PUBLIC HEALTH GOALS FOR CHEMICALS IN DRINKING WATER

GLYPHOSATE

June 2007

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GLYPHOSATE
in Drinking Water

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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 Health Services (DHS) 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 DHS are to consider economic factors and technical feasibility. Each primary drinking water standard adopted by DHS 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 DHS must be at least as stringent as the federal MCL, if one exists.

PHG documents are used to provide technical assistance to DHS, 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 GLYPHOSATE IN DRINKING WATER

SUMMARY

The Office of Environmental Health Hazard Assessment (OEHHA) has reviewed the scientific literature on glyphosate and evaluated risk assessment methods that have been developed since the publication of the original Public Health Goal (PHG) for glyphosate in 1997. The Office has reduced the PHG for glyphosate in drinking water from 1,000 to 900 parts per billion (ppb), based on an updated exposure calculation for adult females, on whom the PHG value is based.

OEHHA chose a developmental study in rabbits as the key study in the development of the updated PHG for glyphosate. At the highest gavage dose, 350 mg/kg-day, diarrhea, nasal discharge, and early mortality were observed in the exposed rabbits. Developmental toxicity was not observed at any dose tested. The next lower dose of 175 mg/kg-day was identified as the No Observed Adverse Effect Level (NOAEL). An acceptable daily dose (ADD) of glyphosate of 0.175 mg/kg-day was derived from this by dividing by an uncertainty factor of 1,000 (100 for inter- and intra-species variation and another factor of 10 to account for the severity of the endpoint (mortality) and the short exposure duration of the rabbit study). The updated PHG of 0.9 mg/L (900 ppb) was developed using a body weight per liter of water consumed of 25.2 kg-day/L, and a relative source contribution of 20 percent. The 25.2 kg-day/L value represents the upper 95 percent confidence limit for relative water consumption by pregnant women (OEHHA, 2000). The relative source contribution is a default value commonly used for chemicals for which drinking water is assumed to be a minor source.

Glyphosate is a non-selective systemic herbicide used in agriculture, rights-of-way and aquatic systems. Exposure to glyphosate may occur from its normal use due to spray drift, residues in food crops, and from runoff into drinking water sources. Following acute exposure, glyphosate has low systemic toxicity to mice and rats. In humans, irritation of the oral mucous membrane and gastrointestinal tract is the most frequently reported effect in suicide attempts with glyphosate-surfactant formulations. In most of the short- and long-term toxicity studies in animals, there were no treatment-related gross or cellular changes except reduced body weights, increased liver weights, and ocular lesions at relatively high doses. Three carcinogenicity studies have been conducted, two in rats and one in mice, and all are considered to be negative. *In vitro* and *in vivo* genotoxicity tests are generally negative. There are a few reports of increased sister chromatid exchange in human and bovine lymphocytes at high concentrations *in vitro*, which could be secondary to oxidative stress, and effects on mouse bone marrow after very large intraperitoneal doses. Based on the weight of evidence, glyphosate is judged unlikely to pose a cancer hazard to humans.

OEHHA's review of the glyphosate toxicity literature includes many new scientific studies, plus comments received from the public. Our evaluation has concluded that a

PHG of 900 ppb provides adequate protection against adverse effects of glyphosate in drinking water for the general population and potential sensitive subpopulations such as pregnant women and their fetuses, infants, and the elderly.

INTRODUCTION

Glyphosate, N-(phosphonomethyl) glycine, is used as a non-selective post-emergence herbicide for controlling weeds in agriculture (cropped and non-cropped), forestry, rights-of-way and aquatic systems. Glyphosate inhibits the 5-enolpyruvylshikimate-3-phosphate synthase activity and blocks aromatic amino acid synthesis. This enzyme is found in plants but not in mammals, thereby providing a selective toxicity to plants. In affected plants, this causes reduced protein synthesis, cessation of growth, and leads to cellular disruption and death. Glyphosate has nonspecific metal-chelating properties; it inhibits enzymes that require transition metal cations for activity, such as 3-deoxy-2-oxo-D-arabino-heptulosonate-7-phosphate synthase and 5-dehydroquinate synthase (NTP, 1992).

Glyphosate was first introduced in 1974 and is sold under various trade names such as Roundup branded herbicides, Rodeo®, and Accord®. The major product is a family of herbicides sold under the trade name of Roundup, which consists of the isopropylamine salt of N-(phosphonomethyl) glycine and a surfactant. The predominant surfactant used is a polyethoxylated tallow amine (POEA), which is a mixture of polyethoxylated long-chain alkylamines (Williams *et al*., 2000). Roundup branded herbicides are sprayed as a liquid with ground and aerial equipment. According to U.S. EPA (2004), glyphosate was the second most commonly used pesticide in both the agricultural and non-agricultural (home, garden, and commercial) market sectors. In the agricultural market sector, it was estimated that 34 to 38 million pounds and 67 to 73 million pounds of glyphosate were used in 1997 and 1999, respectively. In the non-agricultural market sector, it was estimated that the annual usage was approximately 7 million pounds of glyphosate during that period. In 2003, approximately 12 million pounds of glyphosate, isopropylamine salt were sold in California. In the same year, approximately 5.6 million pounds were reported used in California. This would cover primarily agricultural uses.

The California Department of Health Services (DHS, 1989) conducted a risk assessment on glyphosate and set the Proposed Maximum Contaminant Level (PMCL) and MCL for drinking water at 0.7 mg/L (700 ppb). This was based on systemic toxicity in a three-generation rat reproduction study with a NOAEL of 10 mg/kg-day (Bio/Dynamics, Inc, 1981b) and an uncertainty factor of 100. The California MCL was established at that level in 1990.

According to the Integrated Risk Information System (IRIS) (U.S. EPA, 2007), the U.S. EPA chose the same rat study, NOAEL, and uncertainty factor in developing a reference dose (RfD) of 0.1 mg/kg-day (in 1990). Applying default exposure assumptions and a relative source contribution (RSC) of 20 percent, U.S. EPA developed a MCL of 0.7 mg/L (U.S. EPA, 1992a). However, a subsequent two-generation rat developmental study at much higher doses (Monsanto, 1990b) did not confirm the findings of this study.

Despite the availability of some new studies, the oral RfD listed in IRIS has not been updated since 1990.

Another RfD of 2 mg/kg-day is listed in the Federal Register (Fed Reg, 1997) for use in the development of the pesticide tolerance for glyphosate in crops. This RfD is based on adverse health effects observed in pregnant rabbits exposed during gestation (21 days) by gavage (IRDC, 1980b). At the highest dose, 350 mg/kg-day, diarrhea, nasal discharge, and early mortality were observed in the exposed rabbits. Developmental toxicity was not observed at any dose tested. The next lower dose of 175 mg/kg-day was identified as the NOAEL. U.S. EPA derived the RfD of 2 mg/kg-day by applying an uncertainty factor of 100.

In 1997, OEHHA evaluated the glyphosate toxicity literature and developed a PHG of 1,000 ppb for glyphosate in drinking water (OEHHA, 1997). The PHG was based on the same rabbit teratology study that was used by U.S. EPA in deriving the RfD of 2 mg/kg-day. OEHHA used an uncertainty factor of 1,000, an assumed body weight of 60 kg for an adult female, a water consumption rate of 2 L/day, and a relative source contribution of 20 percent.

Several health effects studies and review papers on glyphosate have been published over the past several years. This document provides a brief summary of toxicity studies of glyphosate in the context of the updated review of chemical contaminants in drinking water that is required under Health and Safety Code 116365, including the amendments under AB 2342 (2004) for special consideration of infants and children.

