PUBLIC HEALTH GOALS FOR CHEMICALS IN DRINKING WATER

2,4-DICHLOROPHENOXY-ACETIC ACID

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Prepared by

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PREFACE

Drinking Water Public Health Goals
Pesticide and Environmental Toxicology Branch
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This Public Health Goal (PHG) technical support document provides information on health effects from contaminants in drinking water. PHGs are developed for chemical contaminants based on the best available toxicological data in the scientific literature. These documents and the analyses contained in them provide estimates of the levels of contaminants in drinking water that would pose no significant health risk to individuals consuming the water on a daily basis over a lifetime.

The California Safe Drinking Water Act of 1996 (Health and Safety Code, Section 116365) requires the Office of Environmental Health Hazard Assessment (OEHHA) to perform risk assessments and adopt PHGs for contaminants in drinking water based exclusively on public health considerations. The Act requires that PHGs be set in accordance with the following criteria:

1. PHGs for acutely toxic substances shall be set at levels at which no known or anticipated adverse effects on health will occur, with an adequate margin of safety.
2. PHGs for carcinogens or other substances that may cause chronic disease shall be based solely on health effects and shall be set at levels that OEHHA has determined do not pose any significant risk to health.
3. To the extent the information is available, OEHHA shall consider possible synergistic effects resulting from exposure to two or more contaminants.
4. OEHHA shall consider potential adverse effects on members of subgroups that comprise a meaningful proportion of the population, including but not limited to infants, children, pregnant women, the elderly, and individuals with a history of serious illness.
5. OEHHA shall consider the contaminant exposure and body burden levels that alter physiological function or structure in a manner that may significantly increase the risk of illness.
6. OEHHA shall consider additive effects of exposure to contaminants in media other than drinking water, including food and air, and the resulting body burden.
7. In risk assessments that involve infants and children, OEHHA shall specifically assess exposure patterns, special susceptibility, multiple contaminants with toxic mechanisms in common, and the interactions of such contaminants.

8. In cases of insufficient data for OEHHA to determine a level that creates no significant risk, OEHHA shall set the PHG at a level that is protective of public health with an adequate margin of safety.
9. In cases where scientific evidence demonstrates that a safe dose response threshold for a contaminant exists, then the PHG should be set at that threshold.
10. The PHG may be set at zero if necessary to satisfy the requirements listed above in items seven and eight.
11. PHGs adopted by OEHHA shall be reviewed at least once every five years and revised as necessary based on the availability of new scientific data.

PHGs adopted by OEHHA are for use by the California Department of Public Health (DPH) in establishing primary drinking water standards (State Maximum Contaminant Levels, or MCLs). Whereas PHGs are to be based solely on scientific and public health considerations without regard to economic cost considerations or technical feasibility, drinking water standards adopted by DPH are to consider economic factors and technical feasibility. Each primary drinking water standard adopted by DPH shall be set at a level that is as close as feasible to the corresponding PHG, placing emphasis on the protection of public health. PHGs established by OEHHA are not regulatory in nature and represent only non-mandatory goals. By state and federal law, MCLs established by DPH must be at least as stringent as the federal MCL, if one exists.

PHG documents are used to provide technical assistance to DPH, and they are also informative reference materials for federal, state and local public health officials and the public. While the PHGs are calculated for single chemicals only, they may, if the information is available, address hazards associated with the interactions of contaminants in mixtures. Further, PHGs are derived for drinking water only and are not intended to be utilized as target levels for the contamination of other environmental media.

Additional information on PHGs can be obtained at the OEHHA Web site at www.oehha.ca.gov.

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PUBLIC HEALTH GOAL FOR 2,4-DICHLOROPHENOXYACETIC ACID IN DRINKING WATER

SUMMARY

A Public Health Goal (PHG) of 0.020 mg/L or 20 parts per billion (ppb) has been developed for the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in drinking water. This report provides a brief discussion of 2,4-D and its amine, esters and salts to support the determination of this PHG. The estimated health-protective level is based on a no-observed-adverse-effect level (NOAEL) of 5 mg/kg-day in a chronic rat study (Charles *et al.*, 1996a). Effects observed at the next higher dose of 75 mg/kg-day included decreased body-weight gain, reduced platelet count, an increase in alkaline phosphatase, thyroxine (T4), and cholesterol, and increased thyroid weights in both sexes. Reduced body weight, reduced glucose and globulin levels, reduced blood parameters (RBC count, Hb, HCT, platelet count), reduced ovarian weights, increased thyroid weights, increased hepatocyte size and increased chronic or subchronic inflammation in the lungs were observed only in females, while increased alanine and aspartate aminotransferases and decreased testes weights were observed only in males. These observations were supported by toxic effects in other studies in rats and dogs.

Limitations in renal clearance play an important role in the degree and variety of toxic effects caused by 2,4-D exposures. Available animal and human data indicate that saturation of renal clearance, which prolongs the systemic half-life, leads to 2,4-D accumulation and the most severe effects in animal studies.

A combined 1,000-fold uncertainty factor was applied to the no observed adverse effect level (NOAEL). This updated PHG value is less than the current PHG of 70 ppb, established in 1997 (OEHHA, 1997). The most recent re-evaluation by the United States Environmental Protection Agency (U.S. EPA) is also based on a NOAEL of 5 mg/kg-day with a combined uncertainty factor of 1,000 (U.S. EPA, 2004a,b, 2005).

Justification for the lower value includes qualitative evidence of developmental toxicity and endocrine disruptive effects as well as uncertainty based on data gaps for a developmental neurotoxicity study and a 2-generation reproductive toxicity study with an improved protocol. The new value is judged adequate to protect potentially sensitive subpopulations, including pregnant women and their fetuses, infants, children, and the elderly.

2,4-Dichlorophenoxyacetic acid was not detected in 8,408 drinking water samples analyzed from 1984-2001 at a detection limit of 10 ppb. The state Maximum Contaminant Level (MCL) and the U.S. EPA MCL and MCL Goal (MCLG) are all 70 ppb, set in 1991.

INTRODUCTION

2,4-Dichlorophenoxyacetic acid (2,4-D) is an alkylchlorophenoxy herbicide commonly used to control a variety of broad-leaf weeds (while sparing grasses) in agricultural, nonagricultural (e.g., residential turf, right-of way), forestry, and aquatic sites. It has been widely used in consumer products for weed control on lawns and is formulated as the acid, an ester, or an aqueous solution of an amine salt, and is provided as a concentrate or wettable powder.

For this review, the medical literature was searched with Medline and PubMed and several hundred citations were retrieved. Current U.S. EPA documents, regulatory submissions and evaluations and reviews from the California Department of Pesticide Regulation were also examined. Those documents and articles that appeared to have the potential to affect the evaluation of a health-protective level were retrieved. Among these, the most significant animal studies are acute, subchronic and chronic toxicity studies (Charles *et al.*, 1996a,b; Paulino *et al.*, 1996). The report on chronic tests by Charles *et al.* (1996a) provides cancer bioassays in rats and mice. These studies were conducted by Dow Chemical Company at the request of U.S. EPA to investigate doses of 2,4-D higher than those in the previous studies (Dow, 1983).

2,4-D is one of the oldest pesticides registered in the United States. Its wide agricultural use results also in widespread potential human exposures. Numerous epidemiological studies have connected 2,4-D to non-Hodgkin's lymphoma (NHL) among farmers (Zahm, 1997; Zahm and Blair, 1992; Fontana *et al*., 1998; Morrison *et al*., 1992; McDuffie *et al*., 2001; De Roos *et al.*, 2001). These studies are confounded by exposure to multiple pesticides; earlier formulations often contained congeners such as 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and traces of dioxin contaminants. A preparation containing a mixture of 2,4-D and 2,4,5-T, known as Agent Orange, was widely used as a defoliant in the Vietnam war. Birth defects and other developmental toxicities have been attributed to exposure to this formulation, although there are indications that the dioxin and perhaps 2,4,5-T in the mixture are the more likely cause of the putative effects.

Children, especially farm children, may be particularly vulnerable to exposure to 2,4-D. Children have distinctive exposure patterns and sensitivities to pesticides (NRC, 1993). Per unit body weight, they drink, eat, and breathe more than adults do. They also engage in more frequent hand-to-mouth contact, resulting in higher rates of oral exposure from contaminated objects, dust, or soil. Farm children may come in contact with 2,4-D through residues from contaminated soil in areas where they play, their parents' clothing, dust tracked into their homes, food eaten directly from the fields, drift from aerial spraying, contaminated well water, and breast milk. In addition, farm children may accompany their parents to work in the fields, further increasing their pesticide exposure.

CHEMICAL PROFILE

Chemical Identity

The herbicide 2,4-dichlorophenoxyacetic acid is more commonly known as 2,4-D. It has been used in the form of the sodium salt and various esters and amines, under many trade

names, such as Aqua-Kleen, Barrage, Lawn-Keep, Malerbane, Planotox, Plantgard, Savage, Salvo, Weed-B-Gon, Weedone, and Weedtrine-II. The structure of the 2,4-D free acid is shown below.

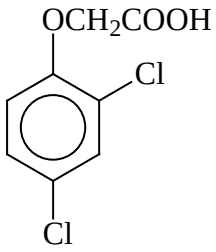

Figure 1. Chemical structure of 2,4-D

Physical and Chemical Properties

The properties of 2,4-D are summarized below in Table 1.

Table 1. Physical and Chemical Properties of 2,4-Dichlorophenoxyacetic Acid

Property	**Value**
Empirical Formula	$C_6H_6Cl_2O_3$
CAS Registry No.	94-75-7
Physical state	Crystalline solid
Molecular weight	221.0
Density/specific gravity	1.416 at 25 ^oC
Solubility in water	569 mg/L at 20 ^oC
Solubility in organic solvents	g/100 g at 25 ^oC: acetone=85; benzene=1.07; diethyl ether=220; ethanol= 130; isopropanol=31.6; toluene=0.067; xylene=0.58
Vapor pressure	1.4 x 10^{-7} mm Hg at 25 ^oC (acid) 2.3 x 10^{-4} mm Hg at 25 ^oC (isopropyl ester) 6.2 x 10^{-5} mm Hg at 25 ^oC (butyl ester)
Henry's Law constant	4.74 x 10^{-10} atm-m^3/mol @ 25 ^oC
Octanol-water partition coefficient (Log K_{ow})	2.81
pKa	2.73

Production and Uses

2,4-D is available on the market in the form of the acid, the sodium salt, several alkylamine salts, and several esters. About 40 million pounds of 2,4-D are used in the U.S. every year (30

million in agriculture, 10 million in residential and 1 million in aquatic settings). The main agricultural uses include applications to pasture land, wheat, corn, soybeans, barley, rice, oats, and sugar cane. 2,4-D is the most widely used residential lawn and garden herbicide in the U.S. Lesser amounts of 2,4-D are used in forestry and rights of way (U.S. EPA, 2004a).

