Ian,

Finally!! Attached are the text, tables, and references. I have sprouted several new gray hairs during the writing of this thing, but as best I can tell, at least they have stayed attached to my head.

As I told you on the phone, the text and tables have been QA-approved. The documents (text and most of the tables) show the numerous changes (in ‘Revisions’ mode) that have been made as part of this lengthy and painful process. There are also a couple of enhancements made in the genetox section - Larry and I feel very strongly about them, so we will need to discuss if you don’t want to add them as is.

Everyone at Monsanto has agreed with adding you as an author - please do so.

A few other notes on the manuscript and references:

1) Douglas told me a week or two ago that he has a few edits from Gary and Kroes that he needs to add.
2) Douglas needs to add Figure 3. I think he has prepared it, but it was not included in the last version he sent me.
3) Douglas needs to supply the full citation for #152 on the reference list.
4) I am not sure about the formatting on some of the EPA references (#151-163) - Douglas may want to take a quick look at them.

If you need to discuss anything with me over the next 3 weeks, note that my availability is quite limited - right now, I plan on being in the office only on Aug 4, 5, 9, and possibly 19. My administrative assistant, Cam Verdin (REDACTED) can reach me most of the time during this period if you need to talk to me. I will be back in the office full time on Monday, August 23.

Best regards, Bill
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SAFETY EVALUATION AND RISK ASSESSMENT OF THE HERBICIDE ROUNDUP® AND ITS ACTIVE INGREDIENT, GLYPHOSATE, FOR HUMANS

MNUSCRPT9.doc

*** DRAFT ***

July 30, 1999

[Version DB sent to experts by 5/20/99 - saved as “Manuscr7”. This is now “Manuscr8” and incorporates changes resulting from: QA review of all sections; WFH/LDK of new, combined Genetox section. Sent to QA 6/21/99. Changed to “Manuscr9” 7/13 and added responses to QA comments from audits. This version has been QA-approved]
Abstract—Reviews on the safety of glyphosate and Roundup® herbicide have been conducted by several regulatory agencies and scientific institutions worldwide and have shown no indication of any human health concern. As the use of glyphosate expands, however, questions regarding its safety are periodically raised. Therefore, this review was undertaken to produce a current and comprehensive safety evaluation and risk assessment for humans. It includes assessments of glyphosate, its major breakdown product [aminomethylphosphonic acid (AMPA)], its Roundup® formulations, and the predominant surfactant [polyethoxylated tallow amine (POEA)] used in Roundup® formulations worldwide. The studies evaluated in this review included those done for regulatory purposes as well as published research reports.

The oral absorption of glyphosate and AMPA are low, and both materials are eliminated essentially unmetabolized. Dermal penetration studies with Roundup® showed very low absorption. Experimental evidence has shown that neither glyphosate nor AMPA bioaccumulates in any animal tissue. As expected based on these properties, no significant toxicity occurred in acute, subchronic, and chronic studies. Direct ocular exposure to the concentrated Roundup® formulation can result in significant irritation, while normal spray dilutions cause, at most, only minimal effects.

The genotoxicity data for glyphosate and Roundup® was assessed using a weight-of-evidence-approach and standard evaluation criteria. There was no convincing evidence for direct DNA damage in vitro or in vivo, and it was concluded that Roundup® and its components do not pose a risk for the production of heritable/somatic mutations in humans. Multiple lifetime feeding studies have failed to demonstrate any tumorigenic potential for glyphosate. Accordingly, it was concluded that glyphosate is non-carcinogenic.

Glyphosate, AMPA and POEA were not teratogenic or developmentally toxic. There were no effects on fertility or reproductive parameters in two multi-generation reproduction studies with glyphosate. Likewise there were no adverse effects in reproductive tissues from animals treated with glyphosate, AMPA, or POEA in chronic and/or subchronic studies. Results from standard studies with these materials also failed to show any effects indicative of endocrine modulation. Therefore, it is concluded that the use of Roundup® herbicide does not result in adverse effects on development, reproduction, or endocrine systems in humans and other mammals.
For purposes of risk assessment, no-observed-adverse effect levels (NOAELs) were identified for all subchronic, chronic, developmental and reproduction studies with glyphosate, AMPA, and POEA. Margins-of-exposure (MOEs) for chronic risk were calculated for each compound by dividing the lowest, applicable NOAEL by worst-case estimates of chronic exposure. Acute risks were assessed by comparison of oral LD50 values to estimated maximum acute human exposure. It was concluded that, under present and expected conditions of use, Roundup® herbicide does not pose a health risk to humans.

[symbol 32 'Symbol' \s 12]
**INTRODUCTION**

**History of Glyphosate and General Weed Control Properties**

The herbicidal properties of glyphosate were discovered by Monsanto Company scientists in 1970. Glyphosate is a non-selective herbicide that inhibits plant growth through interference with the production of essential aromatic amino acids by inhibition of the enzyme enolpyruvylshikimate phosphate synthase. This enzyme is responsible for the synthesis of chorismate, which is an intermediate in phenylalanine, tyrosine and tryptophan biosynthesis (Figure 1). This synthetic pathway for amino aromatic acids is not shared by members of the animal kingdom, making blockage of this pathway an effective inhibitor exclusive to plants. Glyphosate expresses its herbicidal action most effectively through direct contact with foliage, and subsequent translocation throughout the plant. Entry *via* the root system is negligible in terrestrial plants. For example, glyphosate applications will eliminate weeds around fruit trees in an orchard without harming the trees, provided the leaves of the tree are not exposed. Glyphosate is predominantly degraded in the environment by microorganisms and through some limited metabolism in plants (Figure 2); glyphosate ultimately breaks down into natural substances such as carbon dioxide and phosphonic acid.

Roundup® herbicide, which contains glyphosate as the active ingredient, was first introduced in 1974 for non-selective weed control (Franz *et al.*, 1997). During the last 25 years of commercial use, growers, agricultural researchers, and commercial applicators, working in conjunction with Monsanto Company, have expanded the uses of Roundup®. These uses have largely focused on
inhibiting the growth of unwanted annual and perennial weeds, as well as woody brush and trees. Today, a variety of glyphosate-based formulations such as Roundup® are registered in more than 100 countries and are available under different brand names. These products are widely used in agricultural, industrial, forestry, and residential weed control. Although patents for this product held by Monsanto Company have expired in many countries, Monsanto continues to be the major commercial supplier of glyphosate and its formulations, worldwide.

**Purpose and Scope**

Glyphosate and Roundup® herbicide have been investigated for the potential to produce adverse health effects in humans. Government regulatory agencies around the world, international organizations, and other scientific institutions and experts have reviewed the available scientific data and independently judged the safety of glyphosate and Roundup®. Conclusions from three major organizations [Agriculture Canada, United States Environmental Protection Agency (U.S. EPA), and World Health Organization (WHO)] are publicly available (Agriculture Canada, 1991; U.S. EPA, 1993, 1997a, 1998a; WHO, 1994). Those reviews, which have applied internationally accepted methods, principles, and procedures in toxicology, have discovered no grounds to suggest concern for human health. Roundup® and glyphosate are constantly re-evaluated in a science-based process for many reasons including its volume of production and new uses. Nevertheless, questions are raised from time to time regarding the safety of Roundup®.

The purpose of this review is to critically assess the safety of glyphosate and Roundup® and to produce a comprehensive and current safety evaluation and risk assessment for humans. Certain
sectors of the scientific and non-scientific communities have commented on the safety and benefits of pesticide use. With this in mind, parts of this assessment address specific concerns raised by anti-pesticide critics. This review will focus on technical glyphosate acid; its major breakdown product aminomethylphosphonic acid (AMPA); its Roundup® formulations; and the polyethoxylated tallowamine surfactant (POEA), which is the predominant surfactant used in Roundup® formulations worldwide. The review will evaluate data relating to toxicity based on exposure to Roundup® and its components. The sources of information used in this review include studies conducted by Monsanto and published research reports dealing with glyphosate, AMPA, POEA and Roundup®. The scientific studies conducted by Monsanto were done for regulatory purposes and, thus, comply with accepted protocols and Good Laboratory Practices (GLP), according to standards of study conduct in place at the time. Published research reports available in the general scientific literature range in quality from well conducted investigations to those containing serious scientific deficiencies. Other sources of information, primarily reviews from regulatory agencies and international organizations, have also been used to develop this risk assessment. In this effort, the authors have had the cooperation of Monsanto Company which has provided complete access to its database of studies and other documentation. Other studies on glyphosate products have been conducted by other manufacturers, but this information is not generally available, and was not evaluated for this risk assessment.

**Principles of the Risk Assessment Process**

The risk assessment process involves the characterization and estimation of possible adverse outcomes from specific chemical exposures (CCME, 1996; Environment Canada, 1997; NRC,
The determination of hazard is often dependent on whether a dose-response relationship is present (U.S. EPA, 1991). Hazard identification for developmental toxicity and other non-cancer health effects is usually done in conjunction with an evaluation of dose-response relationships. The dose-response assessment evaluates what is known about the biological mode of action of a chemical and assesses the dose-response relationships on any effects observed in the laboratory. At this stage, the assessment examines quantitative relationships between exposure (or the dose) and effects in the studies used to identify and define effects of concern.
The exposure assessment reviews the known principal paths, patterns, and magnitudes of human exposure and numbers of persons who may be exposed to the chemical in question. This step examines a wide range of exposure parameters including the scenarios involving human exposure in the natural environment. Monitoring studies of chemical concentrations in environmental media, food, and other materials offer key information for developing accurate measures of exposure. In addition, modeling of environmental fate and transport of contaminants as well as information on different activity patterns of different population subgroups can produce more realistic estimates for potential exposures. Values and input parameters used for exposure scenarios should be defensible and based on data. Any assumptions should be qualified as to source and general logic used in their development (e.g., program guidance, analogy, professional judgement). The assessment should also address factors (e.g., concentration, body uptake, duration/frequency of exposure) most likely to account for the greatest uncertainty in the exposure estimate, due either to sensitivity or lack of data.

A fundamental requirement for risk characterization for humans is the need to address variability. Populations are heterogeneous, so heterogeneity of response to similar exposures must also be anticipated. Assessments should discuss doses received by members of the target population, but should retain a link to the general population, since individual exposure, dose, and risk can vary widely in a large population.

In contrast to variability, uncertainty arises from a lack of knowledge about factors that drive the events responsible for adverse effects. Risk analysis is characterized by several categories of
uncertainty including measurement uncertainty, uncertainties associated with modeled values, and uncertainties that arise from a simple lack of knowledge or data gaps. Measurement uncertainty refers to the usual error that accompanies scientific measurements as expected from statistical analysis of environmental sampling, and monitoring. The assumptions of scientific models for dose-response, or models of environmental fate and transport also have some uncertainty. Finally, in the absence of data, the risk assessor should include a statement of confidence that estimates or assumptions made in model development adequately fill the data gap.

*Chemical characterization and technical aspects of Roundup® formulations addressed in this review*

Glyphosate is an amphoteric compound with several pKₐ values. The high polarity of the glyphosate molecule makes it practically insoluble in organic solvents. Glyphosate is formulated in Roundup® as its isopropylamine (IPA) salt. Roundup® is supplied as both dry and aqueous formulations at various concentrations; it is commonly formulated with water at 2.13M (3.56 g/L free acid, or 480 g/L IPA salt) with a surfactant added to aid in penetration of plant surfaces, thereby improving its effectiveness.

Technical grade glyphosate acid manufactured by Monsanto Company averages 96% purity on a dry-weight basis. The remaining components are by-products of synthesis, whose individual concentrations are below 1%. This impurity profile has been identified and quantified during the development of the detailed manufacturing process. This information has been provided to and
evaluated by a number of government authorities as part of the information supporting regulatory approval of Monsanto-produced glyphosate. All manufacturers of glyphosate-containing herbicides must meet similar regulatory requirements. This technical grade glyphosate was used as the test material in the extensive toxicological testing discussed in this assessment. The identity of the impurities in technical grade glyphosate has remained relatively unchanged over the course of the history for toxicological testing of the product described in the reports reviewed here. The findings of those studies, therefore, include any effects that could result from the impurities and are therefore embodied in the resulting hazard characterization and risk assessment.