CHEMICAL PROFILE

The structure of glyphosate, N-(phosphonomethyl) glycine, is shown in Figure 1; its properties are summarized in Table 1. Glyphosate is usually formulated as a salt of the deprotonated acid of glyphosate and a cation, e.g., isopropylamine or trimethylsulfonium. Surfactants and inert ingredients are often added to formulations of glyphosate such as Roundup branded herbicides and Vision®. Common surfactants are polyoxyethylene amine, ortho X-77, Li-700, R-11 and Widespread. Other additives that may be found in formulations are sulfuric and phosphoric acids. The amount of glyphosate in these products varies over a wide range. The percentage by weight can be as low as less than one percent in ready to use commercial products to over 40 percent in some concentrates (WHO, 1994). As the subject of this evaluation is glyphosate, and there are many possible compositions of commercial products, most of the data and discussion presented in this analysis are on glyphosate rather than the formulated products. In drinking water, the glyphosate anion is likely to be associated with alkali metal cations such as sodium ion (Montgomery, 1993). Toxicity results for commercial products are included only when they provide additional insights to the health hazards associated with the oral exposure to the active ingredient, glyphosate.

Figure 1. The structure of glyphosate [N-(phosphonomethyl) glycine]

Table 1. Physical and Chemical Properties of Glyphosate

Name	Glyphosate (N-(phosphonomethyl)-glycine)
Trade names	Roundup branded herbicides, Rodeo®, Accord®
CAS No.	1071-83-6
Physical state	White crystalline solid
Melting point	230 ^{o}C (decomposes)
Molecular weight	169.07
Density	1.74 g/mL
Solubility in water	12 g/L at 25^{0}C
Solubility in organic solvents	Insoluble
Vapor pressure	7.50x10^{-6} mm Hg at 25^{o} C
Henry's Law constant	1.39x10^{-10} atm-m^{3}/mol.
Octanol-water partition coefficient (Log K_{ow})	-2.8, -1.6
pKa values	2.32, 5.86, 10.86
pH (1% solution in water)	2.5

(Adapted from Edmund, 1988; Montgomery, 1993; WHO, 1994.)

ENVIRONMENTAL OCCURRENCE AND HUMAN EXPOSURE

Soil

Glyphosate may reach soil in its normal use as a liquid spray, through spillage or accidental discharge. Once in soils, it is strongly adsorbed onto the soil forming insoluble complexes with metal ions. Glyphosate is readily degraded by soil microbes to aminomethyl phosphonic acid (AMPA), which is then degraded to inorganic constituents, including carbon dioxide and phosphate. Based on field experiment data, the dissipation

half-life of glyphosate from soil can range from 3 to 174 days (WHO, 1994), depending on soil and climatic conditions.

Precipitation, soil composition, presence and absence of a soil constricting layer and drainage type may influence the leaching of glyphosate from soil. Field and laboratory studies indicate that glyphosate generally does not move vertically in the soil below the topmost six-inch soil layer (U.S. EPA, 1993).

Air

There are no data available on ambient air concentrations of glyphosate. Air concentrations during silvicultural spraying were mostly below 1.3 $\mu g/m^3$; the highest value observed was 15.7 $\mu g/m^3$ (Jauhiainen *et al*., 1991). Due to the low vapor pressure of the chemical, volatilization of glyphosate from a sprayed area is not expected to be significant. Inhalation of spray droplets by agricultural workers and residents living near agricultural fields can be an important exposure pathway.

Water

Glyphosate may enter water via runoff, from overspray, or from spray drift. In water, it adsorbs strongly to sediment and particulate matter in the water column. It may also form insoluble complexes with metal ions and precipitates. In water, glyphosate does not degrade readily. Under laboratory conditions, no appreciable degradation of glyphosate was observed in dechlorinated tap water via chemical, microbiological or photolytic processes 78 days, with or without aeration (Anton *et al.*, 1993). Sediment adsorption and biodegradation represent the major dissipation processes in aquatic systems (Goldsborough and Brown, 1989). Laboratory experiments showed that the rate of biodegradation varied, depending on the experimental conditions, e.g., availability of oxygen, temperature, and type of sediment. The time needed for 50 percent degradation of glyphosate in a test system with water and sediment was estimated to be less than 14 days under aerobic and 14-22 days under anaerobic conditions (WHO, 1994).

The half-lives of glyphosate in three forest ponds in Manitoba, Canada that were aerially sprayed in August were approximately 1.5 to 2 days; glyphosate was not detected in any sample by day 38 (Goldsborough and Brown, 1989). However in two field studies (Feng *et al*., 1990 and Monsanto, 1990a, as cited in WHO, 1994), it was noted that under certain conditions, glyphosate and its degradation product, AMPA, could persist in the pond sediment for up to one year.

The off-target movement of glyphosate had been studied (Smith *et al.*, 1996) in Newfoundland, Canada. A 2 percent solution of Roundup was sprayed evenly at the rate of about 11.4 to 13 L/hectare to a site called Massey Drive that was located on a fractured lime stonebed. Drinking water wells from the sprayed site were sampled at 1, 2 and 4 weeks after the first spray and at 1, 2, 4, 13 and 32 weeks after the second spray. Glyphosate was detected in well water at the Massey Drive site at levels ranging from 0.0072 to 0.045 mg/L. Levels peaked two weeks post-spray at 0.025 mg/L in well water and then dropped off to 0.004 mg/L by the fourth week of sampling. After the second

treatment, the concentration in the well increased to a maximum of 0.045 mg/L at seven weeks post-spray and again dropped off. This study showed that though glyphosate is known to adsorb strongly to soils, this factor alone did not prevent off-target movement of glyphosate on a limestone bed where the topsoil was replaced with gravel, and thus the potential for off-target movement of chemical was increased.

Food

Glyphosate is not absorbed by a plant's root system because of its strong adsorption to the soil. However, it is easily absorbed by leaves from spray residues and is translocated throughout the plants and fruits. Glyphosate is not metabolized to any significant degree in plant tissues (Ghassemi *et al*., 1982 as cited in NTP, 1992). Therefore, glyphosate concentration may increase in plants immediately after spray. Ingestion of sprayed food material or products from animals fed treated vegetation may lead to glyphosate exposure. Glyphosate residues in cattle, pig, and poultry meat, eggs, and milk were found to be negligible after the animals were fed a diet containing 100 mg/kg glyphosate and AMPA (WHO, 1994).

Bioconcentration factors are low in laboratory tests with invertebrates and fish. In one study, a bioconcentration factor of 0.5 was estimated in bluegill sunfish exposed to 11 to 13 mg/L for 35 days. Maximum glyphosate concentrations in the whole fish, viscera and fillet were 13, 7.6, and 4.8 mg/kg, respectively (ABC Inc., 1989 as cited in WHO, 1994).

In its dietary risk assessment based on a worst-case scenario, U.S. EPA (1993) concluded that the chronic dietary risk from food use is minimal. The calculated theoretical maximum residue contribution for the U.S. population is 0.025 mg/kg-day. The exposure for the most highly exposed subgroup, non-nursing infants less than one-year-old, is 0.058 mg/kg-day. The major dietary contribution is from wheat products. Though the U.S. EPA dietary risk assessment methods have changed since that time, the overall conclusions regarding dietary risk probably would not change.

Biomonitoring

A biomonitoring survey of 48 farmers and their family members who had potential exposure to glyphosate was reported by Acquavella *et al*. (2004). Composite urine samples (24-hr) of the farmer, the spouse and their children were collected the day before, the day of, and for three days after glyphosate application. It was reported that 60 percent of farmers had detectable levels of glyphosate in their urine on the day of application. The geometric mean concentration was 3 ppb, the maximum value was 233 ppb, and the highest estimated systemic dose was 0.004 mg/kg. For spouses, 4 percent had detectable levels in their urine on the day of application. Their maximum urine concentration was 3 ppb. For children, 12 percent had detectable glyphosate in their urine on the day of application, with a maximum concentration of 29 ppb. All but one of the children with detectable concentrations had helped with the application or were present during herbicide mixing, loading, or application.