Mode of Action

2,4-D is a selective herbicide that kills broadleaf weeds by mimicking the plant growth hormone auxin (indoleacetic acid). It is absorbed by plant leaves, stems, and roots and is translocated throughout the plant. It causes little harm to grass crops. Unlike auxins, 2,4-D remains at high levels within plant tissues rather than fluctuating, as the naturally-occurring hormone would. As a result, the plant's transport systems become blocked and destroyed by abnormally fast growth that leads to plant death (National Pesticide Information Center, 2004; Hess, 1993).

ENVIRONMENTAL OCCURRENCE AND HUMAN EXPOSURE

Air

Like other pesticides 2,4-D may be transported away from the target site via spray drift at the time of application and volatilization. This process raises concern for impact to non-target organisms. Spray drift, as a process affecting exposure, is usually not taken into account in drinking water risk assessments due to limited data relevant to population exposures. The tendency of 2,4-D to evaporate is dependent on the chemical form used. Forms with the least tendency to evaporate include acid, inorganic salt, amines and long chain esters; the oil-soluble amines are least volatile. The vapor pressure of the 2,4-D anion (about 1×10^{-7} mm Hg) is so low that it would only be found in the particulate phase in the atmosphere, either as spray droplets or bound to dust particles (HSDB, 2008). The 2,4-D isopropyl ester, if released to air, will exist mainly as a vapor in the ambient atmosphere, with a vapor pressure of about 2×10^{-4} mm Hg, while the higher molecular weight esters and amines will have intermediate properties, with both vapor and particulate phase distribution (HSDB, 2008). The potential for 2,4-D to volatize increases with increasing temperature, increasing soil moisture, and decreasing clay and organic matter content in the soil (Helling *et al.*, 1971).

Occupational inhalation exposure to 2,4-D has been documented, but the inhalation exposure route appeared to be relatively minor, compared to dermal uptake (Kolmodin-Hedman *et al.*, 1983; Libich *et al.*, 1984). 2,4-D concentrations in air of 0.1-0.2 mg/m^3 have been documented in the breathing zone of workers spraying 2,4-D from tractor-driven equipment, with lower concentrations downwind (Kolmodin-Hedman and Erne, 1980; Kolmodin-Hedman *et al.*, 1983; Libich *et al.*, 1984).

Soil

2,4-D has low soil persistence. The half-life of 2,4-D in soil is about 1-2 weeks (Wauchope, 1992; U.S. EPA, 2005). At its highest application rate, detectable levels of 2,4-D persist in soil for up to 30 days. Soil microorganisms are commonly involved in

the rapid degradation of 2,4-D in soil (Howard, 1991). It degrades more rapidly under warm, moist conditions. Some of the amine and ester forms of 2,4-D will evaporate from the soil.

2,4-D can be transported into homes from residential lawns treated with this herbicide. It has been shown that indoor levels of 2,4-D increased 37-fold as a result of track-in by high-activity children wearing shoes indoors and by pets (Nishioka, 1999; U.S. EPA, 1999).

Water

The 2,4-D acid form, the oil-soluble amine salt and the low-volatility esters do not dissolve well in water. Other amine salts readily dissolve in water. 2,4-D has only a limited potential to contaminate ground water. It is unlikely to be a ground-water contaminant due to rapid degradation in most soils and rapid uptake by plants.

Most reported 2,4-D ground-water contamination has been associated with spills or other large releases of 2,4-D. 2,4-Dichlorophenoxyacetic acid was not detected in 8,408 drinking water samples analyzed from 1984-2001 (DHS, 2002), at a detection limit of 10 ppb.

2,4-D applied to surface water was quickly distributed through the water body. The half-life of 2,4-D applied to surface water is 1-3 weeks. Its residues may be detected in still water sediment after six months.

Food

Tolerances and food and feed additive regulations have been established for residues of 2,4-D in a variety of raw agricultural commodities such as apples, pears, and quinces, 5 parts per million (ppm); apricots, 5 ppm; citrus fruits, 5 ppm; and potatoes, 0.2 ppm (U.S. EPA, 2005; HSDB, 2005). In human foods derived from fruits, grasses, grains, nuts, vegetables, sugarcane, cotton, hops, and asparagus tolerances for 2,4-D were set at 0.1 ppm to 5 ppm; processed products of sugarcane (5 ppm) and grains (2 ppm); fish and shellfish at 1.0 ppm and potable water at 0.1 ppm (U.S. EPA, 2004c). Tolerances for residues in livestock feed commodities are currently established for 2,4-D and/or its metabolite 2,4-dichlorophenol (U.S. EPA, 2004d). 2,4-D *per se* is a residue of concern in plant and livestock commodities as well as in drinking water, and the established tolerances refer to residues of 2,4-D, both free and conjugated, determined as the acid.

In general, the amount of residues in commodities treated with pesticides depends on the type and number of applications. In 27 tests for 2,4-D residues in soybeans performed in Arkansas, Illinois, Louisiana, Missouri, and Tennessee, 2,4-D was nondetectable (< 0.01 ppm) in all samples of forage, as well as seeds from soybeans treated with a preplant application of 2,4-D (acid, ester or amine) at 0.5, 1.25 and 2.75 lbs active ingredient per acre (up to 5.5 times the normal rate). Residues of 2,4-D were also nondetectable in the majority of hay samples (21 out of 27) from the same tests (U.S. EPA, 2004d). Testing results for 2,4-D residues in food are broadly discussed in the WHO Pesticide Residues Series 4 (INCHEM, 2008). The data showed that pre-harvest use of 2,4-D on citrus and potatoes resulted in low residues (<0.01 mg/kg on citrus and <0.01 mg/kg in potatoes).

Post-harvest applications on lemons resulted in residues of < 2 mg/kg. Low levels of 2,4-D residues were also found in the milk of cows fed rations containing high levels of 2,4-D (1,000 mg/kg) in their diet.

METABOLISM AND PHARMACOKINETICS

Absorption

Oral absorption of 2,4-D in mammals is nearly complete; essentially all of a single oral dose of 5 mg/kg 2,4-D was absorbed by humans (Sauerhoff *et al*., 1977). The chemical is also readily absorbed through the lungs. The potential for dermal absorption depends on the particular form (free acid, alkylamine salt, or ester). The dermal absorption rate used by the U.S. EPA in its preliminary human health risk assessment for 2,4-D was 5.8 percent (U.S. EPA, 2004a). However, this value may be too low, especially in formulations where interactions with other chemicals are likely. For example, after skin application of DEET, palmar absorption of 2,4-D was found to be 14 percent (Moody *et al*., 1992). Some studies have also shown that commercial sunscreen formulations can enhance the penetration of 2,4-D through hairless mouse skin by 60 to 200 percent (Pont *et al*., 2004; Brand *et al*., 2002).

Distribution

Once absorbed, 2,4-D is rapidly distributed, with the highest concentrations appearing in the kidneys and liver (Johnson and Wattenberg, 1996). Most of the compound is excreted unmetabolized (Sauerhoff *et al.*, 1977; Ibrahim *et al*., 1991). Nearly all of a dose is excreted in the urine.

Metabolism

The metabolism and excretion of 2,4-D have been studied in a number of species including humans. 2,4-D undergoes limited metabolism; esters will be hydrolyzed and a small fraction of the parent acid is conjugated. Oral exposure to 2,4-D in humans, dogs, mice and hamsters resulted in urinary excretion of intact 2,4-D as well as its conjugates. No metabolites of 2,4-D were found in the rat, and only the parent acid was found in rat urine.

Excretion

Once in the body, 2,4-D is distributed rapidly, with the greatest concentrations appearing in the kidneys and liver (Johnson and Wattenberg, 1996). The majority of the compound is excreted unmetabolized; a smaller portion will be in the form of water-soluble conjugates. Due to its solubility in water, 2,4-D does not accumulate in tissues. Like other phenoxy herbicides, it is actively secreted by the renal proximal tubules. 2,4-D has a relatively short half-life in most mammalian species, estimated at between 10 and 36 hours. In the absence of sustained exposure, nearly the entire 2,4-D body burden was cleared within 2 to 4 days (Sauerhoff *et al*., 1977; Pelletier *et al*., 1989; Moody *et al*., 1990, 1991; Knopp and Schiller, 1992), and is nearly completely excreted in the urine

within a week following exposure (Sauerhoff *et al.*, 1977; Shearer, 1980; Johnson and Wattenberg, 1996).

Pharmacokinetics

Renal clearance plays an important role in 2,4-D toxicity. After absorption 2,4-D is rapidly excreted in urine by rodents and humans, but not dogs (Sauerhoff *et al.*, 1977; Gorzinski *et al.*, 1987; Van Ravenzwaay *et al.*, 2003; Timchalk, 2004).

Sauerhoff *et al.* (1977) administered oral doses of 5 mg/kg 2,4-D to five adult male human volunteers, and evaluated plasma and urine levels of 2,4-D at several time points afterward. 2,4-D was eliminated from plasma in an apparent first-order process with an average half-life of 11.6 hours. Excretion was in the urine, about 82 percent as intact 2,4-D and 12 percent as conjugates, with an apparent excretory half-life of 17.7 hours.

Gorzinski *et al. (*1987) gave groups of six adult male Fisher 344 rats single oral doses of 10, 25, 50, 100, or 150 mg/kg [^{14}C]2,4-D. Each rat received approximately 5 μCi of radioactivity. The concentration of ^{14}C in the plasma and the amount of ^{14}C in the urine measured six hours after administration of single oral doses of 10, 25, or 50 mg were proportional to the dose. In animals administered 100 or 150 mg 2,4-D/kg, the concentration of ^{14}C in the plasma was greater than expected and the amount of ^{14}C in the urine was less than expected based on the data from the lower dose levels, indicating apparent saturation of renal excretion at doses above 50 mg/kg. All ^{14}C in the urine collected over 12 hrs from single male rats given 10 or 100 mg/kg 2,4-D eluted in an HPLC peak at the same retention time as 2,4-D, indicating that the 2,4-D was being excreted intact.

Increased sensitivity of dogs to 2,4-D was shown in a study where male and female rats and dogs were orally dosed with either 5 or 50 mg/kg $^{-14}$C-2,4-D (Van Ravenzwaay *et al.*, 2003). The rates and routes of excretion were studied along with plasma toxicokinetics and biotransformation of the compound through blood samples and urine and feces collection over five days. Elimination of the radioactive 2,4-D from plasma was considerably faster in rat than in dog at both doses. The estimated t½s were 1.7 and 1.3 hr for male and female rats at 5 mg/kg, and 99 and 104 hrs for male and female dogs at the same dose. Comparative results were similar at the higher dose. In rats, 2,4-D was excreted unmetabolized, mainly in the urine, and it was basically completed by 24 hr. In dogs, elimination was not finished over the 5-day sampling period with only 50 percent of the dose recovered. The main route of excretion in dog was urine at the low dose, but about equal amounts were eliminated in urine and feces at the high dose over 120 hr; the urinary 2,4-D was mainly in the form of conjugated metabolites. Dogs had a very much larger area under the curve (AUC) of 2,4-D in plasma in units of concentration times time than rodents, which is equivalent to a greatly increased body burden.