Glyphosate acid is usually formulated with the organic base IPA to yield a more water soluble salt. This salt, combined with water and a surfactant, comprise the principal glyphosate formulations sold worldwide under the Roundup® family of brand names. The predominant surfactant used in Roundup® products worldwide is a POEA, which is a mixture of polyethoxylated long-chain alkylamines synthesized from animal-derived fatty acids. This is the only surfactant considered in any detail in this review. Language considerations and differing business needs have resulted in the marketing of this formulation in some countries using a variety of other brand names (such as Sting, Alphee, Azural, Faena, etc.). Roundup® products are sometimes formulated with various amounts of surfactant, possibly containing additional surfactant components as substitutes for, or blends with, POEA. Most often, the concentration of glyphosate, on an acid basis, in these formulations is 360 grams/L. This, however, is not always the case, and for certain markets where smaller quantities are needed, the base formulation is diluted with water to create more dilute products (e.g. 240, 160, 120, or 9 g/L).
For the purpose of this review, the term “Roundup®” will be used to refer to this entire family of formulations, whose ingredients are qualitatively the same but may vary in absolute amounts. In cases where these differences could lead to substantially different scientific conclusions, these instances will be identified in the context of a comparison among different individual formulations and ingredients. Wherever possible, this document has converted measures to metric units of weight, volume, and area. Some reports of field studies have expressed concentrations in pounds, gallons, or acres, using units of acid equivalents (AE) or IPA salt active ingredient (AI). The conversions have been made to simplify direct comparison of exposure and/or fate data whenever applicable.

**Organization of Assessment**

This assessment initially examines the metabolism and pharmacokinetic studies conducted with glyphosate and AMPA. This includes a review of studies conducted using oral and dermal routes of administration, as these are the predominant pathways of exposure to herbicides like Roundup®. In the second section, the results of toxicology studies in animals are presented for glyphosate and AMPA followed by those conducted with Roundup® and POEA. Consideration is then given to specific organ toxicity and other potential effects such as endocrine disruption, neurotoxicity, and synergistic effects. In the next section, the effects of exposures to humans are discussed; both controlled studies and reports of occupational and other exposures are examined. This is followed by a detailed, worst-case exposure analysis for both children and adults. Finally, the results of the toxicological and exposure investigations will be compared to provide an
assessment of safety for humans. An outline of information presented in this assessment is shown below.

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| OVERALL CONCLUSIONS AND SUMMARY                              |                                       |

**METABOLISM & PHARMACOKINETICS: STUDIES WITH GLYPHOSATE, AMPA AND ROUNDUP® HERBICIDE**

[page]
Glyphosate - Oral Dose Studies in Rats

Introduction

Three studies were conducted to investigate the pharmacokinetics of glyphosate following a single oral dose. In the first of two studies with Sprague-Dawley rats, glyphosate was administered at dose levels of 10 and 1000 mg/kg (Ridley and Mirley, 1988; Howe et al., 1988). The second study was done primarily to assess the distribution and nature of glyphosate-derived radioactivity in tissues following a 10 mg/kg dose (Brewster et al., 1991). A third metabolism study was conducted by the National Toxicology Program (NTP) (1992) in the Fischer-344 strain of rat at dose levels of 5.6 and 56 mg/kg.

Two studies have been conducted to evaluate pharmacokinetic parameters in rats following repetitive oral exposure. In the first study, glyphosate was fed to Wistar rats at dietary concentrations of 1, 10, and 100 ppm (mg/kg/dm²) for 14 days; this was followed by a 10 day depuration period (Colvin and Miller, 1973a). The second repetitive dosing study was conducted to determine if repeated administration alters the metabolic fate of glyphosate. In this study, pharmacokinetic parameters were evaluated in groups of Sprague-Dawley rats given glyphosate by oral gavage at a dose level of 10 mg/kg for either one or 15 consecutive days (Ridley and Mirley, 1988; Howe et al., 1988).

Absorption
The absorption of orally administered glyphosate was shown to be incomplete. Following the administration of a single dose of glyphosate at 10 mg/kg, approximately 30 to 36% (males and females, respectively) of the dose was absorbed. This has been determined from measurements of the area under the curve (AUC) for whole blood (as compared to the AUC for rats dosed intravenously) and the urinary excretion of radioactivity. These results were confirmed in the NTP study, which showed that 30% of the administered 5.6 mg/kg dose was absorbed as determined by urinary excretion data. At the high dose of 1000 mg/kg, absorption appeared to be lower (approximately 15% to 29%) based on the percentage of material excreted in the urine at 10 and 1000 mg/kg/day. In the 14-day repeat dose study conducted at dietary concentrations up to 100 ppm, it was estimated that 15% of the administered material was absorbed.

**Tissue distribution**

The tissue distribution of glyphosate was investigated in Sprague-Dawley rats at 2, 6.3, 28, 96, and 168 hours after the administration of a single 10 mg/kg oral dose (Brewster et al., 1991). Tissue retention times were relatively short, and the vast majority of the body burden was unmetabolized parent glyphosate. Significant radioactivity (> 1% of administered dose) was detected in the small intestine, colon, kidney, and bone. Maximum concentrations in the small intestine (associated primarily with cells rather than contents) and blood were observed 2 hours after oral glyphosate administration, while peak levels in other organs occurred 6.3 hours after dosing. Levels of radiolabeled material in the small intestine, colon, and kidney declined rapidly. Radioactivity in bone steadily decreased over time, albeit at a slower rate than that observed in
blood and other tissues. It was suggested that the slower elimination of glyphosate from bone may be due to reversible binding of the phosphonic acid moiety to calcium ions in the bone matrix; this type of binding has been shown to occur with glyphosate in soil (Sprankle et al., 1975). Regardless of the mechanism involved, there has been no histological or hematological evidence of toxicity to bone in any of the toxicology studies conducted. Metabolite analysis showed that a minor metabolite was present in the gut content or colon tissue of a few animals. Analysis indicated that this metabolite was AMPA, but the small amount and transient nature of the material precluded further characterization. Essentially 100% of the radioactivity in all other tissues/samples was shown to be parent glyphosate (Howe et al., 1988).

When glyphosate was fed in the diet for 14 days, steady-state tissue levels were reached within approximately six days of dosing (Colvin and Miller, 1973a). The highest glyphosate concentration was found in the kidneys (0.85 mg/kg tissue wet/dry weight at the 100 ppm dose level) followed in decreasing magnitude by spleen, fat, and liver. Tissue residues declined markedly after dosing was terminated. Ten days after dosing was discontinued, tissue levels ranged from only 0.067 to 0.12 mg/kg at the highest dose tested. Data from the second multiple dose study showed that repetitive dosing at 10 mg/kg/day had no significant effect on the tissue distribution of glyphosate (Ridley and Mirly, 1988).

Metabolism/Excretion

Orally administered glyphosate is poorly metabolized in animals. It was shown to be rapidly and completely excreted unchanged in the urine and feces of rats. For example, in the single dose
study done by NTP, it was reported that more than 90% of the radioactivity was eliminated in 72 hours. The whole body elimination kinetics were evaluated for rats given the single 10 and 1000 mg/kg doses and found to be biphasic. The half-life of the alpha phase was approximately 6 hours at both dose levels. The beta phase half-lives ranged from 79 to 106 and 181 to 337 hours for animals given the 10 and 1000 mg/kg doses, respectively. The feces was the major route of glyphosate elimination at all dose levels tested; approximately 5862 to 7569% of the administered dose was excreted in the feces. Less than 0.3% of an administered dose was recovered as CO₂ in expired air. In rats given glyphosate at 10 and 1000 mg/kg, it was shown that the vast majority (97.5%) of the administered dose was excreted as unchanged parent material.

In the first multiple dose study (1 to 100 ppm (mg/kg/day) for 14 days), urinary excretion accounted for less than 10% of the dose, while 80 to 90% of the administered material was excreted in feces. The excreted material was shown to be essentially all unmetabolized glyphosate. Upon withdrawal of glyphosate, the amount in excreta dropped sharply, but plateaued temporarily after four days. This plateau was attributed to redistribution of mobilized tissue residues. Evaluation of the data from the second repeat dose study conducted at 10 mg/kg/day also showed that repetitive dosing (15 days) had no significant effect on the elimination of glyphosate.
AMPA - Single Oral Dose Study in Rats

AMPA was administered via gavage at a dose of 6.57 mg/kg (Colvin et al., 1973). Only 20% of the AMPA was absorbed, while 74% of the administered dose was excreted in the feces over the 5-day experimental period. The absorbed AMPA was not metabolized and was excreted rapidly in the urine: approximately 65% of the absorbed dose was eliminated in the urine within 12 hours, and essentially 100% was excreted between 24 and 120 hours. Only trace residues (3 to 6 ppb) were detected in the liver, kidney, and skeletal muscle five days after dosing.

Glyphosate and AMPA - Oral Studies in Non-Rodents

Other studies have been conducted in which glyphosate or a glyphosate/AMPA mixture was administered to non-rodent species. Data from these investigations using rabbits, goats and chickens have shown that the absorption and resulting tissue levels were low.

When a single oral dose of glyphosate (6 to 89 mg/kg) was administered to New Zealand white rabbits, more than 80% of the material appeared in the feces, indicating poor oral absorption (Colvin and Miller, 1973b). Tissue levels were less than 0.1 ppm by the fifth day after dosing.

Lactating goats were fed a diet containing 120 ppm of a 9:1 mixture of glyphosate and AMPA for five days (Bodden, 1988a). In a similar study, the same 9:1 glyphosate/AMPA mixture was fed to hens at dietary levels of 120 and 400 ppm for seven days (Bodden, 1988b). The results from both studies indicated that 30% or less of the test material was absorbed. The concentrations of
test material in goat milk ranged from 0.019 to 0.086 ppm at the end of the dosing period and declined to 0.006 ppm 5 days after the last dose.

When glyphosate was included in the diet of at 120 ppm, residues in chicken eggs obtained at the end of the dosing period ranged from 0.002 to 0.24 ppm, and from 0.010 to 0.753 ppm at the 400 ppm dose level. When eggs were obtained 10 days after the last dose (120 ppm), residue levels ranged from non-detectable to 0.019 ppm.

**Glyphosate and Roundup® - Dermal Penetration**

The dermal penetration of glyphosate is very low based on results from studies in Rhesus monkeys and *in vitro* studies with human skin samples. Maibach (1983) studied the *in vivo* dermal absorption of glyphosate when undiluted Roundup® herbicide was applied to the skin of monkeys. Penetration was slow, as only 0.4% and 1.8% of the applied dose was absorbed over 24 hours and 7 days, respectively. A second study in Rhesus monkeys investigated the absorption of diluted glyphosate (1:29) to simulate a spray solution (Wester *et al.*, 1991). Dermal penetration was found to be 0.8% and 2.2% at low and high doses (500 and 5400 [symbol 109 \( \times \)] g/cm², respectively). Wester *et al.* (1991) also reported that the *in vitro* percutaneous absorption of glyphosate through human skin was no more than 2% when applied for up to 16 hours either as concentrated Roundup® or as a diluted spray solution. In another *in vitro* study, glyphosate absorption through human skin was measured during and for up to one day following a 24-hour exposure period. When glyphosate was applied either as formulated Roundup®, a spray dilution of Roundup®, or another concentrated glyphosate
formulation (Franz, 1983), dermal penetration rates ranged from 0.028 to 0.152% for the three materials tested.

Summary

The pharmacokinetics of glyphosate and AMPA have been thoroughly evaluated in several studies. Both of these materials have phosphonic acid moieties with low pHs and, thus, are charged at the physiologic pHs found in the gut lumen and tissues. Only 15 to 36% of orally administered material administered repeatedly, or as a single dose was absorbed, thereby demonstrating that the absorption of glyphosate and AMPA is low. As expected for substances that are not well absorbed orally, the feces was the major route of elimination. Once absorbed, glyphosate and AMPA were rapidly excreted in urine almost exclusively as unmetabolized parent material. The levels of glyphosate and AMPA in peripheral tissues were low. Results from the multiple dose studies demonstrated that repeated oral dosing had no significant effect on elimination (as compared to a single dose), and that glyphosate does not bioaccumulate. The dermal studies using glyphosate show low rates of penetration with Rhesus monkeys in vivo, and human skin in vitro. Therefore, it is concluded that the potential for systemic exposure is limited by low absorption following oral and dermal contact.
Acute Toxicity and Irritation Studies

The acute toxicity of glyphosate and AMPA has been studied in laboratory animals. Oral and dermal LD50 values for glyphosate in rats are greater than 5000 mg/kg (WHO, 1994). The oral LD50 for AMPA in rats is 8300 mg/kg (Birch, 1973). Using the acute toxicity classification system employed by the U.S. EPA, both glyphosate and AMPA are classified in the least toxic category (IV). These results show that the acute toxicity of glyphosate and AMPA is very low.

