paraquat. Syngenta indicated that U.S. EPA guideline compliant studies did not show neurotoxic effects of paraquat and U.S. EPA concluded that there was limited concern for neurotoxicity. Likewise, the 2003 Joint meeting on Pesticide Residues of the World Health Organization (JMPR/WHO) concluded that paraquat's neurotoxicity is of limited concern. Syngenta also provided the reports of an acute investigation and a subchronic study to support the view that paraquat is not neurotoxic.

Response 2: Many U.S. EPA guideline compliant studies do not provide a high resolution in detecting developmental endpoints. In those studies, either critical developmental windows of exposure are not considered, or the most sensitive endpoints are not measured during testing. It seems that U.S. EPA relied heavily on guideline compliant studies in drawing that conclusion. A

simple Pubmed search using paraquat and neurotoxicity, paraquat and dopamine, or paraquat and Parkinson as key words yielded 80, 117, or 109 citations. Further evaluation of the literature has led OEHHA to conclude that paraquat is neurotoxic.

With respect to JMPR/WHO's conclusion that the published neurotoxicity studies are not relevant, that conclusion was based on a dated premise that paraquat does not cross the blood brain barrier (BBB) and on the data that paraquat does not share the same mode of action of MPTP. As discussed in the OEHHA report, there is sufficient evidence that paraquat can penetrate the BBB. While paraquat has a different mode of action than MPTP, available data indicate that it can induce oxidative stress and neuron apoptosis.

The acute and subchronic studies provided by Syngenta do not clearly demonstrate that paraquat does not impact the developing brain. In these studies, the animals were not exposed to paraquat during the critical window of development. Dosing started on at least day 42 rather than during the perinatal or early postnatal period. It is also interesting to note that while certain behavioral endpoints were measured, and the tibial, sciatic, and optic nerves were reviewed microscopically, the nigrostriatal neurons were not examined and dopamine levels were not measured.

Comment 3: Syngenta is particularly concerned about the use of the 1993 Fredikssson et al. study with a developmental neurotoxicity endpoint for establishing the chRD. Syngenta quoted a conclusion of the 2003 JMPR/WHO, which indicated that the findings of Fredikssson could not be reproduced. Syngenta further cited the 2003 Muhammad et al. study, which suggested that in using the Fredriksson study design, the authors were unable to reproduce Fredirksson's the test results on pyrethroids.

Response 3: OEHHA is aware of the work performed by D. E. Ray's group including the Muhammad et al. study. However, OEHHA is not aware that any of Ray's work has been published as a full paper in a peer review journal. JMPR/WHO's knowledge that the Fredikssson study on paraquat could not be replicated was based on a personal communication with Ray. The Muhammad et al. study on pyrethroids cited by Syngenta was published as a letter to the editor. OEHHA cannot accept personal communication as a proper documentation. With respect to Muhammad's publication as a letter, aside from not having the benefit of journal peer review, the brevity of the information rendered does not permit one to follow the experimental set up and discern how the receptor binding and behavioral studies were conducted. The authors also acknowledged that they did not follow Fredriksson and Eriksson's original experimental conditions in its entirety. In particular, the male and female mice were not separately housed, as done in the original study. This condition may influence the outcome of these behavioral studies. In comparing habituation data between the two studies, the Muhammad et al. study noted that the rate of habituation in their controls was markedly slower than the controls in the Eriksson and Fredriksson study. This reduced their ability to detect any delay in habituation in the treatment group. In sum, the demonstration of a failure to replicate the results requires a replication of the experimental conditions. Ray et al. did not follow the protocol of Fredriksson and Eriksson in its entirety. These were not replicate studies.

Comment 4: The 1993 Fredriksson et al. study should not be used to establish the paraquat chRD because of numerous scientific concerns (see Comments 4a-4f, below).

Response 4: We respectfully disagree with these comments, which have taken this scientific publication out of context. *Toxicology and Applied Pharmacology* is a respected journal. If this paper had so many scientific flaws, it would not have been accepted for publication. OEHHA finds it counterproductive to respond to the individual assertions; however, OEHHA will highlight specific comments to illustrate our view on this issue.

Comment 4a: Details on the specific strain were not provided.

Response 4a: Fredriksson et al., in their paper, indicated that these were C57 black mice. We are not sure what other relevant details ought to be added, especially when historic controls were not used in this experiment.

Comment 4b: Details of the specific salt/hydrate of MPTP and paraquat were not provided.

Response 4b: Paraquat was in solution and thus the relevant species was the paraquat cation.

Comment 4c: The use of fat emulsion vehicle.

Response 4c: The fat emulsion vehicle does not provide an adjuvant effect or enhance absorption (paraquat is in the aqueous phase) in this situation; it was used primarily to facilitate oral administration of paraquat to neonatal mice via a PVC tube.

Comment 4d: Although no effects on body weight were noted at the end of the study, body weight changes during the early phase of experiment could not be precluded. Loch et al. (1978) indicated that body weight changes especially during the neonatal period could affect locomotor activity.

Response 4d: The Loch study was in context of the concern that the increased litter size could affect the nutrition status, body weight, and then the locomotor activity. Loch compared the growth rate and the spontaneous activity of mice that were raised in small litters of eight pups or large litters of 16 pups. The conclusion was that mice from large litters were malnourished, had slower growth rate, and were shown to be hypoactive. Fredriksson et al. had observed a good laboratory practice to keep the litter size to 8-10 mice to address this confounder concern in their experiment since spontaneous activity was a key neurobehavioral parameter measured.

Comment 4e: In most situations, no changes of dopamine levels were noted with paraquat treatment. This is in contrast to the observed decrease in dopamine levels with MPTP treatment.

Response 4e: It should be clarified that there was a significant decrease in dopamine levels in the high-dose (0.36 mg/kg-day) paraquat treatment group. There was also a small decrease in dopamine levels in the low-dose (0.07 mg/kg-day) group. As discussed in the OEHHA draft report, the insignificant dopamine decrease in the low-dose group can be explained by a compensatory mechanism. While the overall dopamine levels may not have significantly changed for the low-dose group, localized neuronal death may have affected the critical neural

circuits and impacted the locomotor activity. Regardless of the connection between dopamine and hypoactivity, hypoactivity by itself is still a valid endpoint.

Moreover, much higher doses of MPTP were used (0.3 or 20 mg/kg-day) relative to paraquat (0.07 or 0.36 mg/kg-day). Thus, it is inappropriate to compare MPTP and paraquat effects in this context.

Comments 4f: The sample sizes of 12 and 8 were based on only three litters per group and the statistical analyses used are inappropriate due to the allocation of litters to groups.

Response 4f: The crux of these criticisms pertains to the experimental design and its evaluation. In Fredriksson and Eriksson's design, the pups were individually treated during the neonatal period and the mothers were untreated. Accordingly, the pups were the experimental unit; whereas in traditional behavioral teratology, the mother or the litter was the statistical experimental unit. This issue has come up before. In an analysis, Eriksson showed that in their neonatal animal model, there is no difference whether the litter or the randomly selected individuals are used as the statistical unit, and that this design does not overestimate any effects[2].

Comment 5: There were similar methodological shortcomings in the Brook et al., Thiruchelvam et al., and Ossowska et al. studies, which were used to support the Fredriksson et al. study (see comments 5a-5d, below).

Response 5: Again, these studies have been published in respected journals and have been subject to scientific peer review prior to their approval for publication. OEHHA will address Syngenta's specific comments on these papers below.