METABOLISM AND PHARMACOKINETICS

The absorption of glyphosate from oral administration in various species is about 30 to 36 percent. In a single dose (5.6 or 56 mg/kg) study in F344 rats (NTP, 1992), 30 percent of the oral dose was absorbed. In a comparable study, after a single oral dose of 10 or 1,000 mg/kg body weight, 30 to 36 percent absorption was reported based on percentage excretion in the urine. The remaining total body burden was about 1 percent, which was widely distributed in the body but mainly associated with bone. Only a very small percentage (less than 0.2 percent) of the administered dose was expired as carbon dioxide. The results are summarized in Table 2 (Monsanto, 1988 as cited by WHO, 1994). The dermal absorption from a diluted Roundup herbicide in Rhesus monkeys was about 3.7-5.5 percent after 12 hours of exposure (Wester *et al.*, 1991).

Glyphosate is poorly metabolized in rats and most of the dose was excreted unchanged as the parent compound. AMPA is the only metabolite found in feces and accounts for 0.2 percent to 0.3 percent of a 10 mg/kg administered dose (Brewster *et al.*, 1991).

Table 2. Concentrations of C^{14} label (as mg Glyphosate-Equivalents/kg Fresh Weight) in Selected Rat Tissues 7 Days after a Single Oral Dose

	Dose: 10 mg/kg		Dose: 1,000 mg/kg	
	Male	**Female**	**Male**	**Female**
Blood	0.0045	0.0027	0.33	0.17
Liver	0.030	0.014	1.9	1.3
Kidney	0.022	0.013	1.9	1.4
Spleen	0.012	0.0073	2.6	3.0
Lung	0.015	0.012	1.5	1.1
Thyroid	0.00080	0.00036	1.5	1.2
Nasal mucosa	0.0050	0.023	1.7	1.8
Stomach	0.0080	0.0037	2.4	2.4
Small intestines	0.022	0.018	1.9	1.6
Colon	0.034	0.016	11.0	9.2
Bone	0.55	0.31	30.6	19.7
Bone marrow	0.029	0.0064	4.1	12.5

(Monsanto, 1988, as cited in WHO, 1994.)

After a single oral dose of glyphosate (10 or 1,000 mg/kg) to male and female rats, fecal elimination was 62-70 percent (at both doses) and excretion in urine was 14-18 percent (at the high dose) or 22-29 percent (at the low dose); less than 0.2 percent of the dose was expired as carbon dioxide (Monsanto 1988 as cited in WHO, 1994). The elimination data suggest a two-compartment model. At the 10 mg/kg dose level, the half-life for the α

phase was 5.9 to 6.2 hours and for the β phase was 79 to 106 hours. At 1,000 mg/kg, the half-life for the α phase was 5.3 to 6.4 hours and for the β phase was 181 (male rats) to 337 hours (female rates). Pretreatment with unlabelled compound for 14 days at the low dose level had no effect on whole body elimination rate.

In the National Toxicology Program (NTP) study, a single gavage dose of ^{14}C-labelled glyphosate (5.6 or 56 mg/kg) was given to male F344/N rats. Approximately 50 percent of the radioactivity at both dose levels was eliminated in the feces in the first 24 hours, and urinary elimination of radioactivity was essentially complete by 12 hours. More than 90 percent of the radioactivity was eliminated within 72 hours (NTP, 1992). When glyphosate was administered by intravenous injection at 5.6 mg/kg, the blood radioactivity vs. time plot fitted a two-compartment model with an α phase of about 0.5 hour and a β phase of 13 hours.

In lactating goats, excretion in milk was shown to occur to a minor extent. Concentration of glyphosate in whole milk was equal to or less than 0.1 ppm at a concentration of 120 ppm in diet (WHO, 1994).

TOXICOLOGY

Toxicological Effects in Animals

Acute Effects

The acute lethal dose (LD_{50}) of glyphosate in various species by different routes is given in Table 3. Glyphosate has very low toxicity by the oral and dermal routes, partly due to its limited absorption. It is significantly more toxic by the intraperitoneal (ip) route. The reported toxic effects following acute exposure were hyperemia, severe stress, accelerated breathing and occasional asphyxial convulsion.

Table 3. Acute Toxicity of Glyphosate in Experimental Animals

Species	Administration mode	LD_{50} (mg/kg)
Rat	oral	4,873
Rat	ip	235
Mouse	oral	1,568
Mouse	ip	130
Rabbit	oral	3,800
Goat	oral	3,500
Rat	dermal	>2,000
Rabbit	dermal	>5,000

(Adapted from NTP, 1992; WHO, 1994.)

Most studies reviewed by WHO (1994) reported that the LD_{50} of glyphosate is at or above 5,000 mg/kg. In a study by Knapek *et al.* (1986 as cited in WHO, 1994), a commercial product containing glyphosate showed a LD_{50} of 2,047 mg/kg. Several acute toxicity studies using Roundup branded herbicides indicated that its LD_{50} is at or above 5,000 mg/kg, and the LD_{50s} of other products such as Sting® and Legend® are approximately 2,000 mg/kg (WHO, 1994).

Dermal and Ocular Effects

Glyphosate technical and Shackle®, at various concentrations, were tested for eye irritation in rabbits. Slight irritation was reported in some animals, and the irritation disappeared after a day or more (Monsanto, 1971, 1975, and 1979a; Branch *et al*., 1983). Glyphosate was not found to be a strong dermal irritant. Several irritation studies using rabbit intact or abraded skin showed glyphosate produced a relatively low response (Monsanto, 1979b and 1979c). When a formulated glyphosate was tested at a concentration five-fold higher than the normal field application level, severe local skin reaction, reduced food consumption, body weight loss, mortality, and testicular effects were observed (Heydens, 1988).

Subchronic Effects

Glyphosate (purity 98.7 percent) was administered in the diets of CD-1 mice for 90 days at levels of 5,000, 10,000 or 50,000 ppm (calculated to be 940, 1,890, and 9,710 mg/kg-day in males and 1,530, 2,730, and 14,860 mg/kg-day in females). Liver weights were increased at 10,000 and 50,000 ppm and growth retardation and increased organ weights of brain, heart and kidney were observed at 50,000 ppm (Monsanto, 1979d as cited by WHO, 1994). The authors concluded that the NOAEL was 10,000 ppm.

In a 90-day study, Sprague-Dawley rats were administered glyphosate at 1,000, 5,000 or 20,000 ppm in the diet (calculated to be 63, 317, and 1,267 mg/kg-day in males and 84, 404, and 1,623 mg/kg-day in females). No toxic effects were observed. Hematology, blood chemistry, and organ weights were not affected by the treatment. Limited histopathology revealed no adverse effect in any tissue that was examined. The NOAEL from this study was 20,000 ppm (1,267 mg/kg-day) (Monsanto, 1987 as cited by WHO, 1994).

Glyphosate was administered in the diets of 10 F344N rats or $B6C3F_1$ mice per sex per dose for 13 weeks at concentrations of 0, 3,125, 6,250, 12,500, 25,000 or 50,000 ppm. Ten additional rats per sex were included for evaluation of hematology and clinical pathology parameters (NTP, 1992). In the rats, reduced weight gain was observed in males in the 25,000 (males only) and 50,000 ppm groups (males and females). The treatment had no effect on survival of both sexes. The final body weight of the males in the highest dose group was about 18 percent less than controls. In female rats, only a slight (5 percent) reduction in body weight was observed at the highest dose level. In males, there were slight increases in relative weights of liver at $\geq$ 3,125 ppm, kidney and testes at $\geq$ 25,000 ppm, and a decrease in thymus weight at 50,000 ppm. In females, changes in organ weights were minor and could not be related definitely to treatment. Of

the hematological parameters, there was a mild increase in hematocrit and red blood cell (RBC) count at ≥ 12,500 ppm, hemoglobin at ≥ 25,000 ppm, and platelets at 50,000 ppm. In female rats, significant increases were observed in lymphocytes at ≥ 25,000 ppm and platelet counts at ≥ 3,125 ppm, white blood cells (WBC) at ≥ 12,500 ppm, mean corpuscular hemoglobin (MCH) at 50,000 ppm, and mean corpuscular volume (MCV) at 50,000 ppm.