The potential for eye and skin irritation as well as dermal sensitization in response to glyphosate as the free acid has been evaluated in studies with rabbits and as the IPA salt in guinea pigs. In standard eye and skin irritation studies in rabbits, glyphosate (as the free acid) was severely irritating to eyes but produced only mild skin irritation (WHO, 1994). However, the IPA salt of glyphosate, which is the predominant form of glyphosate used in formulations worldwide, was non-irritating to rabbit eyes and skin (Branch, 1981). Glyphosate did not produce dermal sensitization in guinea pigs (Auleta, 1983a).
Subchronic Toxicity Studies

*Glyphosate*

*Mice studies.* Glyphosate was administered to B6C3F1 mice in the diet at concentrations of 0, 3125, 6250, 12500, 25000, and 50000 ppm (NTP, 1992). Decreased body weight gain was observed at the 2 highest dietary levels in both males and females. At necropsy, the only significant finding was a dark salivary gland in one high dose male. Alteration of parotid salivary glands was noted microscopically at and above the 6250 ppm dose level. This alteration consisted of microscopic basophilia of acinar cells, and in more severely affected glands, cells and acini appeared enlarged with an associated relative reduction in the number of ducts. The nature of this salivary gland effect is further discussed in a later section. The sublingual and submandibular salivary glands were not affected. No treatment-related changes were observed in other organs, including the accessory sex organs.

There were several reasons to conclude that the salivary gland change observed is of doubtful toxicological significance. The changes occurred in the absence of other significant adverse effects, and could not be associated with any adverse clinical or pathological effect. Such changes can not be considered preneoplastic because the tumor rate was not increased in chronic studies (Lankas, 1981, Knezevich, 1983; Stout, 1990a). Salivary gland changes are not known to represent any pathologic condition, and have no apparent relevance to humans. In the light of these considerations, the no-observed-adverse effect level (NOAEL) was based on the
suppression of body weight gain, and was set at 12500 ppm (2490 mg/kg/day - males and females combined).

In a separate study, glyphosate was fed to CD-1 mice for 13 weeks at dietary concentrations of 0, 5000, 10000, and 50000 ppm. The only treatment-related effect was decreased cumulative body weight gain in males and females (24.7% and 48.25% below controls, respectively) at the highest dose tested (Tierney, 1979). When the submandibular salivary gland change was examined in this study, no changes similar to those described above (in the parotid gland) were observed. The NOAEL was 10000 ppm (2310 mg/kg/day).

Rat studies. Glyphosate was administered in the diet to F-344 rats at levels of 0, 3125, 6250, 12500, 25000, and 50000 ppm for 13 weeks (NTP, 1992). The mean body weights of males were reduced in the 25000 and 50000 ppm groups (6% and 18%, respectively, below control); in females, there was only a marginal effect on body weight, as the mean weight of high dose animals was approximately 5% below the control value. Small increases in one or more red blood cell parameters were reported in males at doses of 12500 ppm and above. Increased serum alkaline phosphatase and alanine aminotransferase values were noted at and above dietary levels of 6250 ppm (males) and 12500 ppm (females). These increases that were relatively small, not clearly related to dose, and not associated with any histological changes are of questionable toxicological significance. At necropsy, no gross lesions were observed that were related to glyphosate administration were recorded. Other analyses in reproductive tissues are discussed in a later section. The salivary gland changes seen in B6C3F1 mice were also noted in the parotid and, to a lesser degree, submandibular glands of rats. The sublingual salivary gland was not
affected at any dose level. Because salivary gland alteration was noted at the lowest dose tested (209 mg/kg/day for males and females combined), a no effect level was not established. However, the salivary gland effect is of doubtful toxicological significance for reasons discussed above. Therefore, the low dose (3125 ppm or 209 mg/kg/day) is considered to be an NOAEL based on changes in serum enzymes.

In another subchronic rat study, Sprague-Dawley rats were fed diets containing glyphosate at concentrations of 0, 1000, 5000, and 20000 ppm for 90 days (Stout, 1987). Submaxillary salivary glands were microscopically evaluated in this study and did not show the changes noted in the parotid and submandibular glands in the NTP study. No toxicologically significant effects were noted at any dose level. Therefore, the NOAEL was set at the highest dietary exposure, or 20000 ppm (1445 mg/kg/day - males and females combined).

*Dog study.* Glyphosate was administered by capsule to beagle dogs at doses of 0, 20, 100, and 500 mg/kg/day for one year (Reyna and Ruecker, 1985). There were no treatment-related effects in any of the parameters evaluated: clinical signs, body weight, food consumption, ophthalmoscopy, hematology, clinical chemistry, urinalysis, gross pathology, and histopathology. Therefore, the NOAEL was 500 mg/kg/day, the highest level tested.

*Summary.* Glyphosate has been evaluated in several subchronic toxicity studies in mice, rats, and dogs. The dose levels used in these studies were very high, reaching dietary levels of 20000 to 50000 ppm in rodent feeding studies and a doses of 500 to 2000 mg/kg/day in dog studies. The primary finding was a decreased body weight gain in the rodent studies at the highest dietary
concentrations tested ([symbol 179 \f "Symbol" \s 12] 25000 ppm). This effect may have been due, at least in part, to decreased food intake resulting from dilution of the caloric content of the diet (which contained 2.5 to 5% glyphosate) and/or reduced diet palatability. An alteration in the submandibular and/or parotid salivary glands was observed in some of the rodent studies; the sublingual salivary gland was not affected in any study. The salivary gland alteration (acinar cell hypertrophy and basophilic change) occurred in the absence of any toxicity, indicating that the health of the animals was not adversely impacted. Furthermore, the salivary gland change was not associated with any adverse effect even in chronic studies and is not known to represent any pathologic condition. Therefore, the finding is not considered to be toxicologically significant or adverse. No salivary gland changes occurred in dogs. In summary, there were no treatment-related adverse effects in rats, mice, or dogs following glyphosate administration at extremely high levels for several weeks. Therefore, it is concluded that the subchronic toxicity of glyphosate is negligible.

AMPANP

Rat study. AMPA was administered in the diet to groups of Sprague-Dawley rats at dose levels of 0, 400, 1200, and 4800 mg/kg/day for 90 days (Estes, 1979). Changes that were noted included decreased serum glucose and elevated aspartate aminotransferase, but only at the highest dose tested. An increase in calcium oxalate crystals was observed microscopically in the urine of high dose animals, and urinary tract irritation was noted at the mid- and high-dose levels. Gross and microscopic pathology examinations did not reveal effects in any other organ. The NOAEL was 400 mg/kg/day based on urinary tract irritation.
*Dog Study.* AMPA was given to Beagle dogs *via* oral capsule at dosages of 0, 9, 26, 88, and 263 mg/kg/day for three months (Tompkins, 1991). There was no treatment-related effect at any dose level. Therefore, the NOAEL was 263 mg/kg/day.

*Summary.* The subchronic toxicity of AMPA has been investigated in rats and dogs. Treatment-related effects were observed only at very high dose levels. The NOAEL for rats was 400 mg/kg/day, while no effects occurred in dogs even at the highest dose tested (263 mg/kg/day). Based on these results, it is concluded that the subchronic toxicity of AMPA, like that of parent glyphosate, is low.

**Chronic Toxicity / Oncogenicity Studies**

**Glyphosate**

*Mouse study.* CD-1 mice were administered glyphosate in the diet at concentrations of 0, 1000, 5000, and 30000 ppm for a period of 24 months (Knezevich, 1983). Total body weight gain in males was reduced at the end of the study (~26% below control) at the highest dose tested. Also in males, increased incidences of liver hypertrophy and necrosis were observed microscopically at the high dose level. An apparent increase in the occurrence of epithelial hyperplasia (slight-to-mild) of the urinary bladder in mid- and high-dose males was not considered treatment-related because the incidence and severity of this common lesion showed no correlation with dose. The NOAEL for chronic toxicity effects was 5000 ppm (885 mg/kg/day) based on the effects on body
weight and liver histology. The incidences of renal tubular adenomas in males was 1, 0, 1, and 3 in the control, low-, mid-, and high-dose groups, respectively. The incidence in high dose males was not significantly different by pair-wise comparison to concurrent controls or by a trend test, and there were no related preneoplastic lesions. Based on a weight-of-evidence evaluation, the adenomas are not considered to be treatment-related. This conclusion was also reached by the U.S. EPA and an independent group of pathologists and biometricians under the auspices of U.S. EPA’s Scientific Advisory Panel (SAP) (U.S. EPA, 1992a). The WHO (1994) has also concluded that glyphosate did not produce an oncogenic response in this study. Accordingly, glyphosate is concluded to be non-carcinogenic in the mouse.

Rat studies. When glyphosate was fed to Sprague-Dawley rats at dietary concentrations of 0, 60, 200, and 600 ppm for 26 months, no treatment-related chronic or oncogenic effects were observed (Lankas, 1981). The incidence of interstitial cell tumors in the testes of high dose males was above that of the concurrent control group. However, this effect was not considered to be a treatment-related because: (1) it was not accompanied by an increase in hyperplasia (an expected pre-neoplastic effect); (2) the incidence was within the historical control range; and (3) no increase was observed in the subsequent study conducted at higher dose levels (see below).

In a second study with the same strain of rat, glyphosate was administered at dietary concentrations of 0, 2000, 8000, and 20000 ppm for two years (Stout, 1990a). Treatment-related effects occurred only at the high dose level and consisted of decreased body weight gain (23% below control at 20 months, the time of maximal depression) and degenerative ocular lens changes in females, as well as increased liver weights and elevated urine pH/specific gravity in
males. There was a statistically significant increase in the incidence (9/60 or 15%) of
inflammation in the gastric squamous mucosa of mid-dose females that was slightly outside of
the historical control range (0 to 13.3%). However, there was no dose-related trend across all
groups of treated females, as inflammation was found in only 6 of 59 (10.2%) high dose females.
In males, there was no statistically significant increase in stomach inflammation in any group of
treated animals, and the incidences of this lesion fell within the historical control range. Finally,
it should be noted that there was a very low occurrence of inflammation in treated animals
examined at the end of the study, usually a time when the incidence of such lesions is greatest.
Considering all these factors, it is doubtful that the inflammation is treatment-related. Therefore,
the 8000 ppm dose level (409 mg/kg/day - males and females combined) is concluded to be the
NOAEL for chronic toxicity. This dose was also determined to be the NOEL by the U.S. EPA
(1993) and was considered to be the NOAEL by the WHO (1994).

The incidence of thyroid and pancreatic tumors occurred at rates slightly above background
control values. The occurrence of thyroid and pancreatic tumors were judged to be sporadic and
unrelated to treatment for the following reasons: (1) they were within the historical control range;
(2) they did not occur in a dose-related manner; (3) they were not statistically significant in pair-
wise comparisons and/or trend tests; (4) they showed no evidence of progression; and (5) there
were no increases in preneoplastic changes. Accordingly, glyphosate is concluded to be non-
carcinogenic in the rat.

Summary. The chronic toxicity and oncogenic potential of glyphosate have been evaluated in
one study with mice and two studies with rats. Few chronic effects occurred and were limited to
the highest dietary levels tested (20000 ppm in rats and 30000 ppm in mice). Glyphosate was not oncogenic to either species. The studies and their results have been evaluated by a number of regulatory agencies and by international scientific organizations. Each of these groups have concluded that glyphosate is not carcinogenic. In fact, the U.S. EPA classified glyphosate in Category E, “Evidence of Non-carcinogenicity in Humans” (U.S. EPA, 1992a), the most favorable category possible.

AMP A

Although lifetime studies were not conducted specifically with AMP A, the chronic toxicity and oncogenicity of this metabolite can be assessed by examining results from the second two-year rat study with glyphosate (Stout, 1990a). Analysis of the test material used in that study showed it contained 0.68% AMP A (Lorenz, 1994). On this basis, it can be concluded that AMP A was present at dietary levels of 13.6, 54.4, and 136 ppm at the 2000, 8000, and 20000 ppm target concentrations for glyphosate, respectively. These dietary levels corresponded to dose levels of 0.69, 2.8, and 7.2 mg AMP A/kg/day. In that study, there were no chronic effects at the mid-dose level and no treatment-related tumors at any dose tested. Therefore, it can be concluded that AMP A is not oncogenic at dose levels up to 7.2 mg/kg/day, and the NOAEL for chronic effects is at least 2.8 mg/kg/day.
Genetic Toxicology Studies

Glyphosate

The genetic toxicology studies conducted with glyphosate are reviewed in detail later as part of a comprehensive assessment that includes all work done with glyphosate and glyphosate-containing formulations. A brief summary of the results for glyphosate is given below (also see Table 2).

Gene mutation studies. Negative results were obtained from several in vitro bacterial mutation assays (Ames/Salmonella and WP2 strain of E. coli) and mammalian Chinese hamster ovary (CHO) cells, both in the presence and absence of exogenous metabolic activation. These results demonstrate that glyphosate does not produce point mutations in standard bacterial and mammalian cell tests.