Comment 5a: Brooks et al. administered Fluoro-gold by inserting a needle into the brain and in turn may have damaged the blood brain barrier (BBB), allowing paraquat to gain access. This is a potential confounding factor in the interpretation of the effect of paraquat on dopaminergic neurons.

Response 5a: Fluoro-gold was injected on Day 0 and the first paraquat treatment was given on Day 7. It is possible but not probable that healing has not occurred from any damage created by a 33 gauge (0.2 mm) needle. Moreover, the inherent assumption of this comment is that paraquat cannot cross the BBB. OEHHA's draft report cited evidence to indicate the contrary. Histological evaluation of tyrosine hydrolase (TH)-positive neurons in the substantia nigra and TH-positive pre-synaptic terminals in the striatum in absence of the fluoro-gold procedure by Thiruchelvam et al. and Ossowska et al. also demonstrated the effect of paraquat on the dopaminergic neurons.

Comment 5b: Brooks et al. did not report body weight. It is conceivable that the reduced motor activity was due to generalized toxicity rather than neurotoxicity.

[2] Eriksson, P. (2008) Response to: Use of the Pup as the Statistical Unit in Developmental Neurotoxicity Studies: Overlooked Model or Poor Research Design? *Toxicological Sciences* 103(2), 411-413.

Response 5b: Figure 5A in the Brooks et al. paper illustrates that it is unlikely that the reduced motor activity was due to generalized toxicity. If that were the case, one would have observed hypoactivity in paraquat treatment groups consistently over time when ambulatory activity was measured. Instead, the data show that the low-dose (5 mg/kg) group was hyperactive compared with controls for the first 15 minutes of measurement and the high-dose (10 mg/kg) group's activity was not significantly different from controls at least for the first 10 minutes.

Comment 5c: Thiruchelvam et al. exposed to neonatal mice with higher doses of paraquat than Frediksson et al. did. These higher doses of paraquat did not cause a reduction of dopamine levels or produce hypoactivity. This paper is another example of the inability to replicate findings of Fredriksson et al.

Response 5c: Figure 4 of the Thiruchelvam paper clearly shows that paraquat caused a significant reduction of dopamine levels. While hypoactivity was not produced by paraquat treatment, one cannot, on that basis, conclude a failure to replicate Fredirksson's results. Experimental conditions were not identical. In Thiruchelvam's experiment, mice were habituated to the locomotor activity chambers in three sessions before treatment began. Other factors such as the testing chamber size and time of testing may also affect the results. Again, the demonstration of a failure to replicate the results requires the replication of the experiment, and this is not the case.

Comment 5d: Personal communication with the lead author of the Ossowska et al study indicated that the dosing regimen did produce some mortality, body weight reduction, and potential lung pathology. Thus, the reduction of dopamine and TH-positive neurons could be at least partly attributed to non-specific toxicities.

Response 5d: If non-specific toxicities play any role in the neurotoxicity, one would have observed a generalized neurotoxicity—effects on the dopaminergic system as well as the serotonin and noradrenaline systems. That was not the case; there were no significant effects on the serotonin and noradrenaline systems.

Comment 6: Neurotoxicity studies described in the literature typically used high doses of paraquat. These studies are of limited utility for human risk assessment.

Response 6: The fundamental issue is that studies using high doses of paraquat do not reflect environmentally relevant exposure. OEHHA would like to point out that the nature of toxicity testing usually requires testing at relatively high doses. Testing at high doses are necessary to detect adverse effects when a limited number of animals and animal species are used, which is usually the case to minimize the cost of testing. Testing at environmental relevant doses, which would require large studies, utilizing thousands of animals and at extreme costs, are an infeasible proposition.

Comment 7: Given the questions raised on the Fredriksson et al. study, OEHHA should use the one-year dog study that U.S.EPA and regulatory bodies in other countries used in setting the reference dose.

Response 7: From the perspective of establishing a child-specific reference dose, OEHHA has to consider potential sensitivities in children, including the critical developmental window of exposure. The dog study falls short of this criterion. OEHHA has reviewed the questions raised by Syngenta and has concluded that it is appropriate to use the Fredirksson et al. study as the basis for developing the chRD for paraquat.

# APPENDIX 2: Syngenta Crop Protection, Inc. Comments on Draft

**Paraquat**

**Response to the California OEHHA Proposed Child-Specific Reference Dose (chRD) for Paraquat**

**Response**

**DATA REQUIREMENT(S):** Not Applicable

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**COMPLETION DATE:** February 17, 2010

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**VOLUME 1 OF 1 OF STUDY**

# STATEMENT OF DATA CONFIDENTIALITY CLAIMS

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS
UNDER SPECIFIED FIFRA PROVISIONS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10(d)(1)(A) (discloses manufacturing or quality control processes), (B) (discloses the details of methods for testing, detecting or measuring the quantity of any deliberately added inert ingredient of a pesticide), or (C) (discloses the identity or percentage quantity of any deliberately added inert ingredient of a pesticide).

Company
Syngenta Crop Protection, Inc.

Company Agent: Montague Dixon, M.S.

Jim Wells for Montague Dixon 2-17-2010
U. S. Product Registration Manager Date

Syngenta is the owner of this study. Syngenta has submitted this material to the United States Environmental Protection Agency specifically under the provisions contained in FIFRA as amended and, hereby, consents to use and disclosure of this material by EPA according to FIFRA. Notwithstanding the wording of our marking TRADE SECRET, this marking, by itself, conveys no supplemental claims of confidentiality under FIFRA Sections 10(a) or 10(b) (addressing protection of trade secrets and commercial and financial information). In submitting this material to EPA according to method and format requirements contained in PR Notice 86-5, we do not waive any protection or right involving this material that would have been claimed by the company if this material had not been submitted to the EPA, nor do we waive any protection or right provided under FIFRA Section 3 (concerning data exclusivity and data compensation) or FIFRA Section 10(g) (prohibiting disclosure to foreign and multinational pesticide companies or their agents).

# GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

As this volume contains comments and assessment of information and data in reports submitted separately, a Good Laboratory Practice Compliance Statement is not appropriate.

Study Director: There is no GLP Study Director for this volume.

Timothy Pastoor, PhD, DABT
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17 February 2010
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# 1.0 EXECUTIVE SUMMARY

On December 4, 2009, the California Office of Environmental Health Hazard Assessment (OEHHA) published the draft report "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" for public review and comment.

This document provides the evidence and rationale indicating that paraquat should not be included as a contaminant of concern pursuant to Health and Safety Code (HSC) Section 901(g) due to the lack of potential for exposure to children in or around school sites. The following evidence and rationale is discussed in detail in this document and obviates the need for a paraquat chRD:

1. Paraquat does not meet any of the criteria set forth by OEHHA as necessary for developing a chRD.
2. Since 2008, the Department of Toxic Substance Control (DTSC) no longer considers paraquat a "contaminant of concern" for proposed school sites on former agricultural fields.
3. DTSC's original classification of paraquat as a contaminant of concern was based on an erroneous presumption that the relatively long half-life of paraquat in the soil was related to bioavailability or potential exposure to humans.
4. Paraquat binds tightly to soil and is not bioavailable once bound.
5. There are negligible to no paraquat residues on proposed school sites.
6. Since DTSC's original classification of paraquat, numerous Preliminary Endangerment Assessments (PEAs) have been produced with paraquat as one of the analytes. Paraquat has only been detected in a single PEA, at only slightly above the level of detection, including a sample that could not be duplicated and, most importantly, only after extracting paraquat from the soil using boiling sulphuric acid, the only way to extract the tightly bound paraquat from the soil.
7. Paraquat products are restricted from use around schools.
8. Additional air and water monitoring data reinforces negligible to no exposure.