The changes in clinical chemistry parameters included an increase in alkaline phosphatase at ≥ 6,250 ppm in male and at ≥ 12,500 ppm in female rats. Alanine aminotransferase activity was also increased in both sexes. NTP (1992) noted that these findings likely reflect hepatocellular leakage or single cell necrosis and cholestasis. Increases in absolute and relative liver weights in male rats also indicate the effect of glyphosate on the liver. A significant decrease (20 percent) was observed in sperm density in the 25,000 and 50,000 ppm dose groups. The only histopathological changes found were cytoplasmic alterations in the parotid and submandibular salivary glands of male and female rats. These lesions consisted of basophilic changes and hypertrophy of acinar cells. The magnitude of the effect was dose-dependent in both sexes. Because the effects on the salivary glands were observed at all dose levels, no NOAEL was identified.

In mice, the treatment had no effect on survival of either sex. Body weight gains of male and female mice were depressed at the two highest doses. Increased organ weights of heart, kidney, liver, thymus and testes were not dose-dependent and were not considered compound-related. No effects were observed on sperm motility. Pathological changes in salivary glands were similar to rats but were not observed at the lowest level of 3,125 ppm in the diet (calculated to be 507 mg/kg-day in male and 753 mg/kg-day in female mice). Therefore, the NOAEL for glyphosate in mice appears to be 507 mg/kg-day. The salivary gland lesions were similar to those induced by exposure to high subcutaneous doses of the β-adrenergic agonist isoproterenol and could be partially ameliorated with the β-adrenergic antagonist propanolol. These data suggest that glyphosate may induce the salivary gland lesions by acting as a weak adrenergic agonist (NTP, 1992).

Glyphosate (96 percent) was administered orally by capsule at 0, 20, 100 or 500 mg/kg-day to six beagle dogs per sex per dose for 52 weeks (Monsanto, 1985). No adverse effects occurred with respect to clinical signs, body weight, ophthalmoscopy, hematology, blood chemistry, gross pathology, and histopathology. Changes in pituitary weights (absolute and relative) in the males dosed at 100 or 500 mg/kg were noted. The authors suggested that because there were no concomitant histological changes in pituitaries and similar findings were not observed in other animal studies, the toxicological significance of the change in pituitary weights is questionable; they concluded the NOAEL to be the highest dose tested of 500 mg/kg-day. In its evaluation of the toxicity of glyphosate, California Department of Pesticide Regulation concurred with this interpretation.

In a dermal study, glyphosate at levels of 100, 1,000 or 5,000 mg/kg-day was applied to shaven intact or abraded skin of rabbits for six hours/day, five days/week for three weeks. No effect on survival and growth occurred. At the high dose, a slight erythema and edema was observed in intact and abraded skin. No evidence of systemic toxicity was found (IRDC, 1982 as cited by WHO, 1994).

Chronic Effects and Carcinogenicity Studies

Rat

Glyphosate (98.7 percent) was administered in diet to Sprague-Dawley rats (50 per sex per group) for 24 months at approximately 0, 3.1, 10.3 or 31.5 mg/kg-day for male and 0, 3.4, 11.2 or 34 mg/kg-day for female rats (Bio/Dynamics, Inc., 1981a; Monsanto, 1984). Survival, hematology, blood chemistry, urinalysis, and organ weights were not affected by the treatment. The systemic NOAEL for this study was estimated to be 31.5 to 34 mg/kg-day.

C-cell carcinoma of the thyroid was increased in the 34 mg/kg-day female group (1/47 in the control and 6/47 in the high-dose group) (Monsanto, 1984). However, the authors argued that the finding might not be treatment related because the incidence of hyperplasia and adenoma of the thyroid was greater in the control females than in the high-dose females. Due to the difficulties in differentiating c-cell adenoma from carcinoma, Monsanto argued that one should not compare the incidence of animals bearing only C-cell carcinoma, but should instead compare the combined incidence of animals bearing either C-cell adenoma or carcinoma. The incidence of females with either a thyroid C-cell adenoma or carcinoma is similar for the control and high-dose groups (6/47 and 9/47, respectively). Furthermore, there is no dose-response relationship in terms of females bearing thyroid C-cell adenoma or carcinoma (6/47, 3/49, 8/50, 9/47 for the control, low-, mid-, and high-dose groups, respectively).

A statistically significant increase in interstitial cell tumors of the testes was found in the high-dose males, compared to concurrent controls (incidences: 0/50, 3/50, 1/50, and 6/50; historical control range: 3-7 percent) (Bio/Dynamics, Inc., 1981a). However, this tumor is known to be age-related and primarily occurs in older rats. It has been pointed out that survival of control males was lower than that of high-dose males; the mean survival time of control males (660 days) was shorter than that of the high-dose males (732 days). Also, the significance of this result has been questioned because a similar effect was not observed in a more recent two-year rat study at much higher doses (see the study below).

Glyphosate (purity 96.5 percent) was administered to Sprague-Dawley rats (60 per sex per group) for 24 months at concentrations of 0, 2,000, 8,000 or 20,000 ppm in diet (calculated to be 0, 100, 410, and 1,060 mg/kg-day) (Monsanto, 1990c). The highest dose was considered close to the maximum tolerated dose. An additional 10 rats per sex per group were included for one-year interim sacrifice. No change in survival or appearance was noted in the treated animals. Statistically significant reduction in body weight gain was observed in the high dose female rats. There was a significant increase in the incidence of basophilic degeneration of the posterior subcapsular lens capsule fibers in the eye of male rats in the highest dose group; however, the finding was within the historical control range. No changes were observed in hematology and blood chemistry. Liver weight was also increased in male rats of the highest dose group. No other statistically significant changes in organ weights occurred in a dose-related manner.

There was a statistically significant increased incidence of inflammation of the gastric squamous mucosa in the medium- and high-dose females (0/59, 3/60, 9/60, and 6/59 for the control, low-, mid-, and high- dose groups, respectively; historical range: 0-13.3

percent). Though a similar increase was also observed in males, the increase was not statistically significant (2/58, 3/58, 5/59, and 7/59 for the control, low-, mid-, and high-dose groups, respectively). The lesions were not considered neoplastic by Monsanto (1990c). Because there was no dose-related trend across the female groups and no significant difference among the males, it is questionable if the finding was treatment-related.

There was a statistically significant increase in the incidence of pancreatic islet cell adenomas in the low- and high-dose males (incidences: 1/58, 8/57, 5/60, and 7/59; historical control range: 1.8-8.5 percent). The incidence in the control group was below the historical control range, and the trend test for this tumor was negative. Furthermore, there was no evidence of dose-related pancreatic damage or preneoplastic lesions. One pancreatic islet cell carcinoma was found in a control male, but none was found in the dosed males. No significant increase in this lesion was observed in females (5/60, 1/60, 4/60, and 0/59 for the control, low-, mid-, and high-dose groups, respectively) (Monsanto, 1990c). A modest incidence of a relatively uncommon tumor type (adrenal cortical carcinoma) was found only in the highest dosed females (3/50, none in other groups of either sex). Though the trend test is positive, the increased incidence in the highest-dosed female could be by chance. The biological significance of this finding is unknown.

The NOAEL for this study was estimated to be 8,000 ppm (equal to 410 mg/kg-day) for the reduction in female body weight gain, cataractous lens changes in males, and increased liver weights in males at the highest dose (20,000 ppm).

Mouse

Glyphosate (purity 99.7 percent) was administered for 24 months in the diet of 50 CD-1 mice per sex per dose at concentrations of 0, 1,000, 5,000 or 30,000 ppm (calculated to be 0, 157, 814 and 4,841 mg/kg-day for males and 0, 190, 955 and 5,874 mg/kg-day for females) (Bio/Dynamics 1983). There was a slight decrease in the mean body weights of male mice in the highest dose group.