Timchalk (2004) reviewed these studies and others related to renal excretion of organic acids, including several studies in humans (Gehring *et al.*, 1973; Sauerhoff *et al*., 1977; Kolmodin-Hedman *et al.*, 1983. Timchalk points out that renal excretion of 2,4-D in rodents and humans is facilitated by a saturable organic anion transporter located in the renal tubules. In dogs the organic anion transporter does not function efficiently; therefore this species has a limited capacity for excretion of organic acids. In rats, the

observed dose-dependent non-linear pharmacokinetics of 2,4-D appears to be due to saturation of the renal secretory system, which becomes saturated at above about 50 mg/kg. Because of their limited capacity to excrete organic acids, dogs have higher blood concentrations of 2,4-D and an overall higher sensitivity to 2,4-D than do rats and humans. The conclusion of Timchalk (2004) is that dogs are much more sensitive to the effects of organic acids such as 2,4-D than are humans because of the limited excretory capacity of dogs. He recommended that the data in rats be used for risk assessment extrapolation, in preference to that from the dog studies. We think it should be noted that the human plasma half-life for 2,4-D of 11.6 hours (Sauerhoff *et al.,* 1977) is considerably longer than rats at about 1.5 hours (Van Ravenzwaay *et al.*, 2003) as well as much shorter than that of dogs at about 100 hours (Van Ravenzwaay *et al.,* 2003).

TOXICOLOGY

Toxicological Effects in Animals

Acute Toxicity

The toxicity of 2, 4-D varies depending on its form and the type of exposure. There are several forms of 2,4-D currently in use including acids, amines, and esters. Their oral and dermal acute toxicity puts them in Toxicity Category III (oral LD_{50} 500-5000 mg/kg), dermal LD_{50} 2,000-20,000 mg/kg). For the acid form the oral LD_{50} in rats is 699 mg/kg and via dermal exposure in rabbits the LD_{50} is >2000 mg/kg. The acute inhalation LC_{50} in rats for the 2,4-D acid form is >1.79 mg/L. This indicates Toxicity Category III (from 0.2 thru 2 mg/liter). Other forms of 2,4-D can be assigned Toxicity Category III or IV based on their LC_{50} values as determined in rats (greater than 20 mg/liter). 2,4-D acid, diethanolamine (DEA) salt, dimethylamine (DMA) salt, isopropylamine (IPA) salt and triisopropanolamine (TIPA) salts are severe eye irritants, causing corrosive effects (irreversible destruction of ocular tissue), corneal involvement or irritation persisting for more than 21 days (Toxicity Category I). Primary skin irritation tests performed with rabbits showed that 2,4-D acid, its salts and esters caused only minor skin irritation, which puts them into Toxicity Category IV (U.S. EPA, 2004a).

An acute NOAEL for 2,4-D acid was determined in rats by Mattsson *et al*. (1997). In this study, 10 Fischer 344 rats/sex were gavaged with 2,4-D acid in 10 mL/kg corn oil at doses of 0, 15, 75, or 250 mg/kg. Rats were observed for clinical effects and subjected to a Functional Observation Battery, grip tests, and other motor observations on days 1, 8, and 15. The highest dose caused transient changes in gait and coordination, and decreased motor activity. One female rat appeared to have mild locomotor effects at 75 mg/kg. The authors interpreted the effect in the single 75 mg/kg rat as being compound-related, resulting in a NOAEL for acute behavioral effects of 15 mg/kg.

Subchronic Toxicity

Currently available subchronic toxicity studies of 2,4-D meet U.S. EPA requirements for registration of 2,4-D as a pesticide and are considered acceptable and complete for the

oral and dermal routes of exposure (U.S. EPA, 2004a). The studies include 90-day oral toxicity studies in Fischer 344 rats, three 90-day oral toxicity studies in beagle dogs, a 21-day dermal toxicity study in rabbits, and a 28-day inhalation toxicity study in rats. Conditions and results from these studies are presented in Table 2.

The subchronic toxicity studies provide evidence that the kidney (increased kidney weight, histopathological lesions), thyroid (increased thyroxine, increased thyroid weight, hypertrophy of follicular cells), liver [increased liver weight, increased alanine aminotransferase (ALT), and aspartate aminotransferase (AST), histopathological lesions, including hypertrophy], and adrenal (increased adrenal weight, histopathological lesions) are target organs in the rat following subchronic exposure via the oral route at dose levels above the threshold of saturation of renal clearance, which is in the 50 to 100 mg/kg-day range in the rat (Gorzinski *et al.*, 1987; Timchalk, 2004).

A new 28-day inhalation toxicity study was conducted in 10 or 20 eight-week old Sprague-Dawley rats/sex/group, using the free acid form of 2,4-D as a dust for 6 hr/day, 5 days/week for four weeks (at least 20 exposures) in a nose-only inhalation exposure apparatus (Hoffman, 2008). Ten animals/sex/group were sacrificed after the four-week exposure, with 10 more control and high-dose animals of each sex sacrificed after a four-week recovery period. The target mass median aerodynamic particle diameter was 1 to 3 μm, and measured mean particle diameters were within this range, averaging about 2 μm. Target dust concentrations were 0, 0.05, 0.10, 0.30, and 1.00 mg/L; actual mean concentrations by weight were 0, 0.048, 0.11, 0.34, and 1.00 mg/L. We estimate that doses based on a minute volume for 250 g rats of 0.175 L/min (U.S. EPA, 1988) would be approximately 12, 28, 86, and 252 mg/kg-day. It should be noted that not all of the particles would be retained and systemically absorbed, and the behavior of animals changes when exposed to irritating dusts, so actual doses are likely to be lower. All animals survived till their scheduled sacrifice. All animals were monitored during exposure, necropsied, and evaluated for clinical chemistry and hematological parameters.

Excessive salivation, labored breathing and chromodacryorrhea were noted in some of the highest dose rats beginning at the 12^{th} exposure and continuing through the 28-day exposure period. At the highest dose, body weights were significantly decreased, by 9.9 percent in females and 4.6 percent in males. Body weights recovered in males but not in females over the 4-week recovery period. Food consumption was decreased in females consistent with the decreased body weights. There were slight alterations in clinical pathology parameters at the 0.3 and 1.0 mg/L exposure levels, which were not considered by the author to be adverse effects. Changes included 20-26 percent decreases in reticulocyte counts in both sexes at the two high doses, which reversed in males but persisted in females at 1.0 mg/L after the four-week recovery period. A 40 percent increase in serum alkaline phosphatase and 35 percent increase in aspartate aminotransferase were observed in females at 1.0 mg/L, indicative of liver damage, which reversed after four weeks. There was a 25 percent increase in serum alkaline phosphatase in females at 0.3 mg/L.

Table 2. Summary of 2,4-D Subchronic Toxicity Studies

Study Type/Species	Dosing Levels	Results
90-Day oral toxicity in Fischer 344 rat (Charles *et al.*, 1996b)	1, 15, 100, 300 mg/kg-day	NOAEL = 15 mg/kg-day LOAEL = 100 mg/kg-day based on decreases/gains in body weight, alterations in hematology and clinical chemistry (decreased T3 and T4), and cataracts in females
90-Day oral toxicity in beagle dog (Schultze, 1990)	0, 0.3, 1.0, 3.0, and 10 mg/kg-day.	NOAEL = 1 mg/kg-day LOAEL= 3 mg/kg-day based on decreases/gains body weight and food consumption (males), increased BUN in both sexes and creatinine in males, and decreased testis weight in males
90-Day oral toxicity in beagle dog (Dalgard, 1993)	0, 0.5, 1.0, 3.75, and 7.5 mg/kg-day	NOAEL = 1 mg/kg-day LOAEL = 3.75 mg/kg-day based on decreased body weight gain (both sexes) and food consumption (males), increased BUN, creatinine, and alanine aminotransferase in both sexes, and decreased testes weight and slightly higher incidence of hypospermatogenesis / juvenile testis and inactive / juvenile prostate
90-Day oral toxicity in beagle dog (Charles *et al.*, 1996c)	0, 1.0, 3.75 or 7.5 mg/kg-day free acid, dimethylamine salt and 2-ethylhexyl ester plus 0.5 mg/kg-day free acid; all doses calculated as free acid equivalents	The "overall NOAEL" stated as 1.0 mg/kg. Moderate effects on serum parameters (increased alanine aminotransferase and creatinine) at 1 mg/kg not considered biologically significant. The NOAEL for adverse liver effects observed by histology was 3.75 mg/kg for each chemical. LOAEL for liver pathology was 7.50 mg/kg-day
21-Day dermal toxicity, rabbit (Mizell *et al*., 1990)	10, 100, 1000 mg/kg-day	NOAEL = 1000 mg/kg-day LOAEL = > 1000 mg/kg-day
28-Day inhalation, 6 hr/day, 5 days/week, rats (Hoffman, 2008)	0, 0.048, 0.11, 0.34, or 1.00 mg/L, equivalent to ca. 12, 28, 86, or 252 mg/kg-day	Systemic NOAEL = 86 mg/kg-day Portal of entry LOAEL = 12 mg/kg-day (0.048 mg/L)

Squamous metaplastic and hyperplastic changes with increased inflammatory cells were observed within the larynx at all doses, which are consistent with chronic irritation. These changes were partially resolved during the recovery period. The inflammatory changes were explained by the author as an adaptive response to chronic irritation that "are not considered indicative of significant risk in humans." Hoffman (2008) concluded that the study NOAEL was 0.30 mg/L (ca. 86 mg/kg-day) for systemic toxicity, while the lowest exposure, 0.05 mg/L, would be considered a LOAEL for portal-of-entry toxicity.

In the dog with oral exposure at dose levels above the threshold of saturation of renal clearance, the target organs are kidney (elevated blood urea nitrogen and creatinine, decreased glucose, increased kidney weight, cellular alteration of the proximal tubule) and thyroid (increased thyroid weight and decreased T4). Effects in dogs were observed at lower doses than those observed in rats due to the dog's limited capacity to eliminate organic acids including 2,4-D. In both studies, dogs had hypospermatogenesis/juvenile testis and decreased testes weight (Schultze, 1990; Dalgard, 1993).