Syngenta respectfully requests that OEHHA remove paraquat from its list of chemicals requiring a chRD. Paraquat's original inclusion as a contaminant of concern for agricultural field sites (DTSC, 1999) was based on an erroneous connection by the Department between soil persistence and exposure potential (bioavailability). In fact, in 2008, DTSC stated that paraquat assessments are no longer appropriate for school sites located in agricultural regions due to lack of detected residues (DTSC, 2008).

Therefore, OEHHA does not need to establish a child-specific reference dose since paraquat does not meet any of the criteria for doing so. There is no potential for the occurrence of bioavailable residues of paraquat at school sites, consistent with DTSC experimental findings and recent conclusions. OEHHA should continue to utilize the toxicity endpoint currently used by the majority of the regulatory authorities globally rather than the one currently selected by OEHHA in their draft document. OEHHA placed significant emphasis on one study (Fredriksson *et al.*, 1993) which is of questionable quality, which other independent research groups have been unable to reproduce, and which has not been used by any regulatory agency in setting reference doses.

## 2.0 INTRODUCTION

On December 4, 2009, the California Office of Environmental Health Hazard Assessment (OEHHA) published the draft report "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" for public review and comment. This draft report was published based upon OEHHA having erroneously identified paraquat as a contaminant of concern pursuant to Health and Safety Code (HSC) Section 901(g), which would have 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. Summarized in this draft report is OEHHA's evaluation of paraquat's potential health impact in the context of school site risk assessment as well as the process used by OEHHA for developing a proposed child-specific reference dose (chRD) for paraquat.

The purpose of this document is to describe why a chRD for paraquat is neither required nor necessary. The lack of bioavailable paraquat in any of the State's residue analyses and paraquat's rapid and tight soil-binding characteristics are sufficient reasons not to develop a chRD. Children will not be exposed to paraquat residues. Furthermore, this document will comment on OEHHA's derivation of the proposed chRD.

## 3.0 OEHHA CRITERIA FOR ESTABLISHING A CHILD-SPECIFIC REFERENCE DOSE (CHRD)

OEHHA (2009) employs the following criteria in the selection of chemicals for evaluation in the chRD development process:

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."

Paraquat does not fulfill any of these criteria, obviating the need for a paraquat chRD. Syngenta's consideration of each of these criteria is discussed in more detail as follows.

# 4.0 REASONS WHY CHILDREN WILL NOT BE EXPOSED TO PARAQUAT

## 4.1 Paraquat Does Not Have a "Strong Indication of Presence at School Sites or Proposed School Sites"

The draft OEHHA paraquat document (OEHHA, 2009; p 6) indicates that, "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." Based on the State's decade-long analysis for paraquat at potential school sites on land previously used by for agricultural production, the occurrence of paraquat is negligible and the occurrence of bioavailable paraquat is non-existent (DTSC, 2008). Furthermore, based on the known chemical characteristics of paraquat, soil-bound paraquat is deactivated and is not bioavailable.

The use pattern and soil binding characteristics of paraquat are such that children would not be exposed. Paraquat is tightly bound to soil particles and is therefore biologically unavailable. In addition, there are data to show that paraquat concentrations are negligible or non-detectable in soil samples. Finally, environmental monitoring programs demonstrate that paraquat is not typically detected in California paraquat-usage areas, further validating the lack of potential exposure.

### 4.1.1 Soil Binding Characteristics of Paraquat

Paraquat is very rapidly adsorbed to particles of soil, sediment, or dust. As a consequence, the herbicidal property of paraquat is deactivated, the residues are not bioavailable, and toxicity potential is negligible. In fact, clay is given to individuals who have swallowed paraquat formulations. The reason is that the clay rapidly binds paraquat, reducing absorption into the body.

The deactivation of the biological activity of paraquat in soils has been thoroughly and systematically investigated over many years. There is a wealth of evidence to demonstrate that adsorption is capable of deactivating the equivalent of hundreds or thousands of paraquat applications over a wide range of soil types (Roberts, et al, 2002). The strong adsorption also results in paraquat being effectively immobile in soil, with no risk of leaching to ground water.

The primary rapid adsorption of paraquat is via cation exchange, with the positively charged paraquat molecules being attracted to the negatively charged minerals and organic matter in soil. Other processes have also been reported, namely, van der Waals forces, formation of charge transfer complexes and hydrogen bonding. These processes serve to enhance the adsorption beyond the simple cation exchange reaction. Due to these binding characteristics, specific methods must be used for analyzing soils for paraquat residues.

Paraquat is not easily dislodged from its soil-bound sites. Mordaunt C J *et al*. (2005) investigated a range of crop protection chemicals ($^{14}$C-labelled atrazine, dicamba,

isoproturon, lindane, trifluralin and paraquat), employing a sequential extraction procedure, with each subsequent solvent being less polar. The following extraction system was performed:

- Step 1 0.01 M $CaCl_2$ shake extraction for 24 h
- Step 2 Acetonitrile:water (9:1) shake extraction for 24 h
- Step 3 Methanol shake extraction for 24 h
- Step 4 Dichloromethane shake extraction for 24 h
- Step 5 Added $^{14}C$-activity combusted to $^{14}CO_2$

Step 1 was chosen to simulate the readily available soil fraction, Steps 2 to 4 were chosen to indicate potentially bioavailable soil fractions, and Step 5 gave the un-extracted residue. The extraction methods resulted in release of all chemicals except paraquat in Steps 1-4. But paraquat was only found in Step 5, indicating that it remained bound to the soil matrix and was not available for extraction or mineralization under the conditions investigated.

Since paraquat is strongly adsorbed to soil, various approaches to the analysis of paraquat residues in soils have been developed and used. Chemical extraction is most appropriate for determination of total residues in soil. The chemical extraction method involves refluxing soil with concentrated (6N to 18N) sulphuric acid (Ospenson and Pack, 1964 – see Appendix 1; Chevron, 1970). This method results in destruction of the soil matrix in order to release the very strongly bound paraquat residues.

It is important to emphasize that unless harsh chemical extraction methods are used (i.e. boiling soil samples in sulphuric acid), soil-bound paraquat is not released. Perhaps the best demonstration of this comes from studies on the long-term environmental fate of paraquat (Roberts, et al, 2002). These investigators used the "strong adsorption capacity – wheat bioassay (SAC-WB)" to determine the adsorption capacity of paraquat in soils. This method was validated in field soil situations within a series of long-term trials in different regions of the world, most of which are very long-term, beginning as early as 1971. The authors conclude, "During more than 40 years of use in over 100 countries, covering many and varied agronomic practices, there has been no observation of the reactivation of adsorbed paraquat residues due to desorption." Paraquat is 99.99% soil-bound, does not come off the soil unless refluxed in hot acid, and is therefore biologically unavailable. Any residues of paraquat in soil that may be present from prior agricultural use on land that is now intended for schools will not be bioavailable. On this basis alone, there is no need for a chRD.

### 4.1.2 There are Negligible to No Paraquat Residues on School Sites

The OEHHA draft report indicated that:

> "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 reference provided is a personal communication from a DTSC staff member to OEHHA. It is our understanding that, at the time of that communication, the DTSC was still under the misconception that the relatively long half-life of paraquat in the soil was related to the potential of paraquat to be bioavailable during this period or otherwise relevant to human exposure (DTSC, 1999 and 2002).