Chromosomal aberration studies. The ability of glyphosate to induce chromosome aberrations in vitro and in vivo has been extensively evaluated. There were no clastogenic effects in human lymphocytes when tested in vitro at high concentrations. One report of micronuclei formation in a study using an abbreviated protocol was not consistent with the results of several other well-conducted studies showing no chromosomal effects in rodents. The preponderance of data leads to the conclusion that glyphosate does not produce chromosomal aberrations in mammalian systems.
DNA interaction studies. Glyphosate has been tested for its ability to produce primary DNA damage in standard studies accepted by regulatory agencies. Other nonstandard assays have been done which measure endpoints that do not clearly assess specific DNA reactivity; these studies were done at very high in vitro concentrations or at a perilethal dose level in vivo. Observations from these nonstandard investigations are not considered to be biologically significant. The weight-of-evidence from all studies conducted with glyphosate clearly indicate that exposure does not result in direct DNA reactivity.

Summary. The potential genotoxicity of glyphosate has been thoroughly tested in a wide variety of in vitro and in vivo assays. No genotoxic activity was observed in standard assays conducted according to international guidelines. These assays include the Salmonella typhimurium (Ames assay) and Escherichia coli WP-2 reversion assays, recombination (rec-assay) with Bacillus subtilis, Chinese hamster ovary cell gene mutation assay, hepatocyte primary culture/DNA repair assay, and in vivo micronucleus and cytogenetics assays in rat bone marrow. Recently, investigators have reported evidence of genotoxic effects in a limited number of studies (see section on Roundup® Genetic Toxicity). However as discussed later, these assays used toxic dose levels, irrelevant endpoints/test systems and/or deficient testing methodology. In view of the clear negative responses in relevant, well-validated assays conducted under accepted conditions, it is concluded that glyphosate is neither mutagenic nor clastogenic. On the basis of this evaluation, glyphosate does not pose a risk for production of heritable or somatic mutations in humans.
AMPA

Studies conducted with AMPA to detect possible point mutations, chromosome aberrations, and DNA interactions establish that AMPA is not genotoxic. No mutagenic activity was observed in an Ames test performed at concentrations up to 5000 \( \mu \text{g} \)/plate both with and without exogenous metabolic activation (Shirasu, 1980). Similarly, there was no evidence of micronuclei formation or other chromosomal effects in bone marrow cells of mice administered AMPA by i.p. injection at dose levels up to 1000 mg/kg. (Kier and Stegeman, 1993). No genotoxic effects were observed in an \textit{in vitro} unscheduled DNA synthesis repair assay in rat hepatocytes exposed to AMPA at concentrations up to 5000 \( \mu \text{g} \)/ml (Bakke, 1991). The results of these studies are supportive of the conclusion that AMPA is not genotoxic.

Reproductive Toxicology Studies

\textit{Glyphosate}

\textit{Reproductive toxicity.} In the first of two multi-generation reproductive toxicity studies, glyphosate was administered to mice in the diet over three successive generations at dose levels of 0, 3, 10, and 30 mg/kg/day (Schroeder, 1981). An equivocal increase in unilateral renal tubule dilation was judged to be unrelated to treatment since a more extensive evaluation in the subsequent reproduction study conducted at much higher dose levels did not show the effect. There were no treatment-related effects on mating, fertility or reproductive parameters. The
second study, also in mice was conducted at dietary levels of 0, 2000, 10000, and 30000 for two generations (Reyna, 1990). These concentrations corresponded to dosages of 0, 146, 721, and 2153 mg/kg/day for F₀ adults during the 11-week premating period and 0, 154, 757, and 2383 mg/kg/day for F₁ animals from weaning to mating (approximately 14 weeks). Decreased body weight gains were seen in parental animals at 30000 ppm. Other effects at the high dose level were reduced body weight gain in pups during the later part of lactation and an equivocal decrease in the average litter size. The NOAELs for systemic and reproductive toxicity were 10000 ppm (~694749 mg/kg/day) and 30000 ppm (~2132268 mg/kg/day), respectively.

In the subchronic toxicity study conducted in rats by NTP (1992), reduced epididymal sperm concentrations (~20% below control) were reported in F344 rats at both the 25000 and 50000 ppm levels. However, all values were well within the normal range of sperm concentration values reported by the NTP in an analysis of their historical control data for these rodents (Morrissey et al., 1988). As the apparent reductions were not related to dose nor accompanied by decreases in epididymal weights or testicular sperm numbers/weight, the relationship to treatment is doubtful. It should also be noted that male fertility was not reduced in the reproduction study even at the highest dietary level tested (30000 ppm).

An increase in estrous cycle length from 4.9 to 5.4 days was reported in the high dose female F344 rats (50000 ppm) (NTP, 1992). F344 rats, however, are known to exhibit highly variable estrous cycle lengths (4 to 6 days) leading Morrissey et al. (1988) to conclude that “stages of the estrous cycle are so variable [in F344 rats] that they may not be useful in assessing potential toxicity”. Even if the estrous cycle length data were meaningful, they are of doubtful
significance because the extremely high dose associated with its occurrence. This dose was several orders of magnitude greater than ever likely to be experienced by humans. As no changes in sperm counts or estrous cycling were observed in mice treated at the same extremely high dose levels, it is concluded that glyphosate does not adversely affect sperm concentration or estrous cyclicity at any meaningful dose.

Yousef et al. (1995) reported that subchronic glyphosate exposure produced effects on semen characteristics in rabbits. There were a number of serious deficiencies in the design, conduct, and reporting of this study which make the results uninterpretable. Only 4 rabbits per treatment group were used; this is a very low number of animals, and this limitation alone requires that the data be considered preliminary at best. The rabbits used in this study were small for their age, which raises a question regarding their health status and reproductive maturity. The investigators did not state the actual two dosage levels used (referred to only as 1/10th and 100th of the LD50), the purity or even the composition of the glyphosate or the glyphosate formulation, and it is not clear how often the animals were dosed. With no accurate description of the method of delivery or quantity they received, a meaningful assessment of these studies can not be prepared. A critical issue, however, especially in view of the authors’ conclusions, is that the proper method of semen collection was not used, thereby invalidating any meaningful assessment of sperm viability, activity and/or motility. Multiple ejaculates were not pooled to decrease the inter- and intra-animal variability in sperm number and concentration. Unfortunately, it was also unclear whether control animals were subjected to sham handling and dosing procedures, raising serious questions of indirect non-treatment related effects given the known sensitivity of rabbits to stress. Other points that seriously compromise this study include a lack of data for food consumption in
control and treated animals, failure to report variability in measurements (e.g. no error bars), and the presentation of the data for treated groups as a percent of control, a procedure that masks inherent control variability. Despite the 10-fold difference between the low and high dose groups, dose-dependent responses were not observed. Sperm concentration data from both treated and control rabbits were well-within the normal range of sperm concentration values previously reported for mature New Zealand rabbits (Desjardins et al., 1968; Williams et al., 1990). Based on these limitations and the other considerations, the data from this study cannot be used to support any meaningful conclusions.

*Developmental toxicity studies.* Glyphosate was administered by gavage to Sprague-Dawley rats at dose levels of 0, 300, 1000, and 3500 mg/kg/day on gestation days 6 to 19 (Tasker, 1980a). Severe maternal toxicity, including decreased weight gain and mortality (6 of 25 dams), occurred at the excessive dose of 3500 mg/kg/day and was accompanied by reduced fetal weights, ossification of sternebrae, and viability. The NOAEL for maternal and developmental toxicity was 1000 mg/kg/day.

Glyphosate was tested for developmental toxicity in rabbits following administration by oral gavage at dose levels of 0, 75, 175, and 350 mg/kg/day from gestation day 6 through 27 (Tasker, 1980b). Frequent diarrhea was noted in several high dose animals. Deaths occurred in 1, 2, and 10 dams from the low-, mid-, and high-dose groups, respectively. Clear, non-treatment-related causes of death (pneumonia, respiratory disease, enteritis, and gastroenteritis) were determined for the low dose dam as well as 1 mid- and 3 high-dose animals. In the pilot teratology study conducted immediately prior to the definitive study, there was no mortality at doses of 125 and
250 mg/kg/day, while mortality occurred in 80% of the animals from the 500 mg/kg/day group. When this pilot data is included, and when mortality in the definitive study is refined to eliminate non-treatment-related deaths, the overall mortality frequencies are 0%, 0%, 6%, 0%, 44% and 80% at 75, 125, 175, 250, 350 and 500 mg/kg/day, respectively. Thus, it can be demonstrated that there is no dose-response for treatment-related mortality below the 350 mg/kg/day dose. The death of the single mid-dose (175 mg/kg/day) dam cannot be considered a treatment-related effect given the known vulnerability of rabbits to non-specific stressors and the fact that no deaths occurred at a dose of 250 mg/kg/day in the pilot study. Therefore, the NOAEL for maternal toxicity must be represented by the 175 mg/kg/day dose, based on increased mortality and various clinical signs of toxicity at the highest dose tested. The 175 mg/kg/day dose level was also concluded to be the NOAEL by the WHO (1994), while the U.S. EPA (1993) considers this level to be the NOEL. Although there were no effects in fetuses at any dose level, the NOAEL for developmental toxicity was considered to be 175 mg/kg/day due to the insufficient number of litters available for examination in the 350 mg/kg/day dose group.

Summary. Results from several studies have established that glyphosate is not a reproductive or developmental toxicant. Glyphosate was evaluated in two multi-generation rat reproduction studies and in developmental toxicity studies in rats and rabbits. There were no effects on fertility or reproductive parameters, and glyphosate did not produce birth defects. Based on the lack of reproductive toxicity in two multigenerational studies conducted over a very wide range of doses (~3 to 2268 mg/kg/day), there is no support for low-dose effects. The NOAELs for developmental toxicity are equal to or greater than the NOAELs for maternal effects, and the NOAEL for reproductive toxicity is greater than that for systemic toxicity. Therefore, there is no
unique sensitivity from prenatal exposure, and no special sensitivity for children or infants is indicated (U.S. EPA, 1997a, 1998a). Apparent changes in sperm concentrations and estrous cycle length were reported in the NTP (1992) subchronic rat study at doses of 1684 mg/kg/day (sperm only) and 3393 mg/kg/day (sperm and estrous cycle). The validity of these apparent changes are highly suspect as they are not related to dose, their magnitude falls well-within the normal historical control range, and no such changes were observed in mice even at higher doses. In any event, the reported findings in rats are considered biologically irrelevant because the doses at which changes were reported are several orders of magnitude higher than any possible human exposure. The U.S. EPA has recently evaluated tolerance petitions under the Food Quality Protection Act of 1996 (FQPA) (Public Law 104-170) which includes special provisions to protect infants and children. The U.S. EPA concluded that there is ‘reasonable certainty’ that no harm will occur from aggregate exposure to glyphosate (U.S EPA, 1997a, 1998a). The lowest NOAEL for any reproductive study is 175 mg/kg/day in the rabbit teratology study.

AMPA

Reproduction and developmental toxicity studies. The reproductive potential of AMPA can be assessed by examining the results from the two-generation rat reproduction study with glyphosate (Monsanto, 1990). In this study, the glyphosate test material contained 0.61% AMPA (Lorenz, 1994), allowing calculation of dietary concentrations of AMPA at 0, 12.2, 61, and 183 ppm. Given that no effects were seen at the mid-dose level of this study, the overall reproductive NOAEL for AMPA is considered to be at least 61 ppm (~4.23 mg/kg/day - males and females combined) based on systemic (not reproductive) toxicity. In a developmental toxicity study,
AMPA was administered by oral gavage to pregnant rats at dose levels of 0, 150, 400, and 1000 mg/kg/day on gestation days 6 through 15 (Holson, 1991). Slight decreases in maternal body weight gain and fetal body weights were noted at 1000 mg/kg/day. Therefore, the NOAEL for maternal and developmental toxicity is 400 mg/kg/day.

Summary. AMPA has been evaluated for potential adverse effects in reproductive and developmental studies with rats. In addition, reproductive tissues from the three month dog and rat toxicity studies discussed previously (Estes, 1979; Tompkins, 1991) were examined for organ weight, macroscopic, and microscopic effects. No adverse effects have been observed in any of these evaluations. Therefore, it is concluded that the metabolite, like parent glyphosate, is not a reproductive or developmental toxicant.

TOXICOLOGY STUDIES WITH POEA AND ROUNDUP®

Acute Toxicity and Irritation Studies

The acute toxicity of Roundup® herbicide in rats, like that of glyphosate, is very low. The acute oral and dermal LD50 values (Table 1) are greater than 5000 mg/kg (WHO, 1994). The 4-hour inhalation LC50 value in rats is 3.18 mg/L (Velasquez, 1983a). Based on these values, Roundup® is placed in U.S. EPA’s least toxic category (IV) for acute oral, dermal, and inhalation toxicity. Thus, the Roundup® formulation is considered to be practically non-toxic by all routes of exposure.
The acute toxicity of the surfactant, POEA, is somewhat higher than for Roundup® formulation. Oral (rats) and dermal (rabbits) LD50 values (Table 1) have been reported to be ~1200 and >1260 mg/kg, respectively (Birch, 1977). To put the acute toxicity in perspective, the oral LD50 value for POEA in rats is similar to that of Vitamin A (160 to 2000 mg/kg) and greater than that of aspirin (200 to 400 mg/kg) (NIOSH, 1987). The oral LD50 for POEA would place it in U.S. EPA’s second-least toxic category (III). Based on these considerations, POEA is considered to be only ‘slightly’ toxic and does not represent an acute toxicity hazard.