In another subchronic study on effects of 2,4-D in dogs, Charles *et al.* (1996c) compared the toxicity of 2,4-D free acid, its dimethylamine salt and its 2-ethylhexyl ester over 90 days of administration in the feed (four beagle dogs/sex/group at 0, 1.0, 3.75 or 7.5 mg/kg-day, plus an extra 0.5 mg/kg-day dose for the free acid; all doses calculated as free acid equivalents). The three chemical forms appeared to be toxicologically equivalent. The "overall NOAEL" for the three chemicals is stated by the authors to be 1.0 mg/kg. Some moderate effects were observed on serum parameters (increased alanine aminotransferase and creatinine) at 1 mg/kg, which were not considered to be biologically significant. The NOAEL for adverse liver effects observed by histology was 3.75 mg/kg for each chemical.

The 21-day dermal toxicity study in rabbits (Mizell *et al.*, 1990) revealed no systemic toxicity up to and at the highest dose tested. The inhalation toxicity study in rats (Hoffman, 2008) resulted in airway irritation and potency for systemic toxicity similar to oral exposure. Absorption should have been relatively high by this route.

Genetic Toxicity

The database for mutagenicity of 2,4-D is complete, according to pesticide registration criteria (U.S. EPA, 2004a; DPR, 2004). Ames tests with and without metabolic activation were consistently negative (U.S. EPA 2004a; DPR, 2004). 2,4-D assayed with *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 did not significantly increase the number of revertants (DPR, 2004). The lack of genotoxicity for 2,4-D 2-butoxyethylester, 2,4-D isopropylamine and 2,4-D triisopropanolamine was confirmed in tests using cultured mammalian cells and evaluating induction of chromosomal aberrations in primary cultures of rat lymphocytes and forward mutations at the HGPRT locus of Chinese hamster ovary cells.

According to the U.S. EPA (2004a) Reregistration Eligibility Document, 2,4-D was positive for genotoxicity in *Drosophila* larvae, and negative in adults after feeding or injection. In *in vitro* mammalian cell cytogenetic assays, 2,4-D was negative for structural chromosomal damage up to the limit of solubility, but positive in the presence of metabolic activation at high concentrations.

Available micronucleus assays with 2,4-D and mouse bone marrow polychromatic erythrocytes were negative overall in the assessment of the California Department of Pesticide Regulation (DPR, 2004). However, numerous studies on 2,4-D have shown chromosomal aberrations under various conditions and exposures. Dosing rabbits with 10 mg/kg-day of 2,4-D for four months caused an increase in the number of their brain cells with unusual numbers of chromosomes (aneuploidies) or multiple chromosome sets

(polyploidies) (Atanassov, 1992). Dermal application of 2,4-D increased abnormalities in the nuclei of hair follicle cells in mice (Schop *et al*., 1990).

Pavlica *et al*. (1991) evaluated cytotoxic and mutagenic effects of 2,4-D on shallot root tip cells and on V79 Chinese hamster fibroblast cells. In shallot root tips, 2,4-D caused changes in mitotic activity, chromosome and chromatin structure. In the hamster cells, 2,4-D showed mutagenic and cytotoxic effects at concentrations higher than 10 µg/mL. High concentrations of 2,4-D (2 and 20 mg/L) applied to bovine fetal muscle cells (Basrur *et al.*, 1976) caused cell degeneration and mitotic changes. In most of these studies, the use of high, cytotoxic concentrations, lack of positive controls, and poor dose-response make the results difficult to interpret.

Two *in vitro* rat primary hepatocyte unscheduled DNA synthesis assays were negative (DPR, 2004). There is, however, one positive study showing 29 percent inhibition of testicular DNA synthesis in male mice treated with 200 mg/kg of 2,4-D (Seiler, 1979). The study is evaluated by DPR as "incomplete," but according to their evaluation (which we support), the study cannot be ignored because it is "fundamentally different in design and represents *in vivo* mammalian finding." Another *in vivo* assay with rat hepatocytes found no indication of an increase of unscheduled DNA synthesis (Charles *et al*., 1999). In addition, investigations on induction of chromosomal aberrations in primary cultures of rat lymphocytes and forward mutations at the HGPRT locus of Chinese hamster ovary cells found no indications of genotoxicity (Gollapudi *et al.*, 1999*).*

In human white blood cells, 2,4-D caused chromosome damage at ≥ 50 µg/mL and an increase in sister chromatid exchanges at concentrations greater than 10 µg/mL (Korte and Jalal, 1982; Turkula and Jalal, 1985). Figgs *et al.* (2000) reported in a small study of 2,4-D applicators that replicative index of lymphocytes was increased after a season of spraying. Replicative index of human lymphocytes was also increased after in vitro treatment at concentrations approaching cytotoxicity (Holland *et al.*, 2002).

Garabrant and Philbert (2002), in their recent review of 2,4-D human and animal toxicity studies, underlined non-genotoxic and non-mutagenic effects of 2,4-D and its derivatives. They concluded that 2,4-D and its salts and esters in *in vitro* experiments had no effects in bacteria (Ames test) and did not induce DNA damage and repair in rat hepatocytes.

Overall, both the U.S. EPA and DPR consider 2,4-D as non-mutagenic in spite of a few positive test results. In general, we support this evaluation. Positive results shown in older studies are questionable because these studies had significant experimental deficiencies and do not meet the current GLP standards. However, some positive indications should not be ignored in the qualitative evaluation of 2,4-D.

Developmental and Reproductive Toxicity

There are currently no data gaps for prenatal developmental toxicity studies, as defined by California requirements for pesticide registration. A summary of the 2,4-D developmental and reproductive toxicity studies is presented in Table 3. The U.S. EPA (2005) states in their Reregistration Eligibility Document (RED) that "Developmental toxicity, characterized mainly as an increased incidence of skeletal abnormalities in the rat, was observed following exposure to 2,4-D and its amine salts and esters at dose

levels that were at or above the threshold of saturation of renal clearance. Similarly, developmental toxicity was observed in the rabbit only following exposure to 2,4-D (abortions) and DEA (increased number of litters with fetuses having 7th cervical ribs) at or above the threshold of renal clearance."

Table 3. Summary of 2,4-D Developmental and Reproductive Toxicity Studies

Study Type / Species	Doses	Results
Prenatal, developmental in Fischer 344 rat (Nemec *et al*., 1983)	0, 8, 25, and 75 mg/kg-day	Maternal NOAEL = 25 mg/kg-day, LOAEL = 75 mg/kg-day based on decreased body weight gains. Survival not affected by treatment. Developmental NOAEL= 25 mg/kg-day, LOAEL = 75 mg/kg-day based on skeletal abnormalities.
Prenatal developmental in rabbit (Hoberman, 1990)	0, 10, 30, and 90 mg/kg-day	Maternal NOAEL = 30 mg/kg-day, LOAEL= 90 mg/kg-day for clinical signs (ataxia, decreased motor activity, loss of righting reflex, cold extremities), abortion (2), decreased body weight gains. Survival not affected by treatment. Developmental NOAEL = 30 mg/kg-day, LOAEL = 90 mg/kg-day, based on abortions.
Teratology, CRL: CD BRVAF/Plus rats (Lochry, 1990)	0, 12.5, 50, and 100 mg/kg-day of 2,4-D-DMA	Maternal NOEL = 12.5 mg/kg-day, LOAEL= 50 mg/kg-day for body weight and food consumption. Developmental NOEL= 50 mg/kg-day based on reduction in fetal body weights and increased skeletal alterations.
Two-generation reproduction study, Fischer 344 rat (Wil Research Laboratories, Inc., 1985)	0, 5, 20 and 80 mg/kg-day	Parental systemic NOAEL = 5 mg/kg-day, LOAEL = 20 mg/kg-day, decreased female body weight gain [F_1], and renal tubule alteration in F_0 and F_1 males. Reproductive NOAEL = 20 mg/kg-day, LOAEL = 80 mg/kg-day, increased gestation length and reduced gestational and neonatal survival [F_0 females/F_{1b} pups]. Offspring NOAEL = 5 mg/kg-day, LOAEL = 20 mg/kg-day based on decreased F_{1b} pup body weight. Increase in dead pups at 80 mg/kg-day.
Postnatal study in Wistar rat (Duffard *et al.*, 1996)	0 and 100 mg/kg-day	The most vulnerable developmental phase resulting in myelin deficit in pups nursed by 2,4-D treated dams was the period of rapid myelination (from the 15^{th} to the 25^{th} postnatal days).
Postnatal study in rat (Rosso *et al.*, 2000)	0 (PND* 7 to 25), 70 (PND 12 to 25) and 100 mg/kg-day (PND 7 to 25)	100 mg/kg-day (PND 7 to 25) exposure caused significant decrease in body and brain weight from PND 21, decreased gangliosides and myelin deposition, and alterations in all behavioral tests. 70 mg/kg-day (PND 12 to 25) exposure resulted in alteration in forelimb support and open field tests, and decreased myelin deposition.

* PND- postnatal day

In studies in rats and rabbits, developmental toxicity occurred at the same level as maternal toxicity. In rats the effect was an increased incidence of skeletal variations in fetuses (presence of 7^{th} cervical ribs, 14^{th} rudimentary ribs, retarded skeletal ossification, and mal-aligned sternebrae), and decreased food consumption and maternal body weight gain (Nemec *et al*., 1983). In rabbits, abortions reflected maternal-fetal toxicity and strictly maternal toxicity was expressed as decreased body weight gain and clinical signs such as ataxia, decreased motor activity, loss of righting reflex, and cold extremities (Hoberman, 1990). Developmental toxicity studies in rats (eight studies) and rabbits (seven studies) on 2,4-D and its salts and esters showed that the NOAEL for maternal toxicity in both species was approximately 10 mg/kg-day (Charles *et al.,* 2001). Rats appeared to be more sensitive to 2,4-D than rabbits. Only at maternally toxic acid equivalent dose levels above 90 mg/kg-day did rats show significantly decreased fetal body weights and increased variations. In rabbits, embryonic and fetal development were not affected even at maternally toxic doses (Charles *et al.,* 2001).

In a more recent teratology study in rats, 2,4-D dimethylamine salt (2,4-D-DMA) was administered by gavage with aqueous solutions to presumed pregnant rats at doses of 0, 12.5, 50 and 100 mg/kg-day (free acid equivalent) from days 6 to 15 of gestation. Each group consisted of 25 animals. No adverse effects were observed. The maternal NOEL of 12.5 mg/kg-day was based on minor body decrements associated with a minor decrease in food consumption at 50 mg/kg-day. The developmental NOEL of 50 mg/kg-day was based on statistically significant reduction in fetal body weights; increased skeletal alterations, including wavy and/or incompletely ossified ribs, and/or incompletely ossified sternebrae (Lochry, 1990).