Syngenta was unaware of this historical misperception until recently, when the draft chRD document was released and, as a result, had not been able to correct the original misinterpretation that led to paraquat's classification by the DTSC. However, DTSC has corrected their presumption based on their decade-long attempts to find paraquat at potential school sites. In the most recent revision to their "Interim Guidance for Sampling Agricultural Properties (Third Revision)" (DTSC, 2008) they state:

> "While paraquat does have a longer half-life in soil, it has either not been detected or detected rarely at trace levels at sites which DTSC has had oversight, therefore routine analyses for paraquat is not required for field areas. Analyses for paraquat may be required in storage and mixing/loading areas."

In fulfilling its role in analyzing for pesticides on proposed school sites, DTSC reported that paraquat had only been confirmed in a single study and this was from a soil sample taken from former agricultural land in the Central Valley that was being considered for conversion into a school site. In a listing of the nearly two dozen sites assessed for the presence of paraquat (DTSC, 2010), paraquat was detected in soil samples at one proposed school site, Union Ranch Elementary School. Paraquat was detected at "<1.0 to 1.4 mg/kg (3 samples, 1 duplicate)", and the PEA recommended *no* further action (DTSC, 2006a; p7). DTSC concurred with the PEA conclusions and recommendation, and approved the PEA report (DTSC, 2006b). In fact, in this singular report (DTSC, 2006a) paraquat was only detected above the LOQ in two samples, and one of those was a "duplicate" sample, in which paraquat was non-detectable.

Most notable about this analytical report (DTSC, 2006a; p4) is that they used the method "Chevron RM 8-10" (Chevron, 1970), which is the technique of refluxing soil in hot sulphuric acid. As stated previously, this is an extreme analytical method that destroys the soil structure through the use of hot concentrated acid in order to release bound paraquat. This is the only means by which the extremely tightly bound paraquat can be removed from its adsorption to the soil matrix. Even with such extraordinary methods, the paraquat measurements were just above the level of detection. These results confirm that paraquat residues on former agricultural land are negligible or non-existent and further strengthen the point that paraquat is not biologically available. Therefore, the data indicate that no chRD is necessary due to the lack of bioavailable paraquat in soil.

### 4.1.3 Paraquat products are restricted from use around schools

In addition to its lack of bioavailability from soil, no paraquat will be used around schools. Paraquat dichloride is a restricted use, non-selective herbicide that can only be sold to and

used by certified applicators or persons under their directed supervision and only for those uses covered by the certified applicator's certification. Paraquat dichloride labels clearly state that they are not approved for use in or around schools[1].

1. "Do not use around home gardens, schools, recreational parks, golf courses, or playgrounds."
2. "Do not apply this product in a way that will contact workers or other persons, either directly or through drift."
3. "It is a violation of Federal law to use this product in a manner inconsistent with its labeling."

Therefore, it is illegal to spray or use paraquat around schools.

### 4.1.4 Additional California Monitoring Data

Although not relevant to the specific requirements of the OEHAA criteria for school sites and the development of chRDs, the lack of paraquat's presence in other California environmental monitoring programs further supports the lack of exposure to children on schoolyards. Paraquat has been on sale in California for over 35 years.

#### 4.1.4.1 Air monitoring

The California Air Resources Board has conducted ambient air monitoring of pesticides in support of the California Department of Pesticide Regulation toxic air contaminant program. Monitoring was done in several communities in a County of high use during the month of expected peak use of a particular pesticide in order to assess general population exposure.

Additional sampling was conducted adjacent to specific agricultural applications to assess maximum short-term concentrations to which the public might be exposed. Medium and low volume samplers were used with appropriate collection media (i.e. Teflon filters, XAD-2 adsorbent resin), followed by laboratory analysis. Concentrations measured around specific applications would be expected to be representative of other areas, especially in California, with comparable application rates, crops and weather conditions. Since the program began in 1986, monitoring has been conducted for 22 pesticides.

Specific air monitoring for paraquat was conducted between September and November 1987 in Fresno County, a time coinciding with the use of paraquat as a cotton defoliant. No paraquat residues (limit of quantitation 0.022 $\mu g/m^3$) were detected at the four sites sampled over 31 days (Bakker et al., 1996). These results are not surprising since paraquat has a very low vapour pressure and virtually no ability to evaporate (Vapor pressure < 1 x 10-8 kPa at 25 °C -- value was estimated by extrapolation because the vapor pressure of the pure active ingredient is too low to be measured).

[1] For example, 'Gramoxone Inteon' label, http://www.syngentacropprotection.com/labels/

#### 4.1.4.2 Surface water monitoring

Surface water monitoring data is available for paraquat from the California Department of Pesticide Regulation (CDPR) database. A total of 399 water samples were analyzed from July 2005 to October 2006, and of these, only one sample was found to contain a detectable level of paraquat. However, this single detection (0.24 ppb) was below the quantification limit of 1 ppb (USEPA, 2009). Any paraquat present in surface water would likely be bound to suspended sediments and not biologically available in surface water.

#### 4.1.4.3 Groundwater monitoring

A search was conducted for historical paraquat monitoring data in the United States Environmental Protection Agency's (USEPA) Pesticide in Ground Water Data Base (PGWDB) (USEPA, 1992). The PGWDB contains groundwater monitoring data in which pesticides were included as analytes (1971-1991 compilation).

The Californian groundwater monitoring data included in the PGWDB involved the sampling of 833 wells (a total of 884 samples) taken from 43 Counties in the five year period 1984 to 1989. There were no paraquat detections.

## 4.2 No Other US / California Authoritative Body Has Identified Paraquat as a Concern

Syngenta is unaware of any other US programs that have identified paraquat as a concern. Paraquat is not listed under California's Proposition 65 because the weight of evidence from the animal toxicity studies indicates paraquat is <u>not</u> a reproductive toxicant, developmental toxicant nor a carcinogen. Paraquat's status under OEHHA's Proposition 65 is consistent with the compound's evaluations by the USEPA (an "authoritative body" as defined by Proposition 65) who stated that paraquat is <u>not</u> a reproductive toxicant, developmental toxicant or carcinogen (USEPA, 1997).

## 4.3 Neurotoxicology considerations

Because paraquat is biologically unavailable due to its soil-binding characteristics, there is no need to develop a chRD for paraquat. However, OEHHA is suggesting that there may be a need for a chRD, apparently based on the second of its three criteria:

> "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." (p. 5 of the OEHHA's Draft Report).

Paraquat is not a reproductive or developmental toxicant, not an endocrine modulator, not an immuntoxicant, and did not result in neurotoxic effects in USEPA guideline compliant studies. Acute and subchronic neurotoxicity studies conducted with paraquat (Brammer 2006; Chivers, 2006) showed no indication of neurotoxicological effects.

Syngenta has conducted acute and subchronic neurotoxicity studies in the rat in accordance with OECD Test Gudeline 424. Study endpoints investigated included functional observations, locomotor activity and brain weight. Tissues examined included: transverse sections of the brain (at 7 levels), gastrocnemius muscle, eye (with retina and optic nerve) and spinal cord [at cervical and lumbar swellings and including dorsal root ganglia and spinal nerve roots, dorsal and ventral root fibres], longitudinal sections of spinal cord (at cervical and lumbar swellings), transverse and longitudinal sections of proximal sciatic nerve, proximal tibial nerve and distal tibial nerve (tibial nerve calf muscle branches).