At the highest dose, a number of adverse liver and kidney effects were reported: central lobular hepatocyte hypertrophy in males, central lobular hepatocyte necrosis in males, chronic interstitial nephritis in males, and proximal tubule epithelial basophilia and hypertrophy in females. In addition, increased incidences of epithelial hyperplasia (thickening) in the urinary bladder were observed in male mice in the mid and highest dose groups (incidences: 3, 3, 10, and 8 for the control, low, mid, and high exposures, respectively)(Bio/Dynamics Inc., 1983; DPR, 1992). The increased epithelial thickening was described as minimal to mild. The report suggested that although the incidence was increased in mid and high dose males, the observed changes might not be related to the treatment.

Bronchiolar-alveolar lung tumors, hepatic tumors, and tumors of the lymphoreticular system were responsible for the majority of tumors observed in the study. No clear dose-response relationships were noted for these tumors.

Renal tubule adenoma and carcinoma incidence was increased in the high-dose male group (1, 0, 1, and 3 for the control, low, mid, and high dose, respectively). After reviewing the data, the FIFRA Scientific Advisory Panel noted that age-adjusted tumor incidence data did not demonstrate a statistically significant increase based on concurrent controls; nevertheless the incidence in the highest dosed males was statistically significant compared to historical controls (DPR, 1992).

Genetic Toxicity

Glyphosate was mostly negative in *in vivo* and *in vitro* test systems evaluating gene mutation, chromosomal aberration and DNA damage. By the weight-of-evidence, glyphosate is considered to be neither genotoxic nor clastogenic.

Though most of the tests show glyphosate is not genotoxic, a number of positive results have been reported in the literature. Bolognesi *et al*. (1997) first reported that glyphosate increased sister chromatid exchange in human lymphocytes *in vitro*. This finding was supported by two other *in vitro* studies reported by Lioi *et al*. Lioi *et al*. (1998a) showed that glyphosate increased chromosomal aberration and sister chromatid exchange in human lymphocytes above 1.4 mg/L; similarly, they also reported that glyphosate increased chromosomal aberration and sister chromatid exchange in bovine lymphocytes above 2.9 mg/L (Lioi *et al*., 1998b). Lioi *et al*. found glyphosate at these levels caused increased oxidative stress as well as reduced glutathione level in the lymphocytes, and these events might have contributed to the observed genotoxicity of the compound.

Bolognesi *et al*. (1997) administered glyphosate by intraperitoneal injection (at 2 x 150 mg/kg) to three male mice and found the chemical increased micronuclei in bone marrow cells. Negative results have been reported by NTP (1992) and Rank *et al*. (1993). The discrepancies may be explained by the different exposure routes and the difference in dosage. Bolognesi *et al*. (1997) found that glyphosate at 300 mg/kg by intraperitoneal injection increased DNA damage in mice liver and kidney tissues. Furthermore, they found this treatment also increased oxidative damage in the liver but not in the kidney. It should be noted that the dose used in the studies reported by Bolognesi *et al*. was very high, as the estimated intraperitoneal LD_{50} of glyphosate in mouse is only 130 mg/kg (see Table 3).

Teratogenicity

Glyphosate (purity 98.7 percent) was administered by gavage at levels of 0, 300, 1,000 or 3,500 mg/kg-day to female COBS CD rats on days 6 to 19 of gestation. In the highest dose group, a statistically significant decrease in viable fetuses and mean fetal body weight were noted. The highest dose was also toxic to the dam, because it reduced mean maternal body weight gain and caused early death in several animals. The maternal and developmental toxicity NOAELs were 1,000 mg/kg-day (IRDC, 1980a).

Glyphosate technical (98.7 percent) was administered by gavage to 16 female Dutch Belted rabbits per dose at 0, 75, 175 or 350 mg/kg-day on days 6 to 27 of gestation (IRDC, 1980b). The control group received the vehicle only, 0.5 percent aqueous

Methocel®, on a comparable regimen. Cesarean sections were performed on all surviving females on gestation day 28.

No treatment-related abnormal clinical signs were observed in rabbits dosed at 75 mg/kg-day. A slight increase in the incidence of soft stools and diarrhea was noted in the 175 mg/kg-day group and a definite increase in these signs and nasal discharge were noted in the 350 mg/kg-day group, compared to the controls. The mean maternal body weight gain for each dosed group was comparable to that of the control group. Early mortality was reported in the highest dose group (0, 1, 2, and 10 for the control, low, mid, and high doses, respectively). Causes of death were determined for five of the rabbits dying prior to the scheduled sacrifice; they were pneumonia, respiratory disease, enteritis or gastroenteritis. Causes of death for the other eight rabbits could not be determined at necropsy. Two rabbits in the control group and one each in the 175 and 350 mg/kg-day groups aborted and were sacrificed.

The researchers found no biologically meaningful differences in mean number of viable fetuses, early or late resorptions, total implantations, corpora lutea, fetal body weights, the fetal sex distribution, or the number of fetuses or litters with malformations in any of the treatment groups compared to the control group. The number of fetuses and litters with developmental and genetic variations were also comparable for all groups. A slight decrease was noted in mean fetal body weight of all treated groups compared to the controls. However, mean fetal body weights for all groups were comparable to historical control mean fetal body weight values (IRDC, 1980b).

The maternal NOAEL in this study was 175 mg/kg-day. At the highest dose (350 mg/kg-day), there was a significant increase in early mortality (10/16 in the highest dose group versus 0/16 in the control; Fisher exact test, $p = 1.24 \times 10^{-4}$).

Daruich *et al*. (2001) exposed pregnant rats (eight/group) to glyphosate in drinking water at 0, 0.5 or 1 percent w/v throughout the gestation period. On gestation day 21, fetuses were removed and weighed. Maternal and fetal livers, hearts, and brains were also isolated and processed for enzymatic activity analyses. They reported that rats exposed to glyphosate had decreased water and food ingestion. In the high dose group, there was a significant decrease of maternal body and liver weights compared to the controls. However, there were no differences in fetal body weights. Exposure to glyphosate appeared to affect many enzymes in various organs, although a dose-response relationship was not always observed. For comparison purposes, Daruich *et al*. studied the effect of low water and low food intake in another group of pregnant rats and found there were no significant differences in the enzymatic activities, compared to the control group. They therefore attributed the observed changes in the enzyme activities to the effects of glyphosate.

Dallegrave *et al*. (2003) studied the teratogenic effects of Roundup (consisting of 360 g/L glyphosate and 18 percent (w/v) polyoxyethyleneamine) to Wistar rats. Sixty pregnant rats were divided into 4 groups. The control group received distilled water and the treatment groups received 500, 750, or 1,000 mg/kg-day glyphosate diluted in water. The dosing regimen was based on the NOAEL of 1,000 mg/kg-day for developmental toxicity in rats reported by Williams *et al*. (2000). The rats were treated by gavage from days 6 to

15 of pregnancy, defined as the critical period for the embryonic structural development in rats.

Dallegrave *et al*. (2003) found that Roundup was more toxic than glyphosate. At 1,000 mg/kg, 50 percent of the dams died between day 7 and 14 of pregnancy. In the study reported by IRDC (1980a), no significant fatality was noted in pregnant rats treated with 3,500 mg/kg-day on days 6 to 19 of gestation. Among the dams that survived the treatment, the authors found no significant differences in total weight gain and relative weight of the organs. The number of fetuses, corpora lutea, implantation sites and embryo resorption was similar for all groups.