In the two-generation reproduction study in rats, evidence of reproductive and developmental toxicity was shown as increased duration of gestation of F_0 dams producing F_{1b} litters which had skeletal abnormalities similar to those observed in the prenatal developmental toxicity study (14^{th} rudimentary ribs, reduced ossification of vertebral arches, and mal-aligned sternebrae). Also there were maternal weight losses or weight gain decrements during gestation and lactation as well as reduced gestational and neonatal survival (31.7 percent survival in the F_{1b} litter at 80 mg/kg-day) (Wil Research Laboratories, 1985).

Exposure to 2,4-D through mother's milk during the period of rapid myelination (postnatal days 15 to 25) impaired normal deposition of myelin in the developing brain of Wistar rats (Duffard *et al*., 1996). The purpose of the study was to establish the critical period of exposure that causes myelin deficit in the pup's brain and not to determine a NOAEL. The study had only one exposure level, 100 mg/kg-day of 2,4-D given during different periods of lactation, plus untreated controls. Also, severe neurotoxicity demonstrated by a decrease in myelin deposition and alteration in all behavioral tests was observed in young rats exposed to 2,4-D from postnatal days 12 to 25 at doses of 70 mg/kg-day (Rosso *et al.,* 2000).

In young organisms, exposure to 2,4-D caused delays in brain development and abnormal behavior patterns, including repetitive movements, tremor, decreased social interactions, apathy, and immobility (Evangelista de Duffard *et al.*, 1995). The intensity of the response is sex-dependent; females appear to be more severely affected than males.

Studies with rats have shown an apparent region-specific neurotoxic effect on the basal ganglia, resulting in a variety of effects on critical neurotransmitters and adverse effects on behavior (Bortolozzi *et al.,* 2001). 2,4-D appears to affect the neurotransmitters serotonin and dopamine. In a specific example of such effects, rats exposed to 70 mg/kg-day 2,4-D from gestation day 16 to postnatal day 23, and challenged with amphetamine, showed increased sensitivity in dopamine D_2–like brain receptors (Bortolozzi *et al*., 2002).

In light of the results of the developmental and 2-generation reproductive toxicity studies and evidence of potential endocrine disruptive effects caused by 2,4-D, there is a need for repeating the 2-generation study with more focus on addressing thyroid effects (comparative assessment between the young and adult animals), immunotoxicity, and a thorough assessment of the gonads and reproductive/developmental endpoints. A new reproductive toxicity study designed to address the above issues would decrease the level of uncertainty in understanding adverse developmental and reproductive effects of 2,4-D.

Neurotoxicity

2,4-D produced a variety of neurotoxic effects following both acute and repeated-dose exposures. One of the most common neurotoxic symptoms caused by 2,4-D exposure was myotonia (Mattson *et al.,* 1997; Ramirez and Soza, 1988; Beasley *et al*., 1991; Kenigsberg, 1968). Myotonia is an increased muscle irritability and contractility, with decreased ability of muscles to relax after a voluntary contraction. Iincreased incidence of incoordination, slight gait abnormalities and decreased motor activity observed in acute experiments (Mattsson *et al.,* 1997; U.S. EPA, 2004a) may be related to myotonia.

In one such study (Beasly *et al.*, 1991), dogs given 175 or 220 mg of 2,4-D per kg body weight rapidly developed clinical and electromyographic (EMG) manifestations consistent with a diagnosis of myotonia or pseudomyotonia. Dogs given 2,4-D at 86.7, 43.7 or 8.8 mg/kg-body weight developed subclinical manifestation of myotonia detectable only with an electromyography. The administration of 2,4-D at 1.3 or 1.0 mg/kg-body weight did not produce any detectable EMG changes.

Following repeated-dose exposure to 2,4-D in a chronic rat study (Mattson *et al.*, 1997), there was evidence of neuropathological effects, expressed as an increased incidence of severe, bilateral retinal degeneration at 150 mg/kg-day in both sexes, with a NOAEL of 75 mg/kg-day. Mattsson *et al.* (1997) also reported increased forelimb grip strength at 150 mg/kg-day, with no effect on hindlimb grip strength. They postulated that this forelimb effect was related to the myotonia observed in the acute studies.

Rosso *et al*. (2000) made an effort to get some insights about probable mechanisms causing neurotoxic effects of 2,4-D. They showed that 24-hour exposure to 2, 4-D caused dose-dependent inhibition of neurite extension. This was accompanied by reduction in the cellular content of dynamic and stable microtubules, a disorganization of the Golgi apparatus, and an inhibition in the synthesis of complex gangliosides. These results indicate that the primary toxic effects of 2,4-D on neurons may be due to inhibition of microtubule polymerization. Also a disorganization of the Golgi complex can affect neurite formation and may change the pattern of ganglioside biosynthesis.

Although the currently available neurotoxicity data are limited, acute (gait and motor tonicity) and chronic (retinal degeneration) effects are observed at high doses, above the threshold for saturation of renal elimination. No developmental neurotoxicity studies are available that would allow quantitative assessment of neurotoxicity caused by 2,4-D.

Chronic Toxicity/Carcinogenicity

Several chronic studies in rats, mice and dogs, are available for 2,4-D. The chronic toxicity studies that are most useful for quantitative risk assessment are summarized in Table 4.

Table 4. Summary of the Critical 2,4-D Chronic Toxicity Studies

Study Type /Species	Doses	Results
Combined chronic toxicity and oncogenicity in rats (Dow, 1983; Hazleton, 1986)	0, 1, 5, 15 and 45 mg/kg-day	NOAEL = 1 mg/kg-day LOAEL = 5 mg/kg-day based on kidney tubular cell pigmentation at 5 mg/kg-day. Astrocytomas observed at 45 mg/kg-day were considered incidental.
Chronic toxicity in Fischer 344 rats (Charles *et al*., 1996a)	0, 5, 75, and 150 mg/kg-day	NOAEL = 5 mg/kg-day LOAEL=75 mg/kg-day based on decreased body-weight gain, altered organ weights and hematological parameters, and other biochemical changes.
Chronic toxicity in beagle dogs (Charles *et al*., 1996c)	0, 1, 5, and 7.5 mg/kg-day	NOAEL =1 mg/kg-day LOAEL = 5 mg/kg-day based on decreased body-weight gain and glucose, with increased BUN, creatinine, and alanine aminotransferase (both sexes), decreased food consumption and brain weight (females), and histopathological changes (pigmentation in tubular epithelium in kidneys of both sexes and pigmentation in liver sinusoidal lining cells in females).

The earliest combined toxicity and oncogenicity study on 2,4-D was presented in two reports, one preliminary (Dow, 1983) and the other final (Hazleton, 1986). The study was conducted with Fischer 344 rats, 60/sex/dose. Animals were administered 2,4-D in the diet at doses equivalent to 0, 1, 5, 15 and 45 mg/kg-day for 104 weeks. Kidney changes (tubular cell pigmentation) were observed at all but the lowest dose (1 mg/kg of body weight). Besides these changes, male rats had an increased incidence of astrocytomas - 1, 0, 0, 2, and 6 for the increasing dose groups. However, evaluation of the tumor pathological data suggests that the increased incidence of tumors in the high dose group was incidental, since several basic characteristics commonly observed in treatment-caused astrocytomas were not observed in this study.

Additional cancer bioassays in rats and mice were conducted by Dow Chemical Company (Charles *et al.,* 1996a) to follow up on the earlier equivocal observation of astrocytomas in male rats at 45 mg/kg-day (Dow, 1983). The previous observation of

astrocytomas was not confirmed with rat doses of 0, 5, 75 and 150 mg/kg-day administered in feed for two years. At 75 mg/kg-day there was decreased body-weight gain, reduced platelet count, an increase in alkaline phosphatase, thyroxin (T4), and cholesterol, and increased thyroid weights in both sexes. Reduced body weight, reduced glucose and globulin levels, reduced blood parameters (RBC count, Hb, HCT, platelet count), reduced ovarian weights, increased thyroid weights, increased hepatocyte size and increased chronic or subchronic inflammation in the lungs were observed only in females, while increased alanine and aspartate aminotransferases and decreased testes weights were observed only in males. At 150 mg/kg-day, adverse effects were observed in the eyes (cataracts and retinal degeneration), heart (degeneration), liver and adipose tissue (histopathological changes), lungs (inflammation and histiocytosis) and in the level of circulating thyroxin, which was substantially reduced.

No increases in tumor rates were found in either male or female rats, although cataracts and retinal degeneration were found in both sexes at 150 mg/kg-day. There was also no increase in tumor rates in mice administered 2,4-D in food for two years at 5, 150 or 300 mg/kg-day for females and 5, 62.5 or 125 mg/kg-day for males. In both rats and mice, a chronic NOAEL of 5 mg/kg-day was determined for 2,4-D acid added to food.

In a study by Paulino *et al.* (1996), male rats were exposed to 200 ppm of 2,4-D dimethylamine salt in their drinking water for 180 days, which provided an equivalent dose of about 20 to 25 mg/kg-day. Modest changes observed in serum enzymes suggest some liver and muscle cytotoxicity, but no macroscopic or histopathological lesions were observed at autopsy. These results are also consistent with the acute and subchronic studies in rats of Charles *et al.*, 1996b), which showed modest hematological, kidney and liver effects at the low doses, with a NOAEL of 15 mg/kg-day. Cataracts and retinal degeneration were found in female rats treated at 300 mg/kg-day (Charles *et al.*, 1996b).

Charles *et al.* (1996c) reported on a one-year chronic administration of 2,4-D free acid in feed to five dogs/sex/dose at 0, 1, 5 or 7.5 mg/kg-day. Body weight gains were decreased at all doses but were only significantly decreased at 5 and 7.5 mg/kg-day, except for one time period at 1 mg/kg-day in females. The NOAEL for the most sensitive effects in this one-year study was 1 mg/kg-day for both sexes for liver inflammation and changes in several clinical chemistry parameters (Charles *et al.*, 1996c). Dogs are considered to be more sensitive than most other species, including humans, to organic anions (such as 2,4-D) because of their slow renal excretion of organic anions (Timchalk, 2004; U.S. EPA, 2004a).

Toxicological Effects in Humans

Acute Toxicity

Acute toxicity resulting from exposure to high doses of 2,4-D has been reported after accidental ingestion. Acute effects from occupational exposures during application or manufacture, usually from a combination of high dermal and inhalation exposures, have also been observed. Symptoms include effects on the central and peripheral nervous systems, disturbances in the gastrointestinal tract (such as nausea, vomiting and diarrhea),

direct myotonic effects such as muscular weakness, stiffness, loss of tendon reflexes, muscular spasms and partial paralysis, effects on the kidney, and pulmonary edema (WHO, 1984). A variety of toxicological effects resulting from poisoning due to chlorophenoxy herbicides were summarized by Bradberry *et al.* (2004). In addition to the above listed symptoms, other reported effects included metabolic acidosis, rhabdomyolysis, increased aminotransferase activities, pyrexia and hyperventilation. High dermal exposure to 2,4-D has sometimes led to systemic effects including mild gastrointestinal irritation and progressive mixed sensorimotor peripheral neuropathy. The latter effects have occurred as a result of occupational inhalation exposure. The estimated adult mean lethal oral dose of 2,4-D is 28 g (Sullivan and Krieger, 1992).