In the acute neurotoxicity study (Brammer, 2006) there was no evidence of neurotoxicity at dose levels up to 250 mg paraquat technical/kg body weight (equivalent to 84 mg paraquat ion/kg body weight), the highest dose tested.

In the sub-chronic (90 day, dietary) study (Chivers 2006), neurobehavioural tests and neuropathological examination of the central and peripheral nervous system showed no effects of treatment at doses of up to 150 ppm paraquat ion.

Groups of twelve male and twelve female Alpk:AP$_f$SD (Wistar-derived) rats were fed diets containing 0 (control), 15, 50 or 150 ppm paraquat for at least 90 consecutive days. All animals were observed prior to the study start and daily throughout the study for any changes in clinical condition. In addition, detailed clinical observations, including quantitative assessments of landing foot splay, sensory perception and muscle weakness, were performed in weeks -1, 2, 5, 9 and 14. Locomotor activity was also monitored in weeks -1, 2, 5, 9 and 14. Bodyweights and food consumption were measured weekly throughout the study. An ophthalmoscopic examination was performed on all animals pre-study and on top dose and controls in week 13. At the end of the scheduled period, 5 rats/sex/group were killed by *in situ* perfusion fixation, the brain was weighed and selected nervous system tissues were removed, processed and examined microscopically.

There were no test substance related effects on any of the measured parameters. The no effect level for neurotoxic potential was 150 ppm (equivalent to 10.2 - 11.9 mg paraquat ion/kg bw) for male and female rats. This level is well above the existing lowest NOAEL of 0.45 mg/kg bw (for pneumonitis in the dog) from which the conventional chronic regulatory reference dose of 0.0045 mg paraquat ion/kg bw is established.

The USEPA Reregistration Eligibility Decision (RED) Document for paraquat (EPA, 1997) concluded that paraquat is not a reproductive or developmental toxicant, not an endocrine modulator, not an immuntoxicant, and that there was limited concern for neurotoxicity resulting from potential exposure to paraquat. EPA restated this as recently as 2006 (USEPA, 2006).

The World Health Organization (WHO, 2003) Joint Meeting on Pesticide Residues (JMPR) also reviewed the toxicology of paraquat in 2002 and agreed that paraquat is not a reproductive or developmental toxicant, not an endocrine modulator, not an immuntoxicant,

and there is limited concern for neurotoxicity resulting from exposure to paraquat. The review included published studies relating to neurotoxicity.

Unlike USEPA and JMPR, OEHHA placed significant emphasis on published research articles that indicate evidence of neurotoxicity. However, Syngenta is unaware of any regulatory authority that utilizes the 1993 Fredriksson et al. study for reference dose setting. Perhaps one reason is that at least two other independent research groups have not been able to reproduce the results of Fredriksson. WHO/JMPR goes one step further and makes the following conclusion with regard to the Fredriksson findings (WHO, 2003):

> "Persistent hypoactivity was observed in mice given paraquat by mouth on postnatal days 10 and 11. Reduced striatal content of dopamine and its metabolites was seen, but concentrations of serotonin were not affected. In a similar study of which the Meeting was aware, these findings had not been reproduced."

The WHO/JMPR review goes on to discuss other published neurotoxicity studies:

> "Studies on the effects of paraquat on the central nervous system have used a variety of routes, including subcutaneous or intraperitoneal injection and direct injection into the central nervous system, and end-points observed have been behavioural, morphological and neurochemical. Behavioural effects and loss of neurones in the substantia nigra were observed and, neurochemically, depletion of dopamine was reported in many, but not all of these studies. The design of these studies, however, renders the relevance of these data questionable for the risk assessment of dietary exposure to paraquat residues."

The Fredriksson et al., 1993 publication should not be used for any regulatory decision and the paraquat chRD calculation should not be based on this study for the following reasons:

1) There are several methodological concerns/uncertainties:
   a) Details on the specific strain were not provided.
   b) Details of the specific salt/hydrate of MPTP and paraquat were not provided.
   c) The use of the fat emulsion vehicle (paraquat salts are soluble in aqueous solutions).
   d) It is unclear whether litter effect was controlled
2) Concerns regarding the results:
   a) The author states there were no changes in body weight gain or body weight at the end of the experimental period. Although no effects of body weight gain were noted at the end of the study this does not mean that treatment-related body weight changes did not occur earlier during the study. Body weight changes, particularly during the neonatal period, are known to affect locomotor activity (Loch, *et al* 1978).
   b) No specific comments on the locomotor activity data.
      i) There is no information indicating whether the locomotor activity data was transformed prior to statistical analysis.

c) The MPTP treated mice and the paraquat low-dose group mice were not tested on PND 18, which is an unbalanced test.
d) The changes in dopamine (DA) are not consistent with what has been reported by several investigators in adult mice treated with paraquat. In most cases no changes in DA levels are noted. This is in contrast to the decrease in DA seen after adult C57BL/6 mice are treated with MPTP.

3) Concerns with the Statistics:
   a) The statistical analyses used in the paper are inappropriate due to the allocation of litters to groups and the failure to allow for these in the analyses conducted. More appropriate statistical analyses would lead to less statistically significant differences.
   b) The sample sizes of 12 and 8 for motor activity and 8 for neurochemistry are only based on 2 or 3 litters per group and differences between groups could reflect litter differences.
   c) It is unclear whether the treated groups were balanced in terms of litter size or body weight at the time of treatment and it is unclear whether appropriate randomisation for motor activity monitoring and terminal kills were employed. Consequently, the paper does not provide compelling evidence that the differences observed are treatment-related.
   d) The lack of effect with paraquat at day 18 is not convincing due to the very low power for detecting reduced activity in young mice.

The Medical Research Council (MRC) Toxicology Unit, Leicester, UK, also found that the same Fredriksson *et al*. study design was not reproducible with respect to the developmental toxicity of at least three other compounds tested in the Fredriksson/Eriksson lab, including the pyrethroids (Muhammad, B Y *et al*., 2003). The MRC concluded:

> "Whilst the pyrethroid effects are clearly reproducible under the specific conditions pertaining in the laboratory of Eriksson et al., we believe that our negative results must cast doubt on the general applicability of their findings with regard to pyrethroids."

In addition, a small number of additional publications are cited by OEHHA as supportive of the Fredriksson study, but they also had similar methodological shortcomings, with varied high-dosing regimens and unrepresentative routes of administration (i.e. intraperitoneal, i.p., injection) in non-guideline studies. The additional ('supporting') studies are briefly reviewed in Appendix 2.

The neurotoxicity research studies described in the literature have typically studied the neurotoxicological potential of paraquat by using multiple high (10 mg/kg i.p.) doses of paraquat injected directly into the C57Bl6J mice. Some research groups have reported that such a dosing regimen in this particular strain of mouse causes a loss of tyrosine hydroxylase positive (TH+) staining neurones (dopaminergic neurones) in the substantia nigra *pars compacta* (SNpc). Some of the literature reports also indicate that striatal dopamine levels are depleted to varying degrees, and that there is a reduction in locomotor activity associated with the paraquat exposed animals. Given the excessively high doses systemically

administered directly into the body, these studies are of limited utility for human risk assessment purposes.

Syngenta is unaware of any regulatory authority that utilizes these studies for establishing reference doses.

### 4.3.1 Reference Dose Determination

OEHHA should utilize the toxicity endpoint currently used by the majority of the regulatory authorities globally.