Concerning the fetal variables, Dallegrave *et al*. (2003) found no significant difference among the groups in terms of weight, male:female sex ratio, and external malformation rate. However, they reported that the total percentage of skeletal alterations was significantly increased ($P<0.001$, χ^2-test) in all the groups exposed to Roundup, compared with control, with a clear dose-response relationship. The percentage of altered fetuses was 15.4, 33.1, 42.0, and 57.3 for the control and the 500, 750, and 1,000 mg/kg-day groups, respectively. The most frequent skeletal alterations observed were incomplete skull ossification and enlarged fontanel. The occurrence of multiple alterations was also significantly higher in the treated groups compared with the control, but did not show a dose-response relationship. Because Roundup and not glyphosate was the test material in this study, it is possible that the surfactant, polyoxyethyleneamine, in the commercial formulation might have contributed to the observed teratogenicity.

Reproductive Toxicity

Glyphosate (purity 98.7 percent) was administered to CD rats at doses of 0, 3, 10 or 30 mg/kg-day for three successive generations (Bio/Dynamics Inc, 1981b). The diet was prepared weekly during various growth periods and adjusted to achieve the desired dose levels. Groups of 12 males and 24 females F_0 rats were administered test diets for 60 days. Treatment continued through mating, gestation and lactation for two successive litters (F_{2a} and F_{2b}). Groups of 12 males and 24 females were retained at weaning from the second litters of each dose level as parental animals for the succeeding generation.

Early mortalities appeared unrelated to dose and were not considered to be treatment-related. Adult body weights and food consumption during growth, rest, gestation or lactation were comparable between all treated and control groups for all generations. For the entire study, no consistent, dose-related effect was seen in mating, fertility or pregnancy indices to indicate an adverse effect of treatment. The mean liver to body weight ratios of the F_{2b} parental females for all treated groups were significantly lower than the control values. Slightly reduced liver to brain weight ratios also were noted for all treated groups. These differences did not show a dose-response relationship and similar effects were not observed in treated parents from previous generations and no microscopic lesions attributed to treatment were observed in hepatic tissues.

The report concluded that gross necropsy and histopathologic evaluations did not reveal any evidence of effects related to treatment. However, it has been noted that there was an

increased incidence of unilateral renal tubular dilation in the male pups of the F_{3b} generation at the highest dose (Bio/Dynamics Inc, 1981b).

In a two-generation study, glyphosate was administered to CD rats at 0, 2,000, 10,000 or 30,000 ppm in the diet (calculated to be 0, 150, 720 and 2,200 mg/kg-day for the F_0 animals) for 11 weeks before they were mated to produce the F_1 generation. Litters were culled to 8 pups on lactation day 4 and weaned on lactation day 21. At the time of weaning, 30 F_1 rats/sex/group were randomly selected to continue on the study as parental F_1 animals. Following an approximate 14-week period, these animals were mated twice to produce F_{2a} and F_{2b} generations (Monsanto, 1990b).

The F_0 and F_1 male and female adults had reduced body weights (8 to 11 percent) in the highest dose group. Mating, pregnancy, and fertility indices were not affected by the treatment in both F_0 and F_1 animals. On lactation day 0, the average litter size of high-dose F_0 dams was approximately 2 pups less than controls, and a smaller difference (approximately 1 pup/litter) was noted after the first F_1 mating. However, these differences were not statistically significant and there was no increase in the number of dead pups/litter. No treatment-related decrease in litter size was observed in the F_{2b} generation (Monsanto, 1990b).

Postnatal pup survival was not changed by the administration of glyphosate in all three groups (F_1, F_{2a} and F_{2b}). Body weights of some high dose offspring were 4 to 11 percent below controls on lactation day 14. This effect was more pronounced on lactation day 21, as body weights were reduced 11 to 19 percent in all offspring groups. Smaller reduction in body weight (5.6 to 6.6 percent) was noted in some mid-dose offspring on lactation day 21. However, significant body weight decreases were not observed in these animals before or after lactation day 21. The authors of the report did not consider the body weight decreases in mid-dose pups to be treatment-related as they were small, transient, and did not occur consistently in both sexes from all litters (Monsanto, 1990b).

There were no gross or microscopic pathology changes in parents or offspring attributed to the treatment. In a previous developmental toxicity study, 10 pups/sex/generation were examined, and focal renal tubular dilation was noted in the high dose (30 mg/kg-day) male offspring from the last generation. In this study, the high dose level was 30,000 ppm (approximately 2,200 mg/kg-day), and several more offspring were examined (1/sex/litter). No treatment-related renal effect was found, indicating that the previous finding may not be related to glyphosate exposure.

Based on the reduced body weights in adults and pups observed in the high dose group, the NOAEL in this study was estimated as 10,000 ppm in the diet (720 mg/kg-day) (Monsanto, 1990b).

Yousef *et al*. (1995) studied the effects of glyphosate on semen characteristics in rabbits. Glyphosate was given orally in gelatin capsules to four male New Zealand white rabbits per dose at levels of 0, 1/100 LD_{50}, or 1/10 LD_{50} daily for six weeks. A preliminary six-week evaluation period was followed by a six-week treatment period, followed by a six-week recovery period without pesticide administration. The animals were weighed and semen collected weekly throughout the 18-week period. Semen volume, fructose level in semen, semen osmolarity, sperm concentration and live, dead and abnormal spermatozoa were evaluated. The authors concluded that glyphosate treatment reduced body weight,

ejaculate volume and sperm concentration and increased abnormal and dead sperm at both dose levels. The adverse effects continued into the recovery period. Actual dose or LD_{50} values were not given in the paper, and dose-response relationship cannot be characterized.

In an *in vitro* system, Walsh *et al*. (2000) showed that Roundup decreased steroidogenesis in mouse Leydig tumor cells and has the potential to impact the production of testosterone. However, the researchers also found that glyphosate alone did not alter steroid production in the test system. They postulated that other components of the Roundup formulation are required to disrupt steroidogenesis.

Toxicological Effects in Humans

Case Studies and Human Clinical Studies

A number of studies have reported clinical observations in patients who ingested relatively large quantities of glyphosate surfactant mixtures. Some of these cases were suicide attempts and others were accidents. The importance of these results to environmental exposure is limited because the exposures were many times higher than what is likely to be encountered in the environment and the toxicity of glyphosate might have been increased by the presence of surfactants (Sorensen and Gregersen, 1999; Dallegrave *et al*., 2003).

Talbot *et al.* (1991) reported a number of cases of acute intoxication (suicide attempts) with herbicides containing glyphosate. The reported acute symptoms were: sore throat, dysphagia, gastrointestinal hemorrhage, and erosion of the gastrointestinal tract. Other less commonly affected organs were lung, liver, kidney and the central nervous system. The estimated amount of Roundup (41 percent glyphosate) ingested by non-survivors was 184 +/- 70 mL (range 85 to 200 mL). Most of the deaths occurred within a few hours of the herbicide ingestion. In another study, Tominack *et al.* (1991) estimated a dose of 120 +/-112 mL in survivors and 263 +/-100 mL for non-survivors of suicide attempts. The most common reported symptoms in this study were irritation of mucous membrane and gastrointestinal tract. Minor reported effects were pulmonary dysfunction, metabolic acidosis, hypotension, leukocytosis and fever. The high concentrations of both glyphosate and its constituent surfactant in the formulated product in the suicide cases are not anticipated in drinking water.

Hung *et al*. (1997) studied 53 patients with known ingestion of a glyphosate-surfactant pesticide (Roundup) and found the occurrence and severity of laryngeal injury may be an important factor in determining the degree of morbidity and mortality. They suggested that the surfactant (POEA) rather than glyphosate was the likely cause of the observed acute toxicity. It is also possible that POEA and glyphosate potentiate each other's toxicity. In a similar study, Chang *et al*. (1999) reported that the severity of esophageal injuries in patients exposed to glyphosate-surfactants was associated with increased white blood cell count, length of hospital stay, and the occurrence of serious complications. They suggested the severity of esophageal injuries might be used as a prognostic factor in giving treatments.