POEA was reported to be severely irritating to the skin and corrosive to the eyes when tested in rabbits (Birch, 1977). The irritation potential of POEA is consistent with the surface-active properties of surfactants in general. Surfactants with these properties are intentionally used in consumer products such as soaps, shampoos, laundry detergents, and various other cleaners. By virtue of these intended properties, POEA and the other surfactants in consumer products can interact with and solubilize lipid components characteristic of skin and mucous membranes.

Surfactants used in consumer products are effective at dilute concentration. POEA is not used in concentrated form but rather is formulated at lower concentrations into an end-use product (Roundup®) and later diluted to very low levels, rendering it significantly less irritating. In standard studies with rabbits, concentrated Roundup® herbicide was shown to be strongly irritating to eyes (Blaszcak, 1990) and only slightly irritating to skin (Blaszcak, 1988). When diluted to a concentration commonly used for most spraying applications (~1%), Roundup® was shown to be only minimally irritating to eyes and essentially non-irritating to skin (Table 1) (Blaszcak, 1987a,b). Standard dermal sensitization studies in guinea pigs were negative for both
concentrated (Auletta, 1983b) and diluted (Blaszcak, 1987c) Roundup® formulations. As will be discussed in a later section, controlled studies and other data from humans confirms that Roundup® herbicide does not pose a significant eye or skin irritation hazard to humans.

**Subchronic toxicity studies**

**POEA**

POEA was administered to Sprague-Dawley rats in the diet for one month at concentrations of 0, 800, 2000, and 5000 ppm (Ogrowsky, 1989). Body weight gains were reduced in males at the 2000 ppm level and in both sexes at the high dose level. Prominent/enlarged lymphoid aggregates in the colon of high dose females were associated with direct irritation/inflammatory effect of the test material. In a subsequent 3-month study with rats, POEA was administered in the diet at concentrations of 0, 500, 1500, and 4500 ppm (Stout, 1990b). Among the animals from the high dose group, effects noted included intestinal irritation, decreased food consumption and body weight gain, and some alterations in serum hematology/clinical chemistry parameters. intestinal irritation was also observed in some animals from the 1500 ppm dose level. Therefore, the NOAEL was 500 ppm in the diet (~36 mg/kg/day - males and females combined).

The POEA surfactant was administered in gelatin capsules to beagle dogs for 14 weeks (Filmore, 1973). Dosages were increased during the first four weeks of the study due to gastrointestinal intolerance (as evidenced by emesis and diarrhea) and then maintained at 0, 30, 60, and 90 mg/kg/day for the final 10 weeks of the study. Body weights were reduced in high dose animals;
slight decreases in low- and mid-dose females were not always dose-related and, thus, were of questionable significance. The biological significance of slight reductions in serum calcium and protein in mid- and/or high-dose dogs is also uncertain. While a definitive NOAEL was not established, the single significant finding in this study was the inability of dogs to tolerate surfactant ingestion on a daily basis due to gastrointestinal irritation.

*Roundup®*

Sprague-Dawley rats were exposed to Roundup® herbicide by inhalation using aerosol concentrations of 0.05, 0.16, and 0.36 mg/L for 6 hours/day, 5 days/week for one month (22 total exposure days) (Velasquez, 1983b). The only change observed was evidence of respiratory tract irritation in high dose females. This was considered to be a direct irritant response rather than a systemic effect. Therefore, the systemic no-observed-effect concentration (NOEC) was the highest dose, or 0.36 mg/L. To put this value in perspective, the highest Roundup® concentration measured in air during an applicator exposure study (Kramer, 1978) was $8.7 \times 10^{-6}$ mg/L; this is approximately 40,000 times less than the NOEC from the inhalation study in rats.

The effect of dermal administration of Roundup® to rabbits was examined at dose levels of 76 and 114 mg/kg/day for 21 days (Killeen, 1975). Dermal irritation was observed at the application site, but there was no indication of systemic toxicity at either dose tested.

A sub-chronic study with Brahman-cross heifers was carried out by administration of Roundup® via nasogastric tube at doses of 0, 400, 500, 630, and 790 mg/kg/day for seven days, after which
animals were observed for a further 14 or 15 days (Rowe, 1987). One cow died at the high dose level; a death believed to result from gastric irritation and vomiting, followed by aspiration pneumonia. Diarrhea and body weight loss were observed at dosages of 630 and 790 mg/kg/day, which was reduced to soft feces at the 500 mg/kg/day dose level. The NOAEL was 400 mg/kg/day. It was estimated that Roundup® herbicide would have to be applied to forage at a rate of 57 pounds/acre for a grazing animal to receive a daily dose equivalent to that which caused no effects (400 mg/kg/day) in this study. Thus, exposure to forage sprayed at recommended use rates (generally limited to between 1 and 4 pounds/acre/annum) should present no hazard to ruminant animals.

Summary

The subchronic toxicity of POEA has been assessed in one- and three-month studies with rats and in a 14-week study with dogs. Roundup® herbicide has been evaluated for possible subchronic effects in an inhalation study with rats, a dermal study in rabbits, and an oral study with cattle. It was anticipated most observed effects would be related to the surface-active properties and associated irritation potential of surfactants. These studies confirm that irritation at the site of contact was the primary finding with the test material. In the oral studies with POEA and Roundup®, some secondary effects were noted in addition to the gastrointestinal irritation. These included decreased food intake and body weight gain in rats and dogs, and diarrhea and an associated slight body weight loss in cattle. There was no systemic toxicity in the inhalation and dermal studies with Roundup®. No indication of specific target organ toxicity was observed in any of these studies. Therefore, it is concluded that the only changes produced
were non-specific effects that might normally be expected from repeated daily high-dose exposure to any material with significant surface-active properties.

GENETIC TOXICOLOGY STUDIES

Introduction

The consideration of the carcinogenic potential of Roundup®, its active constituent ingredient glyphosate, or any of its other constituent ingredients can be assessed in a number of ways. Short-term tests for mutation, or for other evidence of genotoxic activity, focus on the identification of alterations in the genome. A primary function of such tests is to provide information relating to the production of heritable changes (mutations) that could lead to adverse consequences. An initial and prominent question that tests for genotoxicity are designed to answer is whether the chemical (or its metabolite) interacts directly with and mutates DNA. Such interactions are thought to bring about changes in gene expression or effect other key biological processes. On the other hand, there is clear evidence that some short-term tests demonstrate effects of toxicity that may or may not support direct interaction with DNA. Finally, some chemical exposures show no effect at low doses, and can be shown to rely on a threshold of exposure to produce an effect. The production of such indirect effects is often limited to conditions of high dose, or chronic exposure that may be irrelevant for the purposes of health risk assessment. Thus, the discussion that follows examines the most relevant endpoints to consider in evaluating evidence and any possible genotoxic action of Roundup® in general and glyphosate in particular in terms of “direct DNA effects” or “indirect effects. The database of results from
short-term tests related to effects on genetic material, and the production of mutational events is presented in Table 2. The following discussion refers to individual results where appropriate, and then evaluates these results in a weight-of-evidence narrative that takes all the data available into account.

Glyphosate and Roundup®

Glyphosate was negative in standard, validated mutagenicity assays conducted according to international guidelines and in GLP compliant facilities. The database is, however, not entirely without some positive results, and these will be addressed below. Data related to endpoints for genotoxicity will be discussed in the following manner: first, in vitro and in vivo test results will be examined, followed by a discussion of evidence for production of DNA reactive species.

Gene Mutation Studies

Technical glyphosate has not been found to be mutagenic in several in vitro bacterial mutation assays using Salmonella and Escherichia coli tester strains. Multiple studies have been conducted in several strains of Salmonella typhimurium at concentrations up to and including cytotoxic levels with and without exogenous source of metabolic activation (Li and Long, 1988; Moriya et al., 1983; NTP, 1992; Wildeman and Nazar, 1982; Shirasu et al., 1984). In Escherichia coli, glyphosate did not induce reversion at the trp locus in strain WP2 (Li and Long, 1988; Moriya et al., 1983). These results confirm the absence of evidence of mutation induction by glyphosate, even in the presence of various activating systems.
In mammalian cells, glyphosate was non-mutagenic at the HGPRT locus in Chinese hamster ovary cells treated in vitro with or without microsomal activation systems, even at doses that were toxic (Li and Long, 1988).

Several studies have tested herbicide formulations including Roundup®, Rodeo® and Direct® for mutation induction in bacteria. Four studies were negative (Kier et al., 1997; Njagi and Gopalan, 1980), but one gave equivocal results (Rank et al., 1993). The difference between herbicide formulations such as Roundup® and glyphosate (usually as the IPA salt) used in genotoxicity assays is generally limited to the inclusion of POEA surfactant. Such surfactants include MON-0818 (CAS number 61791-26-2), POEA that are (a mixture of polyethoxylated long-chain alkylamines synthesized from animal-derived fatty acids) and a similar, longer-chain tallowamine surfactant. Addition of surfactants generally increased the toxicity of the formulation compared to glyphosate alone in the Salmonella strains because these tester strains are particularly sensitive to substances that effect membrane surface tension. Toxicity of the formulations was observed at concentrations at which glyphosate content was only 0.5 mg/plate without S9 activation and 1.5 mg/plate when S9 was added. POEA is inactive in Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537 and concentrations of up to 1.0 mg POEA/plate, both with and without metabolic activation (Stegeman and Li, 1990).

Thus, the report of Rank et al. (1993) that glyphosate produced an equivocal result for mutagenicity in one bacterial assay is not supported by the other data as shown in Table 2. In the report of Rank et al. (1993) the preponderance of the data show clear evidence of toxicity but no
dose response. A single dose exceeded the spontaneous frequency by twofold (without microsomal activation) in TA98. In TA100, a strain that detects base substitution mutations, they also reported a single dose that showed a mutational response, but only with S9. Data were pooled from two separate assays, but neither set taken alone satisfied the widely accepted criteria of a positive response (two consecutive doses to exceed twice the spontaneous frequency). In contrast, the Ames tests completed by Kier et al. (1997) at Monsanto using Roundup®, Rodeo® and Direct® formulations at doses in excess of those reported by Rank et al. (1993) were uniformly negative. These studies were conducted with complete protocols to satisfy international regulatory guidelines for these assays (Kier et al., 1997). Therefore, the findings of Rank et al. (1993) must be contrasted with the clear negative responses found by several other investigators. Whether their results were due to the effects of toxicity is uncertain, but the weight of evidence indicates theirs is a false positive result.

Other endpoints that detect mutation have been used with Roundup® formulations. Differing results were reported for the effect of Roundup® in the dominant lethal assay of Drosophila melanogaster. One assay carried out using exposure conditions routinely used for this type of study showed no effect of Roundup® (Gopalan and Njagi, 1981). A second non-standard exposure scheme that required chronic exposure (up to four days) of larvae until pupation did show a significant elevation of the frequency of sex-linked lethals in spermatocytes (Kale et al., 1995). This was a non-standard form of the Drosophila sex-linked lethal assay in which every chemical tested was evaluated as positive. Some methodological concerns associated with this report include the authors' lack of experience with the assay, inadequate use of negative controls, and high exposures that included treatment with chemical concentrations that were lethal to half
the population (LC50). No firm conclusions can be made for possible mutagenic effects from Roundup® exposure on the basis of these two studies that applied different methodologies.

Chromosomal Aberration Studies

Evaluating the potential for a chemical to introduce structural chromosome aberrations provides valuable information for purposes of health risk assessment since there is a clear association between chromosome rearrangements and cancer (Tucker and Preston, 1996). Virtually all tumors contain structural (and/or numerical) rearrangements (Rabbitts, 1994; Solomon et al., 1991), although these most probably arise late in tumor development. Thus, clear evidence for the production of chromosome abnormalities that are heritable at the cellular level is an important consideration for cancer hazard assessment. As will be discussed later, it is quite clear from the evidence of chronic exposure studies in rats and mice that there is no evidence of tumorigenicity for glyphosate. This is a fact that should be borne in mind when evaluating all of chromosomal aberration studies described below.

Glyphosate was negative in an in vitro mammalian cytogenetic assay using human lymphocytes with or without microsomal activation at concentrations up to 0.56 mg/mL, and at exposures up to 48 hours (van de Waart, 1995). These tests were performed according to OECD and EEC guidelines.