Paraquat-containing products are registered by the USEPA and California DPR. USEPA and DPR thoroughly reviewed the toxicity, exposure and risk characteristics of paraquat dichloride and concluded that the registered uses of paraquat do not pose unreasonable risks to humans or the environment. USEPA concluded that the 10x FQPA uncertainty factor for sensitivity to children can be reduced to 1x due to the lack of reproductive or developmental toxicity, and paraquat is not included on the California Prop 65 list.

In the Draft OEHHA document, OEHHA selected the toxicity endpoints from Fredriksson et al, 1993, to establish the chRD for paraquat. OEHHA selected the LOAEL of 0.07 mg/kg/day and divided this value by an uncertainty factor of 1000 to achieve a chRD of 0.00007 mg/kg/day. The draft OEHHA reference dose is inconsistent with USEPA, EU, JMPR, ATSDR and other regulatory authorities by two orders of magnitude. The majority of reviewers recognize the one-year dog study as the most sensitive endpoint (NOAEL = 0.45 mg/kg/day), and the acceptable daily intake (ADI) or reference dose is calculated to be 0.004 to 0.005 (0.45 mg/kg/day divided by a 100 fold uncertainty factor).

| Country/Organization | Acceptable Daily Intake (mg PQ ion/kg/d) |
|---|---|
| Australia | 0.004 |
| Brazil | 0.004 |
| EU | 0.004 |
| JMPR | 0.005 |
| USA/Canada | 0.0045 |
| **OEHHA** | **0.00007** |

Based on the significant concerns over the reliability and reproducibility of the Fredriksson et al., 1993 publication, Syngenta recommends that OEHHA do not use this study for toxicity endpoint selection or reference dose setting and instead rely on previously established reviews, endpoints, and reference doses.

## 5.0 CONCLUSION

In conclusion, Syngenta has provided the rationale and evidence that paraquat does not meet the OEHHA criteria for inclusion on the list for developing a proposed child-specific reference dose (chRD). A chRD for paraquat is unwarranted in light of several factors that indicate negligible or no potential for exposure to children or adverse effects from biologically available paraquat residues in or around school sites. Paraquat is tightly bound to soil, is not biologically available in DTSC tests, is not allowed to be used in or around schools, and is no longer considered a general compound of concern by the DTSC for school sites located on agricultural lands.

Finally, OEHHA could continue to use the existing toxicity endpoints for paraquat rather than the one currently selected by OEHHA in their draft document. OEHHA placed significant emphasis on one study (Fredriksson *et al.*, 1993) that should not be used because it is of questionable quality and has not been reproduced by other independent research groups.

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# APPENDICES SECTION

## Appendix 1 Recovery of Paraquat from Soil and Clay Samples (Ospenson and Pack, 1964)

CRM 117

F2.1/04

CALIFORNIA CHEMICAL COMPANY
ORTHO DIVISION
RESEARCH AND DEVELOPMENT DEPARTMENT
RICHMOND, CALIFORNIA

PARAQUAT - RECOVERY FROM SOIL AND CLAY SAMPLES

By: J. N. Ospenson and D. E. Pack

FILE NO. 721.10
APRIL 15, 1964
(Supersedes previous report on this subject dated February 6, 1964.)

### INTRODUCTION

One of the major initial problems which has confronted us in our assigned project on the soil metabolism of Paraquat has been the lack of an adequate method for the extraction and detection of Paraquat from soil samples. Such an analytical method must be available before any in vivo studies on the breakdown of Paraquat in various types of soils could be made. For this reason, we have concentrated on developing such an extraction procedure.

We made a preliminary report of a satisfactory extraction procedure in our progress report dated November 26, 1963. The purpose of this report is to present the results of our detailed investigation of this method and its application to a variety of soil and clay types.

### SUMMARY

It has been found that Paraquat can be successfully recovered from all types of soil or clay samples studied to date by refluxing with fairly concentrated sulfuric acid. The concentration of acid required for adequate extraction has been found to vary dramatically, depending upon the nature of the clay or soil sample being studied. For routine work, we have standardized on the use of 18 N sulfuric acid. These recovery tests have been run at concentrations between 0.07 to 11 ppm Paraquat ion in the soil or clay. Further work is in progress and it is believed the method will permit the detection of 0.02 ppm.

Preliminary studies were made on the recovery efficiency obtained by this method on Paraquat samples which were stored in the presence of soil for various periods of time. These tests were not primarily designed for metabolism studies and therefore great care was not taken in controlling the storage conditions. All samples were stored in polyethylene stoppered, white pyrex glass vessels at ambient temperatures. They were exposed to air and to normal laboratory lighting. The results obtained are shown in Table 5 and show no differences in recovery at any interval. This data implies that there is no redistribution of Paraquat between various active sites, in the soil, which invalidate the extraction procedure.

### DISCUSSION

All of our preliminary work on the development of a satisfactory extraction procedure was done on three widely divergent types of soils and on a variety of different types of clays. The composition of the three soil types, called Soils I, II and III, are shown in the attached Table 1. These compositions were chosen so as to represent a wide difference in the ratio of the three components.

Our earliest work involved attempts to elute directly from fortified soil samples with various types of eluting solvents. In addition to the solvents shown in Table 2, we also attempted to use various types of high molecular weight quaternary ammonium salts, as well as dimethyl formamide, dimethyl sulfoxide, etc., etc. None of these solvents gave essentially any recovery

Enclosures: Tables 1 through 5
Figure 1

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of Paraquat under these conditions. In Table 2, it can be seen that water and saturated ammonium chloride also gave no recovery whatsoever, concentrated calcium chloride gave a very small amount of recovery, whereas concentrated aluminum sulfate gave none. Concentrated hydrochloric acid gave approximately 15% recovery and 18 N sulfuric acid gave approximately 24% recovery. The results using Soils II and III were, in general, quite similar to those from Soil I.

The next step was to allow a considerably longer contact time between the liquid and the fortified soil samples. In these cases, the fortified soil sample was added to a quantity of the liquid and allowed to soak overnight. It can be seen in Table 2 that the recoveries were generally somewhat better with this longer contact time, but still were not adequate for our purposes.

The next step was to actually reflux the fortified soil sample with the liquid concerned. In the case of saturated ammonium chloride and saturated calcium chloride, this reflux period had essentially no beneficial effect whatsoever. But in the case of the concentrated sulfuric acid, very excellent recovery of Paraquat was achieved.

The effect of the concentration of sulfuric acid required for this extraction has been studied in some detail, particularly for Soil I, which would be considered to be the easiest from which one could remove Paraquat. These results are summarized in Table 3. It can be seen from this table that one obtains very poor recoveries with the use of 1 or 2 N sulfuric acid, which is the concentration normally employed in our work on the recovery of Paraquat from plant materials. The recovery continues to improve until it reaches greater than 95% for both 12 and 18 N. It can further be seen that this high recovery is obtained for all three types of soil samples at between 0.07 to 11 ppm fortification levels. Further work is still going on and it is hoped that we will have satisfactory recovery at levels of 0.02 ppm.

This extraction method has also been applied to a variety of fortified clays. This work is summarized in Table 4. It can be seen from this table that the concentrations of sulfuric acid required for the three types of soil samples studied in Table 3 are not necessarily adequate for all of the clays studied. 9 N sulfuric acid does a satisfactory job with Attapulgus Clay, but a completely inadequate job with the other four clays reported in Table 4. At 12 N sulfuric acid, 4 of the clays give adequate recovery, whereas Kaolinite still gives a very low recovery. However, 18 N sulfuric acid gives very excellent recoveries both at 11.0 and at 1.5 ppm fortification levels. A plot of these recovery figures for representative members of the clays and soils studied are shown in Figure 1.