Lin *et al*. (1999) reported a case of glyphosate-induced cardiogenic shock in a young man who drank approximately 150 mL of glyphosate with surfactant. It is not clear what was the mechanism of this health effect.

Sorensen and Gregersen (1999) reported two cases of lethal intoxication with the herbicide glyphosate-trimesium (Touchdown). They reported a 6-year-old boy and a 34-year-old woman died within minutes after oral ingestion of the pesticide. The post-mortem examination revealed pulmonary edema, cerebral edema, and dilated right atrium and ventricles of the heart, in addition to some of the symptoms described above. The authors speculated that the surfactant, trimethylsulfonium, in the Touchdown might facilitate the absorption after oral ingestion. Round-Up was identified as the probable toxic agent in the suicide of a California woman in 2005 (DPR, 2007a).

Barbosa and Leite (2001) reported that a 54-year old man accidentally exposed to glyphosate developed disseminated skin lesions 6 hours after the accident. One month later, the subject developed a symmetrical Parkinsonian syndrome. The researchers acknowledged that it is not possible to exclude the coincidence of the illness with exposure to glyphosate, and the magnetic resonance imaging findings were not compatible with Parkinson's disease.

In two dermal irritation studies, diluted and undiluted Roundup solutions were applied to intact or abraded skin sites of volunteers. Using the undiluted solution, Maibach (1986, as cited in WHO, 1994) found erythema in 1/24 subjects for the intact skin sites and erythema in 10/24 subjects for the abraded skin sites. The researcher also noted that 4/24 subjects showed an equivocal reaction. However, some glyphosate products are in toxicity category I and II for primary eye irritation and dermal irritation, based on animal testing of the formulations. Applicator exposures to glyphosate formulations have resulted in many reports of minor skin and eye irritation (U.S. EPA 1993; Bradberry *et al.*, 2004). Glyphosate is among the more common pesticides named in pesticide illness reports in California (DPR, 2007b).

Ecological and epidemiological studies

Goldstein *et al*. (2002) reviewed illnesses reports related to glyphosate exposure for the years 1982-1997. Using the data in the California Environmental Protection Agency Pesticide Illness Surveillance Program, they found most of the cases involved topical irritation of the eye, skin, upper airway or combinations of these sites. They noted 187 cases out of a total of 815 reported illnesses also included systemic symptoms, such as nausea, vomiting, diarrhea, headache, and fever. According to Goldstein *et al*, 140 cases were classified as "possibly" related to exposure and the remaining 47 cases as having probably or definite relationship to exposure. Of the 47 cases, Goldstein *et al*. found only 22 cases as probably or definitely related to glyphosate exposure alone.

Hardell *et al*. (2002) studied the association between exposure to pesticides and non-Hodgkin's lymphoma or hairy cell leukemia in a case-control study. They matched each of 563 Swedish patients diagnosed during 1987-1990 with two or four controls obtained from the general population, and evaluated previous pesticide use over many years with a questionnaire. They reported a significant association for glyphosate (odds ratio of 3.04,

95 percent confidence interval 1.08-8.52). The data set is weakened by the fact that there were only 8 glyphosate-exposed cases, as well as the potential for recall bias in this type of study.

Arbuckle *et al*. (2001) studied the association of pesticide exposure with spontaneous abortion in 2,110 farm couples in Ontario, Canada. Women (44 years old or younger) were asked to recall all their pregnancies, including spontaneous abortions. The study involved a total of 3,936 pregnancies and 395 spontaneous abortions. The researchers obtained pesticide exposure information from the farm operator and the couple to construct a history of monthly agricultural and residential pesticide use. Among the many pesticides investigated, Arbuckle *et al*. found that preconception exposure (3 months before and up to the month of conception) to glyphosate increased the risk of both early (<12 weeks) and late (12-19 weeks) spontaneous abortions (crude odd ratio = 1.4 (95 percent confidence interval 1.0-2.1). The researchers cautioned that the data should be interpreted with care because of several limitations. Dose information was not available and misclassification of exposure is possible. Due to the different ways pesticides were handled and used, there could be significant variability in the degree of exposure among the study population. Also, due to the nature of the study, recall bias and interaction between two or more pesticides might have affected the results.

Savitz *et al*. (1997) used the Ontario Farm Family Health Study data to investigate the relationship between male farm activities and reproductive outcomes such as miscarriage, preterm delivery, and small-for-gestational-age births. The combination of engaging in pesticide activities and reported use of specific chemicals produced some elevated risk estimates. For instance, crop herbicide activity combined with glyphosate yielded an odds ratio of 2.4 (after adjustment for a number of characteristics of the mother). However, the lack of reliable exposure information, the potential of recall bias, and the small number of exposed cases (5) make the interpretation difficult.

A similar study was reported by Garry *et al*. (2002). The researchers conducted a study in 1997-1998 of 695 families and 1,532 children in Minnesota that used pesticides for farming. The subjects were interviewed by phone and by written questionnaire. Parent-reported reproductive health information was confirmed through birth certificate and medical records examination. The researchers investigated the association between pesticide usage and birth defects identified in the first year of life and later. Inclusion of children diagnosed with birth or developmental disorders within the first 3 years of life and later led to a rate of 47.0 per 1,000 (72 children from 1,532 live births). Garry *et al*. reported a tentative association between attention-deficit disorder/attention-deficit hyperactivity disorder and use of glyphosate (an odds ratio of 3.6, 95 percent confidence interval 1.3-9.6), as well as an increased odds ratio (2.48, 95 percent confidence interval 1.2-5.1) for adverse neurologic and neurobehavioral developmental effects among children born to applicators of the fumigant phosphine. However, small number of subjects, exposures to multiple chemicals, difficulties in diagnosis, and the possibility of recall bias limit the interpretation of this study. The researchers also noted that there is little evidence of neurotoxicity of glyphosate other than by intentional ingestion.

De Doos *et al*. (2005) reported a study on cancer incidence among glyphosate applicators in the U.S. They evaluated data in the Agricultural Health Study, a prospective cohort

study of 57,311 licensed pesticide applicators in Iowa and North Carolina. Detailed information on pesticide use and other factors was obtained from a self-administered questionnaire completed at time of enrollment (1993-1997). Incident cancers were identified for the time period from the date of enrollment until 31 December 2001. Among private and commercial applicators, 75.5 percent reported having ever used glyphosate. The authors found glyphosate exposure was not associated with cancer incidence overall or with most of the cancer subtypes that were studied. There was a suggested association with multiple myeloma incidence that should be followed up as more cases occur in the future. However, potential bias in subject selection, small number of cases, known association of multiple myeloma with farming occupation, and the possibility of some unknown confounders decrease the confidence in the result.

DOSE-RESPONSE ASSESSMENT

Carcinogenic Effects

In 1985, glyphosate was first classified as a Group C carcinogen (possible human carcinogen) based on an inadequate rat carcinogenicity study (high dose less than the maximum tolerated dose) and an equivocal renal tumor response in a mouse carcinogenicity study. U.S. EPA re-examined the mouse renal tumor slides and changed the glyphosate classification to Group D (not classifiable as to human carcinogenicity) in 1986. However, U.S. EPA required the registrant to repeat the rat study because of the equivocal cancer toxicity data. Following review of the new rat study, U.S. EPA's peer review committee classified glyphosate as a Group E chemical (evidence of noncarcinogenicity) because the tumors observed (pancreatic islet and thyroid C cell adenomas in rats and renal epithelial cell hyperplasia in mice) were not considered to be compound-related and the studies of glyphosate genotoxicity were negative (Fed Reg, 1997). In its 2004 review of the toxicity of glyphosate, WHO (2004) found the chemical has no genotoxic potential and there is no evidence of carcinogenicity in rats or mice. Therefore, no dose-response assessment was conducted for glyphosate carcinogenicity in developing the PHG.