Lioi et al. (1998a,b), however, have recently reported that glyphosate produced an increased frequency of chromatid breaks as well as other chromosomal aberrations in both cultured human
and bovine lymphocytes. There is reason to question these positive results on several grounds. Lioi et al. (1998a) reported evidence of chromosomal damage at doses three orders of magnitude lower than the van de Waart (1995) study cited above. Although Lioi et al. (1998a) also found that in similar conditions, the fungicide vinclozolin produced similar types and frequencies of chromosomal damage across the same dose range as they reported for glyphosate, vinclozolin is known to produce toxicity by non-genotoxic mechanism(s). This fungicide has failed to produce chromosomal aberrations at twelve-seventy times the dose applied by Lioi et al. (1998a), and has failed to show other evidence of direct DNA damage in a number of tests (Hrelia et al., 1996). The treatment protocol of 72 hours used by Lioi et al. (1998a) was also unusual compared with recognized methodologies. Normally, chemicals that produce chromosomal aberrations in stimulated lymphocytes do so within 48 hours, the time to first mitosis. The observation that glyphosate exposures resulted in a reduced growth rate (thus effecting time to first mitosis) is an indication of a toxic effect, and this can have clear implications for the evaluation of any chromosomal aberration data. For an accurate assessment of induced aberration frequency, the cytogenetic evaluations have to be conducted in a period of time shortly after exposure (Tucker and Preston, 1996). The results with bovine and human lymphocytes were not consistent. Lioi et al. (1998a) found chromosome type breaks in human cells, but few if any with bovine cells (Lioi et al., 1998b). Finally, the authors do not explain why in their hands three different chemicals, atrazine, vinclozolin and glyphosate produced nearly identical responses over exactly the same dose ranges also in human lymphocytes. This is even more remarkable in view of the findings from other laboratories that observed no effects in either glyphosate or vinclozolin at dose levels in excess of ten-seventy times those employed by Lioi et al. (1998a) (Hrelia et al., 1996; van de Waart, 1995).
Glyphosate alone was not active for chromosomal damage (De Marco et al., 1992; Rank et al., 1993). Another study has reported that Roundup® or glyphosate can produce chromosomal aberrations in onion root tip plant cells (De Marco et al., 1992; Rank et al., 1993). Glyphosate alone was not active for chromosomal damage (Rank et al., 1993). These investigators (Rank et al., 1993) postulated that the toxic effect of the surfactant in Roundup® could be responsible for the effects on the plant cell onion root tip chromosomes. Goltenboth (1977) found that glyphosate had an effect on water hyacinth root tips, and concluded that the dose dependent effect on the formation of mitotic figures at prolonged exposure times was due to an effect on the spindle apparatus, leading to disorganized chromosomes at anaphase. Given the intended herbicidal activity of glyphosate, these results are considered secondary to phytotoxic activity, and not relevant to human health.

Of greater relevance than in vitro effects is evidence of in vivo effects. Importantly, administration of glyphosate to rats did not produce an increase in frequency of chromosomal aberrations (Li and Long, 1988). No effects were observed in rat bone marrow at several time periods post treatment following intraperitoneal administration of 1.0 g/kg glyphosate.

The In vivo Micronucleus Assay

A number of studies have used the bone marrow micronucleus assay to examine the effects of exposures to glyphosate and Roundup® on dividing cells. The results of these assays are presented in Table 2a. The micronucleus assay targets the most actively dividing cell population
or polychromatic erythrocytes (PCEs) of the bone marrow. PCEs represent immature cells in the progression of haematopoiesis to normochromatic erythrocytes (NCEs) found in peripheral blood. The toxic effect of a chemical exposure to bone marrow can be assessed by the ratio of PCE/NCE. Different mechanisms may be involved in the evolution of micronuclei, including chromosome breakage (clastogenesis) or effects on spindle organization (aneuploidogenesis). Almost all the results for either glyphosate or Roundup® expressed as micronucleated PCE (MNPCE) per 1000 PCE fall within the range of control (vehicle) values. The frequency of spontaneously (vehicle) produced micronuclei in newly produced polychromatic erythrocytes were within the historical range for the CD-1 strain of mouse (Salamone and Mavournin, 1994).

All but one of the published or unpublished procedures that have examined the effect of glyphosate or Roundup® on the bone marrow have used intraperitoneal (i.p.) injection as the route of exposure. While less relevant for purposes of assessing risks for human exposure, i.p. injection assures distribution of chemical into the circulatory system of the target species and exposure of target cells in bone marrow with maximum potential for observation of genotoxic events. In the only study done using the more relevant oral route of exposure (NTP, 1992), glyphosate did not produce micronuclei following 13 weeks of dietary administration at dose levels up to 50,000 ppm (11,379 mg/kg/day).

Three studies (Kier et al., 1997) examined the different herbicide formulations containing glyphosate. Rodeo® herbicide contains only glyphosate as the IPA salt, while Roundup® and Direct® are formulations that also contain surfactant systems. These bone marrow micronucleus studies were performed according to accepted EC/OECD guidelines, using i.p. as the route of
exposure. OECD (1998) guidelines require exposed and control animals (5 per sex at each dose, and for each time period of exposure) for doses examined. At least 1000 immature erythrocytes per animal were scored for the incidence of micronucleated immature erythrocytes. In each case, Kier et al. (1997) found no evidence of clastogenic effect of the herbicide formulation as measured by an increase in the frequency of PCE-containing micronuclei.

Since Rodeo® contains no surfactant, it is therefore less acutely toxic and could be tested at higher dose levels than the other two formulations containing surfactants. The LD$_{50}$ for i.p. exposures to Rodeo was calculated to be 4239 mg/kg in CD-1 mice during range-finding experiments. Rodeo exposures for bone marrow micronucleus assays included doses of 3400 mg/kg, 1700 mg/kg, and 850 mg/kg. There was no evidence of micronucleus induction in either males or females at any dose or time point tested, including up to 72 hours post treatment (Kier et al., 1997).

For Roundup®, i.p. exposures in CD-1 mice were up to 86% of the LD$_{50}$ (643 mg/kg), and bone marrow samples were prepared at 24, 48, and 72 hours post treatment were negative for micronucleus induction (Kier et al., 1997). Roundup® exposures at all doses tested up to 555 mg/kg (single dose, i.p.) failed to produce a significant increase in the number of MNPCE per 1000 PCE in bone marrow of exposed mice.

A third herbicide formulation using glyphosate and a surfactant was tested in the bone marrow micronucleus assay using CD-1 mice (data not shown in Table B2). The herbicide Direct® contains tallowamine surfactant with a longer carbon chain length than MON-08482, the
surfactant used in Roundup®. Male and female CD-1 mice were given single i.p. injections of Direct at three doses; the highest exceeded 80% of the LD_{50} (436 mg/kg). The doses were 365 mg/kg, 183 mg/kg and 91 mg/kg of formulation. Bone marrow samples were prepared at 24, 48 and 72 hours post exposure were negative for micronucleus induction (Kier et al., 1997). Direct exposures at all doses tested up to 365 mg/kg (single dose, i.p.) failed to produce any increase in the number of MNPCE per 1000 PCE in bone marrow of exposed mice when compared to control mice that received saline.

Bolognesi et al. (1997) reported that glyphosate and Roundup® were weakly positive in the bone marrow micronucleus test (Table 2). GlyphosateRoundup (i.p.) reduced the frequency of PCEs in male mice compared to controls, suggesting some evidence of systemic toxicity. This is in contrast to the results of Kier et al. (1997) who reported no increased micronucleus formation (even at much higher doses than Bolognesi tested) and a change in total PCE/NCE ratio among females, but only at the highest dose (3400 mg/kg) when the IPA salt of glyphosate (Rodeo) was used. The protocol used by Bolognesi et al. (1997), however, varied from the standard acute bone marrow micronucleus assay and only 3 or 4 animals per dose group were used. Two i.p. injections, each representing half the final dose were administered 24 hours apart. Animals were sacrificed at either 6 or 24 hours after the final dose (approximately 48 hours after initial exposure). The results reported by Bolognesi et al. (1997) are at direct variance with those observed in much larger studies carried out under conditions of accepted GLP. First, they report a significant toxic effect on the bone marrow from exposure to glyphosate compared to controls.

The ratio of PCEs to NCEs was 73% in controls, but was reduced to 50% with glyphosate and 30% with Roundup. This elevated frequency of PCE production in control animals is unusual for [page ]
this strain (Crebelli et al., 1999). Kier et al. (1997) found approximate ratios for PCE/NCE were similar for control and treated animals, and this is the general experience for results of a well conducted test (OECD, 1998). Bolognesi et al. (1997) compensated for the use of fewer animals by increasing the total number of cells examined per animal. Thus Bolognesi et al. (1997) relied on counts from 3000 PCE examined per animal in fewer animals to calculate the frequency of micronuclei /1000 polychromatic erythrocytes in pooled data. This may have skewed results, for example because one outlier animal would be disproportionately represented. The accepted methodology includes counting PCEs for five animals. Unfortunately, Bolognesi et al. (1997) did not provide micronucleus data for individual animals, and presented only summary totals, pooled for all animals.

Rank et al. (1993) observed no evidence of significant induction of chromosomal effects in mice exposed to either glyphosate or Roundup® using i.p. injection. Three concentrations of glyphosate (100, 150, and 200 mg/kg body weight) were administered to NMRI-Born male and female mice (5 per sex at each dose) at dose levels up to 200 mg/kg body weight. Bone marrow was examined 24 and 48 hours after exposure, and cells were scored for normo- and polychromatic erythrocytes (NCEs and PCEs) as well as for the frequency of micronucleated polychromatic erythrocytes. The weighted mean for spontaneous micronuclei/1000 PCE in this strain is 2.06 (range 0.4 to 7.0) for NMRI mice (Salamone and Mavourin, 1994). For glyphosate, there was no evidence of increased frequency of micronuclei in the bone marrow, and no change in the relative frequency of PCE/NCE. This result is in general agreement with Kier et al. (1997).
In summary, there are a large number of in vivo bone marrow micronucleus assays that depend on i.p. exposure to (1) the herbicide Roundup®; (2) or its active ingredient glyphosate; or (3) the more soluble form of glyphosate as the IPA salt. These exposures range up to 80% of the LD50 in mice, but have failed to show significant genotoxic effects on replicating bone marrow cells. The bone marrow micronucleus assay is a simple yet reliable method capable of providing evidence for in vivo genotoxicity resulting from different mechanisms (Crebelli et al., 1999). The conclusion that must be made from this information is that there are no genotoxic events that occur in vivo in the absence of overt bone marrow toxicity. Therefore, it is important to bear this fact in mind when evaluating the results of other in vivo and in vitro results.

**Sister Chromatid Exchange**

Analysis of SCE frequency can be an unreliable indicator of genotoxic effect. The frequency of SCE can fluctuate based on osmotic balance. Sodium and potassium chloride concentrations have been implicated in SCE production (Galloway et al., 1987). While somewhat more sensitive than assays of clastogenic activity or chromosomal aberrations, the SCE assay does not indicate mutagenic effect. Therefore it is not appropriate to suggest that increases in SCE could be indicative of cancer risk, primarily because of the lack of an associated cellular outcome (Tucker and Preston, 1996). The utility of the in vitro SCE assay is questionable, because hazard can be more readily assessed using any number of in vitro assays for mutation. The sister chromatid exchange assay (SCE) monitors direct exchange between sister chromatids that suggest recombination. They are the cytological manifestation of interchanges between DNA replication products at apparently homologous loci. The exact nature of these exchanges, and
their relevance to toxicological or genetic endpoints is a matter of some debate (Tennant et al., 1987; Zeiger et al., 1990). The mechanism of SCE formation has not been established, but it has been suggested that they may involve events closely associated with replication (Tucker and Preston, 1996). Several studies have examined the effects of glyphosate and Roundup® on the frequency of SCE in cultured human or animal lymphocytes (Bolognesi et al., 1997; Lioi et al., 1998a,b; Vigfusson and Vyse, 1980).

Lioi et al. (1998b) reported increases in SCE per cell for bovine lymphocytes exposed to several low doses of glyphosate (up to 29 mg/L). However, changes were not related to exposure over a greater than ten fold range of dose. Similarly, Lioi et al. (1998a) failed to detect a dose response for SCE production in human lymphocytes after treatment with glyphosate. In addition, all of the SCE data reported by Lioi et al. (1998a) using either human or bovine lymphocytes were characterized by an extremely low frequency of spontaneous (background) events (e.g. ranging between 1.9 and 2.2 in the human lymphocyte study). More normal values for base SCE frequencies in human lymphocytes range around six per cell. Various values based on data from larger populations have been recorded by Anderson et al. (1991) (6.6/cell); Bender et al., 1989 (8.0/cell); and the Nordic Study Group (1990) (5\textsuperscript{33} [f "WP MathA" \textbackslash s 12]-14/cell). This suggests that Lioi et al. (1998a,b) could have performed the assay without sufficient scoring experience, or that they saw no statistically significant change at any dose.