Studies were also made in which the time of reflux was varied between 5 and 12 hours. This increase in reflux time had a relatively minor effect upon the recoveries observed. Thus, it would appear that the actual higher normality of acid was required for obtaining satisfactory recovery of Paraquat.

It was considered desirable, even in these early studies, to get some idea as to the effect upon recovery, if any, of storage of Paraquat on the soil or clay sample being studied. Results of such a test would both answer the question as to whether there was any apparent metabolism or breakdown of Paraquat on the soil or clay samples under the conditions of storage employed and, also, whether or not there was any change in the absorption characteristics which might modify the recovery that could be obtained by the method under study. For this reason, a series of the three soil samples, as well as Kaolinite and Attapulgus clays, were fortified at 11 ppm. Some of these fortified samples were immediately extracted by means of the sulfuric acid, while the remaining samples were stored in the laboratory under ambient



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conditions. Additional samples were extracted after 5 days', 3 weeks' and 7 weeks' storage. These results are shown in Table 5. It can be seen that one obtained essentially the identical recovery at all intervals indicating that there is no breakdown under these conditions but, more significantly, that there is no change in the bonding characteristics which effect the extraction efficiency.

The general extraction procedure that has been employed in the majority of these tests can be briefly summarized as follows:

A 10 gram sample of the soil or clay was fortified with the required amount of Paraquat dichloride from an aqueous solution. 100 ml. of the proper normality sulfuric acid was then added to the sample and boiled for 5 hours. After this period, it was cooled and filtered through glass filter paper, using a Buchner filter. The filtrate was diluted to about 1 N sulfuric concentration and transferred to a separatory funnel. This diluted solution was then percolated through an ion exchange column (Dow AG 50W-X-8, 100/200 mesh) which had been prewashed with 25 ml. of saturated sodium chloride, then rinsed free of excess NaCl with water. After all the material had passed through the column, the column was washed with 50 ml. of 2 N HCl and 25 ml. of 1/10th saturated $NH_4Cl$. The Paraquat was then eluted from the column with saturated $NH_4Cl$ into a 25 ml. volumetric flask. 2 ml. of 0.2% sodium dithionite solution in 0.3 N NaOH was added to a 10 ml. aliquot of the $NH_4Cl$ eluate and read on a Beckman DB Spectrophotometer, following residue procedure, RM-5.

J. N. OSPENSON

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TABLE I

COMPOSITION OF SOIL SAMPLES

| Code | Composition (%) | | |
|---|---|---|---|
| | Sand | Peat | Loam |
| Soil I | 98 | 1 | 1 |
| Soil II | 60 | 10 | 30 |
| Soil III | 30 | 10 | 60 |

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TABLE 2

ATTEMPTED EXTRACTION PROCEDURES*

(Fortification at 11 ppm)

| Sample | Solvent | % Recovery | | | |
|---|---|---|---|---|---|
| | | Immediate Elution From Column | | Soaking Overnight | 5 Hour Reflux |
| | | Cut 1** | Cut 2** | | |
| Soil I | $H_2O$ | 0 | 0 | 0 | - |
| | Sat. $NH_4Cl$ | 0 | 0 | 1.0 | 2.0 |
| | $CaCl_2$ (750 g/l.) | 8.0 | 2.0 | 2.3 | 2.0 |
| | $Al_2(SO_4)_3$ (600 g/l.) | 0. | 0. | 2.0 | - |
| | Conc. HCl | 15.7 | - | 21.6 | - |
| | 18 N $H_2SO_4$ | 24.1 | - | 36.5 | >95.0 |
| Soil II | $H_2O$ | 0 | 0 | - | - |
| | Sat. $NH_4Cl$ | 0 | 0 | - | - |
| | $CaCl_2$ (750 g/l.) | 17.0 | 2.0 | - | - |
| Soil III | $H_2O$ | 0 | 0 | - | - |
| | Sat. $NH_4Cl$ | 0 | 0 | - | - |
| | $CaCl_2$ (750 g/l.) | 6.0 | - | - | - |

* 10 g. soil samples used.

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TABLE 3

SULFURIC ACID EXTRACTION - SOILS*

| Soil | ppm Added | % Recovery | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 N | 2 N | 5 N | 9 N | 12 N | 18 N |
| Soil I | 11.0 | 0.6 | 7.3 | 49.3 | 88.3 | 95.7 | - |
| | 1.5 | - | - | - | - | - | 107, 106 |
| | 0.07 | - | - | - | - | - | 102, 113 |
| Soil II | 11.0 | - | - | - | 77.0 | 94.3 | - |
| | 1.5 | - | - | - | - | - | 108, 109 |
| | 0.07 | - | - | - | - | - | 113, 75 |
| Soil III | 11.0 | - | - | - | 74.7 | 91.5 | - |
| | 1.5 | - | - | - | - | - | 97.5, 102 |
| | 0.07 | - | - | - | - | - | 93, 93 |

* 5 hour reflux period.

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TABLE 4

SULFURIC ACID EXTRACTION – CLAYS*

| Clay | ppm Added | % Recovery | | | |
|---|---|---|---|---|---|
| | | 5 N | 9 N | 12 N | 18 N |
| Attapulgus | 11.0<br>1.5 | 18.9<br>- | 77.6, 76.7<br>- | 81.3<br>- | 87.7<br>97.5, 95.5 |
| Kaolinite | 11.0<br>1.5 | -<br>- | 0.7<br>- | 33.4<br>- | 89.3<br>97.5, 103.0 |
| Montmorillonite | 11.0<br>1.5 | -<br>- | 0.3<br>- | 70.6<br>- | 89.3<br>98.0, 102.0 |
| Blue Slate Flour | 11.0<br>1.5 | -<br>- | 15.1<br>- | 86.5<br>- | 95.8<br>97.5, 103.0 |
| Mica Powder | 11.0 | - | 5.7 | 76.3 | 74.9 |

* 5 hour reflux period.

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TABLE 5

RECOVERY OF PARAQUAT FROM SOILS AND CLAYS AFTER VARIOUS STORAGE PERIODS(1)

| Sample | % Recovery | | | |
|---|---|---|---|---|
| | 0 days | 5 days | 3 weeks | 7 weeks |
| Soil I (2) | 89.2 | 87.7 | 90.1 | 88.0 |
| Soil II (2) | 82.1 | 75.4 | 83.0 | 81.9 |
| Soil III (2) | 78.1 | 75.1 | 88.3 | 78.9 |
| Kaolinite (3) | 97.1 | - | 96.7 | - |
| Attapulgus (3) | 94.6 | - | 93.6 | - |

(1) Fortification at 11 ppm.

(2) Extraction 5 hours reflux with 9 N $H_2SO_4$.

(3) Extraction 5 hours reflux with 18 N $H_2SO_4$.