Subchronic Toxicity

Neurotoxicity is the principal effect of acute short-term inhalation and oral overexposure to 2,4-D, with symptoms including stiffness of arms and legs, incoordination, lethargy, anorexia, and coma (U.S. EPA, 1994). Eyes, thyroid, kidneys, adrenals, and ovaries/testes are the main target organs following subchronic oral exposure at doses of 2,4-D above the threshold of saturation for renal clearance (U.S. EPA, 2004e). Short-term dermal exposure to 2,4-D may cause skin rashes. Prolonged dermal exposures can result in dermatitis (Mackison *et al.*, 1981).

Chronic Toxicity/Epidemiology

Multiple epidemiological investigations have suggested an association of herbicide use or potential exposure with various tumor types. However, the tumor types increased have been inconsistent among studies. Several studies indicated a possible increase in non-Hodgkin's lymphoma (NHL) and soft tissue sarcomas associated with agricultural applications of phenoxyacetic acid herbicides (Hoar *et al.*, 1986; Kelly and Guidotti, 1989; Zahm *et al.*, 1990; Hardell and Eriksson, 1999; Hardell *et al.*, 2002), while others did not (Cantor *et al.*, 1992; De Roos *et al.*, 2003). The pooled analysis of three National Cancer Institute (NCI) farmworker studies which was conducted by De Roos *et al.* (2003) attempted to provide a more rigorous analysis of the association of NHL with pesticide exposures among male Midwestern farmers. This analysis found no association of NHL with use of 2,4-D, basically because the slight positive association in two of the studies was negated by the lack of association in the third. However, De Roos *et al.* pointed out that changes in immune parameters reported among farmers who use phenoxyacetic acid herbicides are consistent with mechanisms of action which could result in NHL.

Davis *et al.* (1993) found that increased brain cancer among children was strongly associated (odds ratios up to 6.2) with household use of pesticides, including 2,4-D, the herbicide most widely used around homes. Leiss and Savitz (1995) found odds ratios of about four for treatment of yards with pesticides among children aged 0 to 14 with soft tissue sarcomas, compared to case-controls. This analysis was conducted on a database of childhood cancer cases in the Denver metropolitan area from 1976 to 1983 collected for an electromagnetic field exposure study. The actual number of cases is small (24 cases, 216 controls) and the stated odds ratios cannot be readily derived from the values

provided in the paper. Attempts to communicate with the authors to substantiate the calculations were unsuccessful. All of these studies are complicated by exposures to multiple chemicals, especially 2,4,5-T and its contaminant, 2,3,7,8-TCDD. It should be noted that the registration of the active ingredient 2,4,5-T has now been canceled, and TCDD is claimed not to be a contaminant of 2,4-D. Hardell suggests that a declining incidence of NHL in Sweden and other European countries is likely to have resulted from decreased exposure to both TCDD and phenoxyacetic acids (Hardell and Eriksson, 2003; Hardell, 2004). However, the studies of Hardell *et al*., the NCI, and other workers provide no compelling evidence of a link between exposure to 2,4-D and increased rates of any tumor type.

Reproductive/Developmental Toxicity

Garry *et al.* (1996) conducted an analysis of birth defects among offspring of pesticide applicators in Minnesota. This analysis shows a positive correlation of birth anomalies and altered sex ratio of births between 1989 and 1992 with increased regional use of chlorophenoxy herbicides and fungicides. To quote the abstract, "The pattern of excess frequency of birth anomalies by pesticide use, season and alteration of sex ratio suggests exposure-related effects in [pesticide] applicators and the general population of the crop-growing region of western Minnesota." This is consistent with earlier studies which show various abnormal health outcomes to be increased in rural areas compared to urban areas, and vice-versa, but does not clearly point to specific causes of the differences.

Exposure of farmers to 2,4-D resulted in measurable amounts of this herbicide in their urine and semen (Arbuckle *et al*., 1999). Preconceptional exposure to chlorophenoxy herbicides was also associated with a moderate increased risk of early abortions (odds ratio 1.5, 95 percent confidence interval 1.1-2.1) (Arbuckle *et al.,* 2001).

Other Human Toxicity data

Available human data indicates that 2,4-D may be cytotoxic and mutagenic. A study of pesticide applicators showed an 11-14 percent increase of lymphocyte replicative index directly related to absorbed dose of 2,4-D (Figgs *et al.,* 2000). This finding was confirmed in another study *in vivo* and *in vitro* showing that higher-dose exposures may cause a direct cytotoxic effect on lymphocytes that results in a decreased replicative index and an inverted U-shaped dose-response curve (Holland *et al.,* 2002). A commercial formulation of 2,4-D tested on human lymphocytes *in vitro* showed a treatment-related increase in the number of chromatid and chromosome breaks, as well as acentric fragments and aberrant cells at a concentration of 0.4 μg/mL (Zeljezic and Garaj-Vrhovac, 2004). At this level there were also significant increases in the number of micronuclei and nuclear buds. The frequency of chromatid and chromosome breaks was significantly increased by metabolic activation. 2,4-D was also shown in *in vitro* tests to cause oxidative stress at the cellular level by changing antioxidant enzyme activities (Bukowska, 2003). This study involved treatment of human erythrocytes with 2,4-D at 250 and 500 ppm, which resulted in decreased levels of reduced glutathione, decreased activity of superoxide dismutase, and increased levels of glutathione peroxidase. The

cytotoxicity of 2,4-D was also expressed as increased rates of apoptosis related to breakdown of mitochondrial membrane, induction of DNA strand breaks, and a loss of membrane integrity in human hepatoma cells treated with this chemical (Tuschl and Schwab, 2003). Induction of apoptosis was also shown in cerebellar granule cells treated with 2,4-D (De Moliner, 2002).

DOSE-RESPONSE ASSESSMENT

Noncarcinogenic Effects

The studies most relevant to the 2,4-D quantitative risk assessment were three chronic studies in rats and dogs, as summarized in Table 4. In a combined chronic toxicity/oncogenicity study in Fischer 344 rats (60 per sex per dose), animals were given 2,4-D in the diet at doses equivalent to 0, 1, 5, 15 and 45 mg/kg-day (Dow, 1983; Hazleton, 1986). No dose-related effects were observed in clinical, hematological and biochemical parameters. An increased incidence of brown pigment was observed in kidney tubular cells of both males and females at doses of 5, 15 and 45 mg/kg-day, which could be used to derive a NOAEL for this effect of 1 mg/kg-day. However, it has been argued that this pigmentation is "not part of any apparent continuum of tubular epithelial findings in response to dose" (DPR, 2006), which makes this effect of somewhat questionable value for risk assessment.

In another 2-year chronic toxicity study, Fischer 344 rats were administered 2,4-D in the diet at doses equivalent to 0, 5, 75 and 150 mg/kg-day. The NOAEL established in this study was 5 mg/kg-day. A LOAEL of 75 mg/kg-day was based on decreased body-weight gain and food consumption, hematological changes, enzymatic changes, changes in various organs including kidney, and changes in the blood levels of T4, glucose, cholesterol and triglycerides (Charles *et al*., 1996a). The robust study design and evaluation make it highly credible for risk assessment.

The third study relevant to the 2,4-D quantitative risk assessment is a 1–year chronic toxicity study in dogs (Charles *et al.*, 1996c). In this study animals were maintained on a diet containing 2,4-D, receiving doses of 0, 1, 5, or 7.5 mg/kg-day. A NOAEL was established at 1 mg/kg-day. The LOAEL was 5 mg/kg-day, based on decreased body-weight gain and food consumption, clinical chemistry changes, decreased brain weight in females, and histopathological lesions in liver and kidneys. Dogs are considered to be more sensitive to the effects of 2,4-D than rats because of their limited capacity to excrete organic acids (Timchalk, 2004; U.S. EPA, 2004a).

All of these studies showed that kidney is a sensitive organ for effects of 2,4-D with chronic exposures. Because the recent chronic toxicity study in rats (Charles *et al.,* 1996a) failed to confirm the mild kidney effects (increased pigment) found at 5 mg/kg-day in the earlier study, the rat NOAEL of 5 mg/kg-day is considered to be most credible. This NOAEL was based on a variety of clear toxic effects at the next higher dose of 75 mg/kg-day, including renal lesions. The dog study of Charles *et al.* (1996c) is also relevant, but because of the prolonged half-life and a greater potential for kidney damage (and other effects) in dogs compared to humans, an intra-species correction factor of 10

for extrapolation from dogs to humans is judged to be inappropriate. Without this factor, a health-protective level estimated from the dog study yields a higher value than that based on the chronic rat study of Charles *et al*. (1996a).

In conclusion, the study that is most appropriate for deriving a health-protective value is the rat chronic toxicity study of Charles *et al.* (1996a), with a NOAEL of 5 mg/kg-day. The study design and results seem appropriate and credible, including failure to confirm the low-dose renal pigmentation changes observed in the earlier rat study with a more rigorous design and analysis.

Issues Related to Protection of Sensitive Subpopulations

Under the Food Quality Protection Act (FQPA), U.S. EPA is required in setting tolerances for pesticides in food to use an extra 10-fold safety factor to take into account potential pre-and post-natal developmental toxicity and the completeness of the data base unless U.S. EPA determines, based on reliable data, that a different margin would be safe (U.S. EPA, 1997). In addition, the evaluation of the tolerance must take into account:

1. aggregate exposure from all non-occupational sources;
2. effects from cumulative exposure to the pesticide and other substances with common mechanisms of toxicity;
3. effects of *in utero* exposure;
4. potential for endocrine disrupting effects; and
5. completeness of the available toxicological data base.

The new California law AB 2342 (2004) requires OEHHA to assess the following while preparing PHGs, to the extent of the available information:

1. Exposure patterns, including, but not limited to, patterns determined by relevant data, among bottle-fed infants and children that are likely to result in disproportionately high exposure to contaminants in comparison to the general population.
2. Special susceptibility of infants and children to contaminants in comparison to the general population.
3. The effects on infants and children of exposure to contaminants and other substances that have a common mechanism of toxicity.
4. The interaction of multiple contaminants on infants and children.