Bolognesi et al. (1997) reported SCE in cultured human lymphocytes after exposure to glyphosate (1.0 to 6.0 mg/mL) or Roundup® (0.1 mg/mL). Glyphosate as the free acid is soluble in this range, and has a pH of 2.5. The investigators offered no explanation of precautions taken
to insure against the strong acidity of glyphosate in solution. Glyphosate produced a weak response of about 3 SCE per cell (estimated from the figure presented) after a 48 hour exposure. These results were produced from 2 donors whose data were pooled (50 metaphases per dose). Normally, protocols for analysis of cytogenetic data would not permit pooling of data from different individuals or from different experiments. Confidence in results, and statistical analysis is only valid when expressed on the basis of the variation of response among the individuals tested. Bolognesi et al. (1997) failed to provide the tabulated SCE values for individuals or experiments, so it is quite possible that the variation within the data set explains the apparent increase. According to Bolognesi et al. (1997) Roundup® was more toxic to lymphocytes, and only doses approximately ten fold below those tolerated for glyphosate could be tested. Once again, the responses described by these authors are well within the spontaneous SCE frequencies in the human population (see discussion above).

Vigfusson and Vyse (1980) were the first to report on the frequency of SCE in human lymphocyte cultures exposed to Roundup®. The authors acknowledged that cytotoxicity was a confounding factor for their results. They observed very minor changes in SCE in lymphocytes from two donors, but only two doses were reported because the highest dose was toxic and no cell growth occurred. Cells from one donor appeared to show a moderate response, but the other did not. Therefore, the results are not internally consistent. Because of this lack of dose response, it is not possible to apply statistical analysis to determine whether or not an observable effect could be described.
*In vivo*, glyphosate has been shown to be devoid of genotoxic activity in a dominant lethal assay in mice (Wrenn, 1980). This result confirms that there is no reason to suspect that glyphosate could act to effect genetic changes in actively dividing reproductive tissues.

**Mutation Studies with AMPA**

The available data on AMPA indicate it to be non-genotoxic and non-mutagenic. No mutagenic activity was observed in an Ames test performed on AMPA at concentrations of up to 5000 μg/plate, both with and without an exogenous source of metabolic activation (Shirasu *et al.*, 1980). Similarly, no genotoxic effects were observed in an *in vitro* unscheduled DNA synthesis repair in rat hepatocytes exposed to AMPA at concentrations of up to 5000 μg/mL (Bakke, 1991). *In vivo*, no evidence of micronuclei induction or other chromosomal effects was found in the bone marrow of CD-1 mice treated with AMPA by *i.p.* injection at doses of 100 to 1000 mg/kg body weight (Kier and Stegeman, 1993). The results support the weight-of-evidence conclusion that AMPA is non-genotoxic.

**DNA Reactive Species from Glyphosate or Roundup®**

Glyphosate is not a DNA reactive chemical. Experiments *in vivo* were carried out in which Swiss CD-1 mice treated by *i.p.* administration of glyphosate as the isopropyl-ammonium salt at perilethal doses of 130 and 270 mg/kg (Peluso *et al.*, 1998). Glyphosate administered *i.p.* is considerably more toxic than either dermal exposure or by ingestion, and the doses utilized by Peluso *et al.* (1998) should be considered extraordinary. No evidence of DNA adducts was
found on examination of kidney and liver from these mice as measured by the $^{32}$P postlabeling assay. The route of administration should be considered unusual, since intraperitoneal injection (i.p.) is a route of exposure of little relevance for humans. In mice, the LD$_{50}$ values are 134 to 545 mg/kg body weight (WHO, 1994).

When CD-1 mice were treated i.p. with a formulation identified as Roundup® (600 mg/kg of a 30.4% IPA salt, or a dose equivalent to 182 mg/kg body weight) that also contained a surfactant, Peluso et al. (1998) reported what they described as evidence for DNA adducts in tissues isolated after exposure. There are a number of problems with the procedure that led to this conclusion. First, there is no evidence for a dose-response over the narrow range of doses examined. Second, the level of adducts reported is very low that it is well within the range reported for normal endogenous adducts (Gupta and Spencer-Beach, 1996). In addition, it was not determined if the adducts were derived from the formulation ingredients. There is no evidence that direct DNA reactive intermediates are produced by the surfactants commonly utilized in field formulations of Roundup®. The solvent system used to resolve the potential adducts was suitable for the characterization of large, bulky nonpolar polycyclic aromatic hydrocarbon type nucleotide adducts (Randerath et al., 1994), which are unlike adducts that would be generated from molecules like glyphosate or the surfactant. There is no evidence that direct DNA reactive intermediates are produced by the surfactants commonly utilized in field formulations of Roundup®. The poorly resolved adduct spots of the type reported by Peluso et al. (1998) are commonly observed in tissues from animals exposed to complex environmental mixtures. In general, exposures to a limited number of chemical components (as might be expected in Roundup®) produce well defined radioactive products on chromatography, unlike the diffuse
zones reported. All these considerations suggest that the adducts may have been derived from sources other than the formulation ingredients (i.e., naturally occurring molecules or endogenous metabolites). Indeed, Peluso et al. (1998) were unable to provide any chemical characterization of the product(s) that they identified as adducts, and it should be concluded that the observations of Peluso et al. (1998) are not supportive of a biologically relevant response.

Others have reported that i.p. injection of Swiss CD-1 mice with glyphosate and Roundup® could result in an increased incidence of alkali labile sites in DNA in kidney and liver (Bolognesi et al., 1997). Alkali labile sites are generally produced at abasic sites in DNA, and may be revealed in conditions that denature DNA secondary structure. The type of assay used by Bolognesi et al. (1997) could not differentiate between true abasic sites such as are generated by DNA lyase enzymes, sites produced by excision repair, or natural interruptions in DNA found at points of arrested DNA replication. The effects reported by Bolognesi et al. (1997) were observed at doses of 300 mg/kg glyphosate (900 mg/kg Roundup®, corresponding to 270 mg/kg glyphosate) that is close to, or in excess of the i.p. LD_{50} for mice (WHO, 1994). DNA breaks could be detected for a brief time after initial exposure, but at 24 hours post-exposure, there was no evidence of an excess number of alkali labile sites. There are several reasons to question the interpretation of the results from this assay. These include the interpretation of evidence for an increase in single strand or alkali labile sites. Such breaks might indicate, but could not differentiate between, events due to the increased number of cells arrested in S phase rather than an increase in the number of excision sites. Cytotoxic effects can also be responsible for introduction of single strand breaks.
Bolognesi et al. (1997) reported a dramatic increase in the number of 8-hydroxylguanine (8\(\text{OH}dG\)) residues in DNA of liver cells from mice treated with glyphosate, but not Roundup\(^\circledR\). Opposite results were found for exposures to kidney cells that appeared to accumulate oxidative damage after treatment with Roundup\(^\circledR\), but not glyphosate. Products of reactive oxygen species including 8-\(\text{OH}dG\) are stable, and tend to form adducts with protein and crosslink DNA at lower frequency (Randerath et al., 1997a,b). The findings in the reports of Bolognesi et al. (1997), or Peluso et al. (1998) are not consistent with a specific mode of action. Increased levels of 8-\(\text{OH}dG\) residues is not by definition an indicator of chemical-DNA interaction. These products can result from secondary effects associated with chemical induction or inhibition of repair of spontaneous lesions due to toxicity. The solvent system utilized by Peluso et al. (1998) could not detect oxidation products in DNA (Randerath et al., 1997a). Metabolism studies in rodents have shown that glyphosate is poorly metabolized, therefore it is unlikely that products of oxidation could be produced directly in the tissues identified as a result of glyphosate exposure as suggested by Bolognesi et al. (1997). Finally, the lack of increased 8\(\text{OH}dG\) in the same organs with both glyphosate and Roundup\(^\circledR\) containing the equivalent amount of glyphosate suggests that glyphosate is not causing the change observed.

Other assays have been used to indirectly demonstrate the possibility of DNA reactive species from exposure to Roundup\(^\circledR\). Direct reaction with purine or pyrimidine nucleotides could lead to loss of an altered base on exposure to alkali. Alkali sensitive sites resulting from depurination or depyrimidation events can be detected in the Comet assay. Clements et al. (1997) used the Comet assay to examine DNA in erythrocytes from tadpoles exposed to various herbicides including Roundup\(^\circledR\). The Comet assay is a methodology to demonstrate DNA strand breaks.
Clements et al. (1997) reported evidence of a treatment-related increase in DNA breaks as measured by migration in an electrophoretic field. Tadpole erythrocytes were unaffected at the lowest concentration of Roundup® diluted in water (1.7 mg/mL), but at greater concentrations (6.75 mg/mL or 27 mg/mL) did produce evidence of single strand breaks (SSB) in alkaline Comet assays. The dose of Roundup® formulation used in these assays was considerably greater than would be expected at environmental concentrations. Tadpoles were bathed in the exposure concentrations for a period of 24 hours prior to testing. Other tests have clearly shown that glyphosate does not interact with DNA directly, so the effects observed may be from secondary effects of cytotoxicity. Although efforts were taken (trypan blue exclusion) to select cells not undergoing necrosis or autodigestion of DNA, cytotoxicity may have been unavoidable at the doses utilized in the assay.

Rat primary hepatocyte cultures showed no evidence of an increase in unscheduled DNA synthesis (UDS) after a wide range of exposures to glyphosate in vitro. Doses examined ranged over 3 orders of magnitude but failed to produce evidence of DNA repair (Li and Long, 1988). These observations indicate an absence of DNA reactivity, either direct or following hepatocellular biotransformation.

Evaluating Genotoxicity Data: Weight-of-Evidence Approach

When evaluating data for genotoxicity, a primary goal is to determine (a) the likelihood of occurrence of a key event; and (b) whether that event might lead to heritable changes associated
with a number of adverse effects including cancer. The basis upon which a weight-of-evidence evaluation can be constructed include the following:

- Are statistically significant observations also biologically significant?
- What are the dose response relationships for effects?
- Are effects permanent, and progressive or do they reverse upon cessation of chemical dosing?
- Are direct or indirect DNA effects produced?
- Are there inconsistencies in the database on the chemical, and can they be adequately explained?
- Are the effects produced in an assay relevant to humans?

A central objective of the weight-of-evidence is to avoid conditions that could permit one experimental test result to take precedence over others. A conceptual approach to the relative weighting of genotoxicity testing data in the final assessment of mutagenic or carcinogenic potential is shown in Figure 43. This model is based on the National Research Council guidance to evaluating sources of data for risk evaluation (NRC, 1983), and is similar to procedures recommended by several regulatory agencies (e.g., U.S. EPA, 1996, Proposed Guidelines for Carcinogen Risk Assessment) for mutagenicity risk assessment.

The key features of the weight-of-evidence scheme described in Figure 43 are its ability to accommodate results from multiple testing protocols, and its tendency to place a premium on consistency and coherence of results. Greater weight is given to results from laboratories using accepted, well-validated protocols employing GLP procedures. The scheme can also function as
a tool for analysis of a specific protocol, evaluating internal consistency of results from testing similar endpoints. On the other hand, a result from a novel procedure might be acceptable because it is deemed to provide key evidence of a chemical mode of action.

The weight-of-evidence analysis is also significantly affected by the relevance of the data available. Short-term assays disclose evidence of genotoxic events in *in vitro* or *in vivo* that can be compared to more comprehensive examinations of animals such as by the two year rodent cancer bioassay. For purposes of human hazard assessment, greater confidence should be placed in those test systems that examine possible genetic effects from chemical exposure of animals than in tests that rely on homogeneous cell populations raised and tested *in vitro*. Chemical exposures of biological systems carried out *in vitro* are much less realistic, and results of such tests can be determined by effects of toxicity. Such toxicity can occur at unusually high exposure concentrations and/or be dependent on metabolic and detoxification capabilities. Finally, a weight-of-evidence evaluation seeks to establish a dose-response relationship. Greater attention should be given wherever there is a clear association between increased exposure and a genetic effect. Once again, however, conditions of exposure as well as the genetic target are relevant to the evaluation.

**Weight-of-Evidence Narrative**

The database for genetic effects of glyphosate and Roundup® is both large and heterogeneous. Such extensive data sets are sometimes problematic to interpret, but this is not the case for glyphosate. Sporadic positive responses (*i.e.*, non-reproducing) are inherent within assays used
to detect mutagenicity or genetic alterations, particularly *in vitro* tests (Brusick *et al.*, 1998; Kirkland and Dean, 1994). Scientific objectivity precludes emphasis on a few of positive responses rather than the overall response pattern and trend of the results.

Many testing schemes for mutagenicity and other short-term assays are conducted using acute exposure protocols designed for purposes of cancer hazard identification. In the case of glyphosate, there appear to be no tumorigenic endpoints in rodents, or other animals that have been tested, and hence there is no cancer hazard to attribute to any genotoxicity finding.