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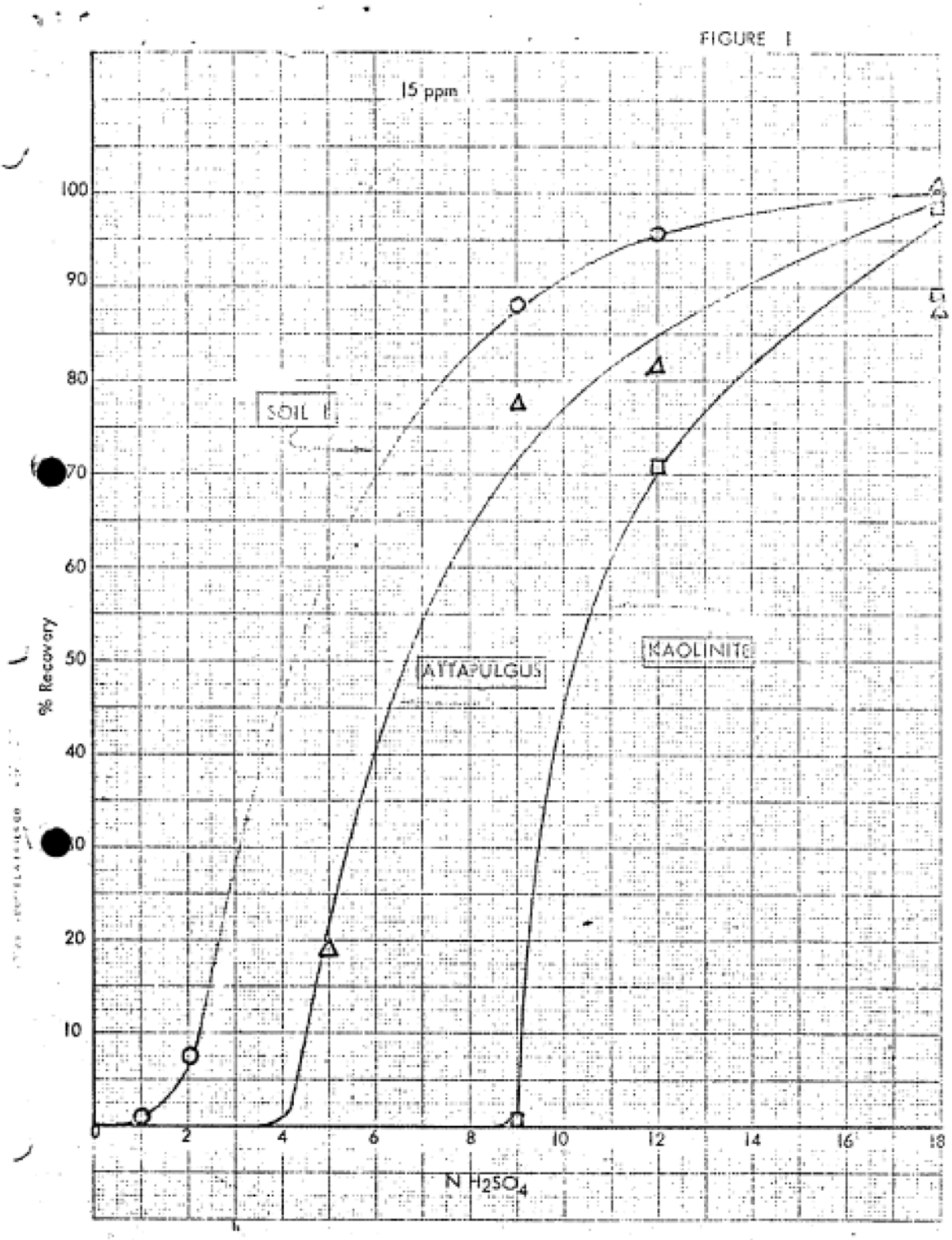


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## Appendix 2 Key Summary Points Regarding Three Referenced Paraquat Publications

**1) Brooks, A I; Chadwick, C A; Gelbard, H A; Cory-Slechta D A; Federoff, H J (1999) Paraquat elicited neurobehavioral syndrome caused by dopaminergic neuron loss, Brain Research, 823, pp 1-10**

The major technical issue with this paper is the potential confounding factor from inserting a needle into the brain to administer the Fluoro-gold and the blood-brain barrier being breached. Although the damage to the barrier will reseal, the injury will need to be repaired, and this will take time. Although the authors show that large protein molecules are excluded from the brain, small molecular weight compounds such as paraquat may still gain some access.

We know from the work of several other groups that following direct injection into the brain, paraquat can be toxic to neurons. There is confusion regarding the exact time paraquat was given after administration of the Fluoro-gold. Fig 1 of the publication showing the dosing regimen states paraquat was first given 7 days after surgery while in the Discussion the authors talk about the first dose of paraquat being given 5 days after surgery.

The clinical condition of the animals is not reported in the Brooks *et al*., study with no indication of body weight, so it is difficult to tell if the reduced motor activity is due to generalised toxicity, e.g. lung damage, as well as the reported effects in the brain. We would expect motor activity to be reduced at toxic doses.

**2) Thiruchelvam, M; Richfield, E K; Goodman, B M; Baggs, R B; Cory-Slechta, D A (2002) 'Developmental exposure to the pesticides paraquat and maneb and the Parkinson's disease phenotype' Neurotoxicology, 23, pp 621-633**

These data show that neonatal $C_{57}Bl_6$ mice exposed to much greater doses of paraquat than in the *Fredriksson et al., 1993* study (including days 10 & 11) do not exhibit deficits in locomotor activity or striatal dopamine levels. Interestingly no deficits in these endpoints were observed in adult mice either when exposed to 10 mg/kg paraquat i.p. (twice a week for 3.5 weeks – total of seven doses). The only endpoint where paraquat alone did produce a small reduction was in the substantia nigra *pars compacta* (SNpc) neuronal cell counts which were reduced by approximately 15% when compared to control.

This paper provides another clear example of the inability to effectively replicate the findings reported by *Fredriksson et al., 1993* even with a much greater exposure to paraquat. Although the dosing regimen was not identical (the i.p. route was used instead of the oral route; dosing was on days 5-19 rather than just 10 & 11; and the doses of paraquat were greater at 0.3 mg/kg oral rather than 0.07 & 0.36 mg/kg i.p.), all three of these components relating to the dosing regimen would be expected to have lead to a substantially greater exposure to paraquat than the oral neonatal exposure on days 10 & 11 reported in the

*Fredriksson et al., 1993* paper, including the period of days 10 & 11. This casts further doubt on the reproducibility of the findings reported by *Fredriksson et al.* already expressed by Dr. Ray at the Medical Research Council (MRC) Toxicology Unit, Leicester, UK.

**3) Ossowska, K; Wardas, J; Smialowska, M; Kuter, K; Lenda, T; Wieronska, J M; Zieba, B; Nowak, P; Dabrowska, J; Bortel, A; Kwiecinski, A; Wolfarth, S (2005) 'A slowly developing dysfunction of dopaminergic nigrostriatal neurons induced by long-term paraquat administration in rats: an animal model of preclinical stages of Parkinson's disease?' European Journal of Neuroscience 22, pp. 1294-304**

Dosing 10 mg/kg paraquat dichloride once a week for a number of consecutive weeks, the authors report a loss of tyrosine hydroxylase positive neurons in the substantia nigra of the male Wistar rat following 4, 8, 12 and 24 weeks of dosing. The loss (approximately 37% loss of $TH^+$ neurones) however, only reaches statistical significance 7 days after 24 weeks of dosing.

A total dose of 240 mg/kg over 24 weeks is required to produce a statistically significant reduction in the number of $TH^+$ neurones in the substantia nigra. No comment is made in the paper regarding whether the dosing regimen that produces a statistically significant loss of $TH^+$ neurones (10 mg/kg once a week for 24 weeks) induces general toxicity, lung pathology or even mortality. However following a personal communication with the lead author we know that this dosing regimen did produce some mortality, body weight reductions and potential lung pathology (animals prone to lung infections).

This raises the question as to whether a component of the effects observed at the higher total doses is at least partly due to non-specific toxicity associated with the prolonged exposure to high doses of paraquat.