Children, in general, have unique exposure patterns. Calculated on a body weight basis, children eat more, drink more, and inhale more air than adults. They also engage in more frequent hand-to-mouth contact, and therefore have higher rates of oral exposure from objects, dust, or soil. The skin surface area of an infant per unit of body weight is double that of an adult (NRC, 1993). Infants and toddlers are of special concern for dermal exposure because of the increased surface area and their behavior patterns, including crawling on lawns, patios and interior floors. Dermal exposure to pesticide residues is often the major exposure route for infants.

Hands moist with saliva collect about 100 times more pesticide residues than dry hands, and children's hands are much more likely to be moist (Camann *et al.*, 1996). The potential for children's residential exposure to 2,4-D residues was evaluated in a U.S. EPA report (1999) on indoor exposures from the transport of lawn-applied herbicide into the home. Opening and closing of doors and windows as well as post-application air intrusion from closed house ventilation increased the indoor background level of 2,4-D six-fold. Children's and pets' high activity and wearing shoes indoors, increased 2,4-D indoor levels by 37-fold. These levels were shown to increase continually over a one-week period. The increase in indoor air levels paralleled the increased levels in floor dust, suggestive of resuspension of house dust by human activity. Considering this exposure pathway and the potential for pulmonary portal-of-entry effects, U.S. EPA has expressed concern about the lack of an inhalation study for 2,4-D (U.S. EPA, 2004a).

U.S. EPA in its 2,4-D Human Health Risk Assessment for the Reregistration Eligibility Decision memorandum (U.S. EPA, 2004a) concluded that "…no special FQPA Safety Factor is needed [1X] since there are no residual uncertainties for pre- and/or postnatal toxicity." However, they then chose to add an additional 10-fold database uncertainty factor, "based on the need for a developmental neurotoxicity study in the rat, and a 2-generation reproduction study with special emphasis on thyroid and immunotoxic effects" (U.S. EPA, 2004b).

For the chronic risk assessment resulting from dietary exposure to 2,4-D, U.S. EPA used a NOAEL of 5 mg/kg-day from the long–term toxicity study in Fischer 344 rats (Charles *et al*., 1996a) and applied a 1,000-fold uncertainty factor to calculate the reference dose (RfD) of 0.005 mg/kg-day. U.S. EPA refers to this as providing a residential margin of exposure (MOE) of 1,000 (U.S. EPA, 2004a,b, 2005); the occupational MOE is typically set at 100.

Because a considerable amount of controversy about the risk assessment of 2,4-D revolves around the relative renal excretion rates for organic acids (especially among the various test species), OEHHA has also considered the potential for human variability in organic acid secretions. Variations are well documented (i.e., Xu *et al.*, 2005; Robertson and Rankin, 2006), but the transporters appear inadequately studied for full characterization regarding potential sensitive subpopulations and consideration of potential interactions among chemicals. For all these reasons, OEHHA is utilizing an uncertainty factor of 1,000 to calculate the PHG as described below, using the NOAEL of 5 mg/kg-day established in a combined chronic toxicity and oncogenicity in rats (Charles *et al.*, 1996a).

Evidence of developmental toxicity

Currently available developmental and reproductive toxicity studies provided evidence of developmental toxicity caused by 2,4-D exposure. Adverse developmental effects were observed in the prenatal developmental toxicity studies in rats and rabbits, and in the 2-generation reproduction study in rats. The effects consisted of skeletal abnormalities in rats and abortions in the prenatal toxicity study in rabbits (also evidence of susceptibility of developing offspring) (Nemec *et al*., 1983; Hoberman, 1990). In the prenatal developmental toxicity study in rats, skeletal variations including ossification delays

consisted of presence of 7th cervical ribs, 14th rudimentary ribs and mal-aligned sternebrae observed at a dose that also caused maternal toxicity (decreased body weight gain and food consumption) (Nemec *et al*., 1983). In the 2-generation reproduction toxicity study, abnormalities included presence of 14th rudimentary ribs, reduced ossification of vertebral arches, and mal-aligned sternebrae observed in the F_{1b} pups (Wil Research Laboratories, 1985). Further evidence of developmental toxicity was a myelin deficit in rat pup's brains produced as a result of 2,4-D exposure through mother's milk during the period of rapid myelination (postnatal days 15 to 25) (Duffard *et al*., 1996).

Evidence of endocrine disruptive effects

2,4-D was found to cause suppression of thyroid hormone levels in rats, accompanied by increases in thyroid gland weight and decreases in ovary and testes weights at 75 and 150 mg/kg-day (Charles *et al.*, 1996a). Suppression of thyroid hormone level by 2,4-D was also found in ewes (Rawlings *et al*., 1998). In an *in vitro* study, 2,4-D caused small decreases in testosterone release and significant increases in estrogen release from testicular cells (Liu *et al*., 1996). 2,4-D endocrine disruptive action is not limited to the androgen and thyroid hormone systems. In rodents, 2,4-D was shown to increase levels of progesterone and prolactin, and caused abnormalities in the estrus cycle (Duffard *et al*., 1995).

A potential for harmful endocrine disruptive effects was also postulated in humans. Male farm sprayers exposed to 2,4-D had lower sperm counts and more spermatic abnormalities than men who were not exposed (Lerda and Rizzi, 1991). In Minnesota, higher rates of birth defects have been recorded in the areas of the state with the highest use of 2,4-D and other herbicides of the same class. The increase in birth defects was highest among infants who were conceived in the spring, the time of greatest herbicide use (Garry *et al.*, 1996).

Data gaps

The toxicological database for 2,4-D is extensive and its scientific quality seems to be relatively high. However, the available data do not permit a thorough assessment of toxicity to the developing brain and nervous system, endocrine system, reproductive system and immune system, as well as risk from inhalation exposure. Current data gaps according to U.S. EPA include: a developmental neurotoxicity study, a repeat 2-generation reproductive toxicity study that would use a new protocol, and a 28-day inhalation toxicity study (U.S. EPA, 2004a, 2005). The new reproductive toxicity study would address concerns for thyroid effects (comparative assessment between the young and adult animals) and immunotoxicity, and provide a more thorough assessment of the gonads and reproductive/developmental endpoints. The inhalation toxicity study would allow better assessment of health risk (or toxicological hazards) resulting from aggregate exposure to 2,4-D. The U.S. EPA has added a 10-fold factor for database uncertainty in its most recent review of 2,4-D toxicity (U.S. EPA, 2004a, 2005).

Besides children, populations who would be assumed to be at special risk from 2,4-D exposure include persons suffering from liver disease, kidney disease, cardiovascular

disease, skin disease, convulsive disorders or neuropathy (Mackison *et al*., 1981), because these are the organs, tissues, or functions affected by 2,4-D. However, no information was found on any effects of 2,4-D under such conditions, so it is not possible to incorporate any specific consideration of such potential sensitive groups.

Carcinogenic Effects

Due to lack of conclusive findings in the epidemiological data, and the lack of evidence in animal studies for carcinogenicity, carcinogenicity is not used as the endpoint for the PHG.

CALCULATION OF PHG

Noncarcinogenic Effects

For estimation of a health-protective concentration of a chemical in drinking water, an acceptable daily dose (ADD) of the chemical from all sources is first calculated. This involves incorporation of appropriate estimates of uncertainty in the extrapolation of the critical toxic dose from human or animal studies to the estimation of a lifetime daily dose that is unlikely to result in any toxic effects. For this purpose, the following equation will be used:

$$\text{ADD} = \frac{\text{NOAEL/LOAEL in mg/kg-day}}{\text{UF}}$$

where,

ADD = an estimate of the maximum daily dose which can be consumed by humans for an entire lifetime without toxic effects;

NOAEL/LOAEL = no-observed-adverse-effect level or lowest-observed-adverse-effect level in the critical study;

UF = uncertainty factor.

Calculation of a public health-protective concentration (C, in mg/L) for a chemical in drinking water will use the following equation for noncarcinogenic endpoints:

$$\text{C} = \frac{\text{ADD mg/kg-day x RSC}}{\text{DWC L/kg-day}}$$

where,

RSC = relative source contribution (usually 20 to 80 percent, 0.20 - 0.80);

DWC = drinking water consumption, in which an upper 95th percentile value for the general population of 0.044 L/kg-day (U.S. EPA, 2004f) is judged to be most relevant for this analysis.

Due to lack of conclusive findings in the epidemiological data, and the lack of evidence in animal studies for carcinogenicity, mutagenicity, or any low-dose reproductive effects of 2,4-D, the PHG is calculated from the noncarcinogenic systemic effects in animals. For the calculation of the acceptable daily dose, a NOAEL of 5 mg/kg-day is selected from a chronic rat study (Charles *et al.*, 1996a). An uncertainty factor (UF) of 1,000 is utilized, to account for interspecies extrapolation (10), probable variability among humans (10), and potential susceptibility of infants and children associated with the developmental effects noted in the limited available studies, with a lack of more in-depth studies (10). This combined uncertainty factor is equivalent to the latest U.S. EPA risk assessment, which expressed the need for a developmental neurotoxicity study and a 2-generation reproductive study (U.S. EPA, 2004a,b, 2005) in arriving at an uncertainty factor of 1,000. Thus,

$$\text{ADD} = \frac{5 \text{ mg/kg-day}}{1{,}000} = 0.005 \text{ mg/kg-day}$$

For calculation of the public-health protective concentration, two exposure parameters are considered: the relative source contribution (RSC) and the water consumption rate. The RSC is based on an estimate of the contribution of drinking water relative to other sources of exposure to the chemical contaminant. The other sources are food, air, soil contact, occupational exposures and exposure through smoking. Often food is the most significant source of exposure after drinking water exposure. U.S. EPA's RSC range is 20 to 80 percent, depending on the available data. For pesticides that are currently in use, OEHHA and U.S. EPA have customarily used an RSC of 20 percent, reflecting the assumption that drinking water is likely to be a minor exposure route, unless data or relevant information are available to support an alternative value.

For the widely-used herbicide 2,4-D, there may be widespread dietary exposures to trace levels of the chemical in crops, although it has a relatively short environmental half-life and does not bioaccumulate in soil, plants or animals. Pesticide tolerances for 2,4-D are generally less than 1 ppm in edible portions of food, and actual detected residues average much lower (HSDB, 1996; U.S. EPA, 2005).

Exposures to 2,4-D in air should be low-level and infrequent for the general population, caused mostly from overspray during application of the product to lawns for weed control. Exposures to 2,4-D in drinking water are also expected to be low, overall. 2,4-D has not been detected in recent years in California drinking water samples, at a detection limit of 10 ppb. However, low exposures from food can be expected (U.S. EPA, 2005). The RSC from drinking water will therefore be set at 20 percent, based on exposure to trace levels of 2,4-D in both air and food.