Noncarcinogenic Effects

In the absence of adequate human data, a reference dose (RfD) is generally calculated by U.S. EPA from the most sensitive endpoint in a long-term mammalian toxicology study. An RfD, as defined by the U.S. EPA, is an estimate of a daily exposure to the human population that is likely to be without appreciable effect. It is calculated by dividing a NOAEL by an uncertainty factor (UF). A factor of 100 is used as the default, representing one factor of 10 to account for the extrapolation of animal data to humans and another factor of 10 to account for human variability in susceptibility to toxic chemicals.

The U.S. EPA RfD of 0.1 mg/kg was based on the three-generation rat reproduction study (Bio/Dynamics Inc., 1981b) with a NOAEL of 10 mg/kg and an UF of 100. The

NOAEL was based on renal tubular dilation in F_{3b} pups at the next higher dose of 30 mg/kg. This RfD is the basis for U.S. EPA's drinking water equivalent level (U.S. EPA, 1992a) and the current Maximum Contaminant Level Goal (MCLG) and MCL (U.S. EPA, 1996) of 700 ppb. In an earlier California risk assessment, the Department of Health Services (DHS) used the same RfD and critical study in calculating a proposed California MCL (PMCL) (DHS, 1989).

In a more recent two-generation rat reproduction study (Monsanto, 1990b), no histopathological effects on kidneys of F_{2b} pups were observed at a much higher dose level (30,000 ppm in diet). The NOAEL from this study was 10,000 ppm (approximately 720 mg/kg-day) based on decreased body weights and soft stool in the next higher dose group. Therefore, the results from this study suggest that the renal changes in the three-generation rat reproduction study were not compound-related. In addition, other toxicity studies do not support that the renal effects are compound-related.

U.S. EPA's most recently-developed RfD of 2 mg/kg (Fed Reg, 1997) is based on a maternal NOAEL of 175 mg/kg and an UF of 100 in a rabbit study (IRDC, 1980b). The NOAEL is based on maternal mortality at the next higher dose. A recent review of glyphosate considered the rabbit teratology study with a NOAEL of 175 mg/kg-day as the appropriate basis for toxicological evaluation in humans (WHO, 1994). The RfD of 2 mg/kg-day used by the U.S. EPA Office of Pesticide Program is also based on this study.

The OEHHA evaluation has also concluded that the rabbit teratology study of IRDC (1980b) provides the most appropriate endpoint for our risk assessment for glyphosate in drinking water. The maternal NOAEL in this study was 175 mg/kg-day. At the highest dose (350 mg/kg-day), there was treatment-related diarrhea, nasal discharge and early mortality. No teratological effects or other significant toxicity was observed in offspring.

CALCULATION OF PHG

For estimation of a health-protective concentration of glyphosate in drinking water, an acceptable daily dose of the chemical from all sources will first be 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 acceptable daily dose (ADD) 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.

For glyphosate, the no-observed-adverse-effect-level of 175 mg/kg-day for diarrhea and increased maternal mortality from the IRDC (1980b) rabbit teratology study is used. The combined uncertainty factor is 1,000, which includes 10-fold for inter-species variation, 10-fold for human variability and 10-fold for the severity of the endpoint (mortality) and the short exposure duration. Thus,

$$\text{ADD} = \frac{175 \text{ mg/kg-day}}{1{,}000} = 0.175 \text{ mg/kg-day}$$

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

$$C = \text{ADD mg/kg-day} \times \text{BW/WC} \times \text{RSC}$$

where,

BW/WC = the ratio of body weight (kg) and tap water consumption rate (L/day) for the 95th percentile of the pregnant woman population, estimated to be 25.2 kg-day/L (OEHHA, 2000); and

RSC = relative source contribution (usually 20 to 80 percent (0.20 to 0.80), and the lower default value of 0.2 in this case;

Therefore,

$$C = 0.175 \text{ mg/kg-day} \times 25.2 \text{ kg-day/L} \times 0.2$$
$$= 0.88 \text{ mg/L} = 900 \text{ ppb (rounded)}$$

Based on the results of this calculation, OEHHA has derived a public health goal of 900 ppb for glyphosate in drinking water. This PHG is slightly lower than the value published by our office in 1997 of 1,000 ppb, and slightly higher than the U.S. EPA MCL of 700 ppb. The value is judged to be protective of potential sensitive subpopulations, including pregnant women and their fetuses, infants and children, and the elderly.

RISK CHARACTERIZATION

Glyphosate is relatively low in toxicity. In most of the short-term and long-term toxicity studies, reduced body weight, increased liver weights, ocular lesion, and cytoplasmic

changes in the parotid and submandibular salivary glands were observed. These effects were observed at ≥ 350 mg/kg-day dose levels. Glyphosate is not considered to be a mutagen; currently, it is identified as a Group E chemical (evidence of no carcinogenic effects for humans) by U.S. EPA (Fed Reg, 1997). Glyphosate is not a teratogen or a reproductive toxicant, but early maternal death was observed at 350 mg/kg-day in the rabbit teratology study on which the PHG is based. The increased mortality in female rabbits may be due to species-specific sensitivity to glyphosate and/or an increase in sensitivity during pregnancy. Mortality was not observed at much higher dose levels in chronic studies in rats and mice.

The other endpoint of concern is reduced sperm concentration as observed in the subchronic study of Yousef *et al.* (1994). In this study, reduced sperm concentrations were observed at both of the levels tested (1/100 LD_{50} or 1/10 LD_{50}) and therefore no NOAEL was identified. This study had only four rabbits per dose group and the LD_{50} value on which the doses were based and the actual doses administered were not specified. Due to these limitations, the study was not selected for the development of the PHG. Significant reduction in the sperm concentration (20 percent) was also identified in the NTP (1992) study at the high doses of 1,678 and 3,393 mg/kg-day in rats. This toxic effect in the male reproductive system warrants further study.

There are no human data on which to develop a PHG for glyphosate. The human epidemiological studies do not substantiate any effects of population exposures to glyphosate in its use as an herbicide. The PHG for glyphosate is based on diarrhea and increased mortality observed in pregnant rabbits in a teratology study, with a NOAEL of 175 mg/kg-day. In estimating a PHG from animals for application to humans there is an inherent assumption that the data obtained in animals are relevant to humans. An UF of 100 is used to account for inter- and intra-species variation. An additional UF of 10 is added because of the use of a severe endpoint (mortality) from a short-term exposure study (teratology). It should be noted that toxicity tests have been conducted in young and developing laboratory animals and no extra sensitivity, relative to adults, has been observed. No other more susceptible subgroups have been identified in laboratory or epidemiological studies.

In derivation of the PHG, the upper 95th confidence limit for ratio of body weight to drinking water consumption rate of a pregnant female (OEHHA, 2000) was used in the calculation because the critical study involves adverse health effects observed in pregnant females. Relative source contribution was assumed to be 20 percent because glyphosate-containing herbicides are commonly used in residential, commercial, and agricultural settings. Thus it is expected that drinking water will be a relatively minor proportion of total exposure to glyphosate. The RSC value we used is identical to that used by U.S. EPA in deriving the glyphosate MCLG, and is also consistent with current U.S. EPA policy recommendations (U.S. EPA, 2000).

OTHER REGULATORY STANDARDS

The federal MCL of glyphosate in drinking water is 700 ppb (U.S. EPA, 1992a). This value has not been updated to make it consistent with the U.S. EPA's revised RfD (Fed

Reg, 1997). The states of California, Arizona, and Maine all have a drinking water regulatory level of 700 ppb (HSDB, 2005), based on the federal level.

The U.S. EPA has completed a reregistration eligibility document for glyphosate isopropyl amine use as an herbicide (U.S. EPA, 1993). The allowable tolerances of glyphosate and its metabolites in or on produce range from 0.2 ppm to 200 ppm (HSDB, 2005).

WHO (2005) reviewed the toxicological information on AMPA, a major biodegradation product of glyphosate, and derived a health-based drinking water value of 0.9 mg/L or 900 ppb.

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