The other exposure factor in the equation, water intake, must acknowledge the amount of tap water that an individual consumes as drinking water, as well as mixed with beverages

and used in cooking. For this analysis, an upper 95th percent confidence limit consumption value for the general population is used, because the critical endpoint and exposure are whole-life. The consumption value, expressed in L/kg-day, is derived from a recent U.S. EPA analysis of data from an NHANES national survey (U.S. EPA, 2004f). For 2,4-D, dermal absorption and other incidental exposures to the chemical in drinking water should not contribute significantly to the total dose because 2,4-D does not penetrate skin well, and is not volatile enough to provide a secondary inhalation exposure from other household water uses.

Calculation of a public health-protective concentration (C, in mg/L) for 2,4-D in drinking water is then calculated as follows:

$$C = \frac{0.005 \text{ mg/kg-day} \times 0.2}{0.044 \text{ L/kg-day}} = 0.023 \text{ mg/L} = 20 \text{ ppb (rounded)}$$

Based on the results of the above calculations, OEHHA has calculated a public health goal of 0.02 mg/L (20 μg/L or ppb) for 2,4-D in drinking water. This value is judged to be adequately protective of the identified potentially sensitive subpopulations such as pregnant women and their fetuses, infants, children, and the elderly.

RISK CHARACTERIZATION

Authors of some epidemiological studies have postulated that there may be a potential for tumorigenic and developmental effects of phenoxy herbicides, but the association of increased tumor rates or birth defects with 2,4-D has been weak and inconsistent. The reported adverse effect rates in epidemiological studies have not, in general, been corrected for other risk factors. Association of a variety of tumors and reproductive disruption with overall use of pesticides may show correlation with the most frequently used pesticide types, such as chlorphenoxy herbicides, but this does not prove a cause and effect relationship. The inconsistency among the various studies as to tumor types increased may reflect multiple competing risk factors or, perhaps, chance association with 2,4-D.

The Minnesota birth defect study of Garry *et al.* (1996) illustrates the problem with interpretation of the epidemiological data; there are many conditions beside exposure to chlorphenoxy herbicides that differ among the agricultural and urban regions of the state, such as other agricultural-related exposures or activities. The data on increased tumor rates may also reflect earlier exposures to dioxin, a contaminant of phenoxy herbicides, especially 2,4,5-T. Because these data cannot be used for risk assessment, the animal data are used to derive the PHG.

For this chemical there are two relevant chronic studies in rats of the same strain with discordant results (Hazleton, 1986; Charles *et al.,* 1996a). The latter study supersedes the earlier study, because effects on the kidney seen in the earlier study were not observed at the 5 mg/kg-day level in the latter study. Effects in dogs were not used for calculation of the PHG because several pharmacokinetic studies showed that the dog has increased

sensitivity to phenoxyacetic acid herbicides and related organic acids because of a lower relative capacity to secrete them from the kidney than both rats and humans, leading to a prolonged half-life (Timchalk, 2004). Therefore an interspecies correction factor of 10 for extrapolation from dogs to humans would be inappropriate, and a health-protective level based on the dog NOAEL of 1 mg/kg-day would be higher than the level derived from the rat data. The effects in dogs are still relevant and informative for human risk assessment, confirming the significance of the adverse renal effects.

An uncertainty factor of 1,000 has been incorporated into the PHG to help protect humans, including sensitive populations, and potentially more susceptible infants and children from any adverse effects. This combined uncertainty factor is 10-fold greater than that used in the earlier PHG (OEHHA, 1997), and represents a concern over potential effects in infants and children, related to lack of a developmental neurotoxicity study and a 2-generation reproductive study (U.S. EPA 2004a,b, 2005). Variations in renal organic anion transport which could lead to differences in human pharmacokinetics of 2,4-D have been documented, but not analyzed with regard to potential sensitive subpopulations (Xu *et al.*, 2005; Robertson and Rankin, 2006). The 1,000-fold uncertainty factor is consistent with that utilized by the U.S. EPA in their latest risk assessment for 2,4-D (U.S. EPA, 2004a). OEHHA has also chosen the same NOAEL as used by the U.S. EPA for the point of departure.

The total exposure from food containing trace levels of 2,4-D plus the exposure from drinking water that meets the 2,4-D PHG will be much lower than the occupational exposures to 2,4-D (U.S. EPA, 2005). OEHHA's updated value for the PHG is less than the current PHG of 70 ppb, established in 1997 (OEHHA, 1997). The World Health Organization estimated that average daily doses of 2,4-D to the general population are about 0.3 to 2 μg/kg from air, food and water combined (WHO, 1984). WHO (1996) developed an Acceptable Daily Intake of the combined salts and esters of 2, 4-D (expressed as 2,4-D) of 0.01 mg/kg-day, based on their determination of a NOAEL of 1 mg/kg-day in the dog and rat chronic studies, with an uncertainty factor of 100.

U.S. EPA recently estimated a maximum exposure from food for the general U.S. population of 12 μg/kg-day (U.S. EPA, 2005). Comparing this to the maximum water exposure at the PHG level of 20 ppb, the relative source contribution to total 2,4-D exposure from water for a 60 kg adult drinking 2 L/day of tap water would be less than 10 percent ((20 μg/L x 2 L/day)/((12 μg/kg-day x 60 kg)+(20 μg/L x 2 L/day)) = 5.3 percent). The highest estimated maximum exposures from food were 22 μg/kg-day for children 1-2 years old. If a 1 year old child weighing 10 kg were exposed to a dose of 22 μg/kg-day from food and also drank 1 L/day of water at the PHG level of 20 μg/L, the relative source contribution from drinking water would be 8 percent (20/(220+20) = 0.08). Based on the upper 95th percentile water consumption level of an infant <1 year old, or 0.185 L/kg-day (U.S. EPA, 2004f), consumption of 2,4-D from water would be 37 μg; the relative source contribution for this infant would be 14 percent (37/(220+37) = 0.14). Average contributions from both food and water would undoubtedly be much lower, and cannot be accurately estimated from the available data.

The water consumption value used for the PHG calculation is higher than that used in the earlier PHG, which was 2 L/day for a 70 kg person (equivalent to 0.029 L/kg-day). The

new value, representing the upper 95th percentile water consumption, averaged over a lifetime, is based on the newest U.S. EPA evaluation of drinking water consumption data (U.S. EPA, 2004f), and, we feel, more adequately protects the entire population. The earlier water consumption value has been estimated to represent about the 70th percentile of tap water consumption (Ershow *et al.*, 1991). According to the U.S. EPA analysis, the 95th percentile of drinking water intake for the general population (all ages) is 30 percent higher (2.6 L/day) than the previous default value of 2.0 L/day. The difference is even greater for infants, children, and pregnant women. Since AB 2342 (2004) amended the California Safe Drinking Water Act (HSC section 116365.2) to mandate consideration of the greater exposure of susceptible populations including infants and children, OEHHA has begun to use these new, more health-protective consumption values in our updated drinking water risk assessments, such as in the revised glyphosate PHG, published June, 2007. OEHHA believes that using the 95th percentile drinking water consumption value is the best approach for protecting the health of the entire population. A similar approach for estimating exposures to toxic air contaminants, utilizing upper 95th percentile breathing rates, has been incorporated into OEHHA's Toxic Hot Spots program, and has been approved by the Scientific Review Panel (OEHHA, 2003).

Intermittent exposures to other phenoxy herbicides are expected from dietary and occupational sources, but concurrent exposures to significant levels of two or more phenoxy herbicides should not be common. Thus, no extra margin of safety to allow for concurrent exposures is considered necessary.

OTHER REGULATORY STANDARDS

The International Agency for Research on Cancer (IARC) judges that chlorphenoxy herbicides as a group are in category 2B, or "limited evidence of carcinogenicity to humans." This category is explained as follows: "A positive association has been observed between exposure to the agent, mixture or exposure circumstance and cancer for which a causal interpretation is considered by the Working Group to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence" (IARC, 1987). 2,4-D is specifically characterized as having inadequate evidence for carcinogenicity to animals (IARC, 1987). U.S. EPA (2004a; 2005) classifies 2,4-D as a Group D chemical (not classifiable as to human carcinogenicity). The U.S. EPA IRIS database provides no evaluation of 2,4-D carcinogenicity (IRIS, accessed 2008, last updated 1989).

In addition, U.S. EPA has just published a "Decision Not to Initiate Special Review" of 2,4-D and related herbicides (U.S. EPA, 2007), with the explanation as follows:

> "This notice announces EPA's Decision Not to Initiate a Special Review for 2,4-D, 2,4-DB and 2,4-DP. Based on extensive scientific review of many epidemiology and animal studies, the Agency finds that the weight of the evidence does not support a conclusion that 2,4-D, 2,4-DB and 2,4-DP are likely human carcinogens. The Agency has determined that the existing data do not support a conclusion that links human cancer to 2,4-D exposure. This conclusion applies to 2,4-DB and 2,4-DP because they were considered for Special Review based solely on their similarity to 2,4-D. In

addition, because they are used significantly less than 2,4-D, their contribution to exposure is minimal relative to 2,4-D. Because the Agency has determined that the existing data do not support a conclusion that links human cancer to 2,4-D exposure, the Agency is not initiating a Special Review of 2,4-D, 2,4-DB and 2,4-DP."

U.S. EPA's MCL and MCLG of 70 ppb were derived from a NOAEL of 1 mg/kg-day based on hematologic, renal and liver effects at 5 mg/kg-day in 90-day and chronic rat studies (Dow, 1983; IRIS, 2008, last updated 1989). These studies were summarized and published by Munro *et al*. (1992). The MCL and MCLG were derived from this NOAEL using an uncertainty factor of 100, assuming that an adult drinks two L/day of water and that drinking water contributes 20 percent (0.2) of the total exposure (U.S. EPA, 1991). The MCL of 70 ppb is expressed as the concentration of the free 2,4-D acid. In the Reregistration Eligibility Decision for 2,4-D, U.S. EPA (2005) calculated a chronic RfD of 0.005 mg/kg-day using the long-term toxicity study in Fisher 344 rats (Charles *et al.* , 1996a). Calculation of this RfD used a NOAEL of 5 mg/kg-day and an uncertainty factor of 1,000.

WHO (1996) developed an Acceptable Daily Intake of the combined salts and esters of 2,4-D (expressed as 2,4-D) of 0.01 mg/kg-day, based on their conclusion of a NOAEL of 1 mg/kg-day in the dog and rat chronic studies, with an uncertainty factor of 100.

The free acid is relatively quickly formed in the environment by hydrolysis of the ester and amine forms, so the toxicity of the different 2,4-D products is considered equivalent (on a free-acid basis) for setting public health protective levels in drinking water. U.S. EPA's MCL is identical to the current California MCL, established in 1994.

The reference exposure level (REL) set by the National Institute of Occupational Safety and Health and the Permissible Exposure Level (PEL) set by the federal Occupations Safety and Health Administration for 2,4-D is 10 mg/m^3 averaged over an eight hour work shift (NIOSH, 2005).

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