The information in Table 2 clearly shows that in diverse test systems, glyphosate alone, or as a formulation in Roundup® fails to produce any evidence for mutation induction. Effects of glyphosate on chromosomal organization *in vivo* have been almost wholly negative. The micronucleus data (Table 2a) and those for chromosomal effects in bone marrow (Li and Long, 1988) are consistently negative. The micronucleus data from Bolognesi *et al.* (1997) must be viewed with circumspection until a more complete description of the data is available. The remainder of animal studies carried out *in vivo* show no effect of either glyphosate or Roundup®.

On the other hand, the results of *in vitro* chromosomal aberration tests are more mixed. For reasons described above, it is difficult to give equal weight to the studies based on the quality of the study data presented. In particular, the two studies on bovine and human lymphocytes presented by Lioi *et al.* (1998a,b) are inadequate, and have many problems relating to the internal consistency of the data for other pesticides tested. These studies should not be weighted equally with the assay carried out under GLP conditions (van de Waart, 1999).
There is evidence for the production of effects such as single strand breaks in DNA, but none of these have been linked to the presence of stable adducts, and are therefore most likely due to secondary effects of toxicity. Metabolic studies in rodents plainly show that greater than 99% of glyphosate is rapidly excreted unchanged, and there is very little evidence that chemical residues are associated with any tissue. Bolognesi et al. (1997) have reported evidence of accumulation of $8\text{OHdG}$ adducts in livers of mice treated with glyphosate i.p., but other data indicates that glyphosate is not metabolized. There has been absolutely no evidence produced anywhere, that shows glyphosate or Roundup® is directly responsible for these events. It may be that the injection of such a large quantity of glyphosate ($2 \times 150 \text{ mg}$) creates stress related events that lead to accumulation of these oxidative adducts, which do occur spontaneously. Similarly, the apparent production of single strand breaks in liver or renal tissue DNA (Bolognesi et al., 1997; Peluso et al., 1998) after alkaline elution experiments could also be indicative of events cytotoxicity that reduces or retards rates of DNA replication, giving the appearance of breakage events. The fact that these events were transitory, being no longer evident 24 hours after exposure also suggests an indirect effect of exposure. The fact that there is a negative UDS assay (Li and Long, 1988) would tend to confirm that the SSB of Peluso et al. (1998) likely occur in S phase. Finally, Clements et al. (1997) also appear to have found a weak effect of Roundup® on integrity of tadpole erythrocyte DNA in the Comet assay. Once again, the nature of the exposure conditions, and the concentrations used were considerably greater than might be expected from environmental exposures. Peluso et al. (1998) could detect no evidence of DNA adducts or covalently bound residues in DNA from tissues of mice exposed to glyphosate alone. The weak production of SSB shown by alkaline elution and by the alkaline Comet assay (Clements et al., 1997; Bolognesi et al., 1997; Peluso et
al., 1998) are all suggestive of secondary effects of glyphosate exposure and probably arise from cytotoxicity rather than any direct effect of exposure.

Additional data relating to SCE production presented by Lioi et al. (1998a,b) and Bolognesi et al. (1997) are also questionable on both methodological and scientific grounds. The spontaneous frequency of SCE in untreated cells was extremely low compared with the norm for human lymphocytes, the number of individuals whose lymphocytes were examined does not meet any standard for determining statistical significance and the size of the increases observed were variable and not always dose related. Finally, any changes observed were well within the accepted variation for the incidence of SCE in the human population.

It must concluded that on a weight-of-evidence analysis of the data for glyphosate, and for Roundup® that they are neither mutagenic nor genotoxic in any direct way. The assay systems used in short-term genotoxicity tests are extremely sensitive, but no single test is sufficient to form the basis for conclusive proof for evidence of a genotoxic effect. In the case of these compounds, there is evidence that in circumstances that lead to cytotoxicity(i.e. high dose experimental conditions), as would be predicted for any chemical that can produce such an effect, some effect may be observed such as the production of single strand breaks. The balance of the credible data from in vitro and in vivo test results confirm the safety of glyphosate and Roundup® as non-genotoxic, and conform to the fact that glyphosate is noncancerogenic.

Summary
The mutagenic potential of Roundup® herbicide and the POEA surfactant have been evaluated in several Ames assays. While a marginal response was reported in one limited investigation, results from other full, replicated studies conducted according to international guidelines and Good Laboratory Practices show that these materials are not mutagenic in bacterial systems. Glyphosate herbicide formulations and the POEA surfactant have been evaluated for the ability to produce chromosomal aberrations in several mouse micronucleus assays as well as investigations with onion root tip cells and Drosophila. It is concluded that these materials were not mutagenic in mice. Results from the non-mammalian assays were confounded by various factors and provided no biologically-relevant evidence of genotoxicity. DNA interaction studies with Roundup® herbicide have been reported in the literature. While most of these studies reported positive effects, the assays measuring these effects are not considered acceptable for regulatory decisions. The effects were observed only at cytotoxic concentrations in vitro and at perilethal doses in vivo administered by an irrelevant route of exposure (i.p. injections). Thus, the changes occurred only under extreme conditions of exposure in assays that do not directly assess mutagenicity and are known to produce effects that are secondary to toxicity. It is believed that the high, unrealistic dose levels used in these studies were sufficiently toxic to produce secondary effects rather than direct genotoxicity. In view of all this information, Roundup® is not considered to be mutagenic under conditions that are relevant to animals or humans.

Developmental and Reproductive Toxicity

Developmental Toxicity
POEA was administered by gavage to pregnant Sprague-Dawley rats on gestation days 6 through 15 at doses of 0, 15, 100, and 300 mg/kg/day (Holson, 1990). Significant maternal toxicity was noted at the highest dose tested, while minimal effects (decreased food consumption and mild clinical signs) occurred at the mid-dose level. There were no effects in fetuses at any dose. The NOAELs for maternal and developmental toxicity were shown to be 15 and 300 mg/kg/day, respectively. The POEA surfactant is not a teratogen or a developmental toxin in rats.

Summary

The developmental toxicity of POEA has been evaluated in rats. Subchronic toxicity studies with the surfactant and/or Roundup® herbicide have also been conducted in rats, rabbits, and dogs. In these studies, gross and microscopic pathology examinations were conducted on several reproductive tissues including ovaries, uterus, testes, and epididymis. No developmental effects or changes in reproductive tissues were found in any of these evaluations. Therefore, there is no evidence that the surfactant or Roundup® herbicide adversely impacts reproductive function.

EVALUATION OF POTENTIAL SPECIFIC ORGAN/SYSTEM EFFECTS

Salivary Gland Changes

When salivary gland alterations were observed in rats and mice following subchronic glyphosate administration, additional research was undertaken to investigate the mechanism by which this
change occurred (NTP, 1992). It was hypothesized that glyphosate produced the alterations via weak [symbol 98 \f "Symbol" \s 12]-adrenergic activity. However, careful examination of the data and consideration of other factors do not support this hypothesis.

In a follow-up study conducted by NTP (1992), male rats were fed glyphosate for 14 days at a dietary level of 50000 ppm, which was the high dose level from the subchronic study, while other rats were given isoproterenol (a [symbol 98 \f "Symbol" \s 12]-adrenergic agonist). Both compounds produced increased salivary gland weights. When isoproterenol was given with propranolol, a [symbol 98 \f "Symbol" \s 12]-blocker, there was no increase in salivary gland weight. In contrast, salivary gland weights remained elevated when propranolol was administered along with glyphosate, although the elevation was not as high as that seen when glyphosate was administered alone. The inability of a [symbol 98 \f "Symbol" \s 12]-blocker to significantly inhibit the effects of glyphosate indicates that does not act as a [symbol 98 \f "Symbol" \s 12]-agonist.

Other factors were considered to help resolve questions of salivary gland effects and causality. First, if glyphosate was a [symbol 98 \f "Symbol" \s 12]-agonist material, its effect would be to stimulate [symbol 98 \f "Symbol" \s 12]-receptors in other effector organs and produce a characteristic set of cardiocirculatory effects such as increased heart rate and cardiac output as well as decreased blood pressure and peripheral resistance. None of these effects were noted in two pharmacology studies in which glyphosate was administered intravenously to dogs and rabbits (Tai et al., 1990; Takahashi, 1992). Similarly, it is known that isoproterenol and other [symbol 98 \f "Symbol" \s 12]-agonists cause myocardial necrosis (Lockett, 1965) and
enlargement of heart ventricles (Schneyer, 1962) following prolonged treatment. However, glyphosate did not produce any effects in heart tissue even after chronic exposure at very high doses, providing additional support to the argument that glyphosate does not act as a $\Sigma$-agonist. Furthermore, glyphosate is not structurally related to known $\Sigma$-agonists. It is concluded that glyphosate has no significant $\Sigma$-adrenergic activity, and therefore could not produce salivary gland changes via $\Sigma$-agonist activity.

Indeed, there are a number of other potential mechanisms of salivary gland alteration, including non-chemical modes of action. For example, salivary gland secretion has been shown to be affected by the texture and moistness of feed (Jackson and Blackwell, 1988), and salivary gland enlargement has been caused by malnutrition. Glyphosate could be acting by such a non-chemical mechanism. Because glyphosate is a strong organic acid, dietary administration at relatively high levels may cause mild oral irritation leading to increased salivary gland size and flow. In the chronic exposure studies of glyphosate there were several salivary gland changes. These changes were: (1) most pronounced in the parotid gland, responsible for secretion of serous fluid in response to such stimuli as acidic materials; (2) absent in the sublingual gland, that releases mucous fluid in response to other stimuli; and (3) observed to an intermediate degree in the submandibular gland, that contains a mixture of mucous and serous secreting cells. This pattern is consistent with the hypothesis that the acidic nature of glyphosate is responsible for the salivary gland changes observed.

**Potential for Endocrine Modulation**
In Vitro Assays

A number of in vitro assays have been developed to assess potential endocrine modulating effects of a chemical. The primary use of these in vitro assays in hazard identification is to screen large numbers of chemicals to determine which ones should be further studied in more definitive in vivo testing. As with any screening strategy, these assays are generally designed such that any errors are likely to be false positives rather than false negatives. When a positive result is reported in these assays, in vivo work is indicated to confirm, characterize, and quantify the true nature of the endocrine-modulating properties of the chemical. The recent concern over endocrine modulation and the availability of inexpensive screens is leading to the testing of chemicals in these in vitro assays regardless of the size and reliability of the more definitive in vivo database.

Petit et al. (1997) tested glyphosate and 48 other chemicals in two complementary assays: one measuring activation of the estrogen receptor from rainbow trout in a yeast system, and the other evaluating vitellogenin production in a trout liver cell culture system. Glyphosate had no estrogenic activity in either assay.

In Vivo Studies

The repeat-dose in vivo toxicology studies required by the U.S. EPA and other key worldwide regulatory agencies detect modulation of endocrine system activity (Carney et al., 1997; Stevens
et al., 1997, 1998). These studies are more apical and predictive than in vitro screening assays as they assess a variety of endocrine-sensitive endpoints in animals that are capable of metabolic activation and/or detoxification. These studies also use extended exposure periods encompassing various stages of endocrine development. Endocrine active substances affecting a single or multiple endocrine target sites invariably initiate direct or compensatory biochemical, cellular, and/or histopathological processes which will be detected in standard toxicology studies required for pesticide registration in Canada, Europe, Japan, and the United States. A comprehensive histopathological assessment of endocrine tissues combined with gross organ pathology and organ weight data should detect all adverse endocrinopathies.

The standard toxicology studies that provide valuable information on potential endocrine-modulating effects include subchronic, chronic, developmental, and reproduction studies. The multi-generation rat reproduction study is the most definitive study for evaluating the potential of substances to produce endocrine-modulating effects in humans and other mammals (U.S. EPA, 1998b). This study evaluates effects on gonadal development/function, estrous cycles, mating behavior, fertilization, implantation, in utero development, parturition, lactation, and the offsprings’ ability to survive, develop, and successfully reproduce. A comprehensive histopathological assessment of all major organ systems also is a prominent feature of these studies. Developmental toxicity studies evaluate effects on many of these same processes, while subchronic and chronic studies incorporate numerous direct and indirect evaluations of endocrine and reproductive tissues such as target organ weights and a comprehensive assessment of endocrine organ pathology.
There were no findings in the subchronic, chronic, developmental, or reproductive toxicity studies indicating that glyphosate or AMPA produced any endocrine-modulating effects (see Tables 3 and 4). Histopathological observations of endocrine and reproductive tissues from animals in a chronic and a 2-generation toxicity study are presented in Tables 3 and 4 to illustrate the magnitude and comprehensive nature of these assessments for those not familiar with standard long-term toxicology studies. The data clearly indicate that glyphosate exposure had no adverse histological consequence on any reproductive or endocrine tissue from either male or female rats even at exaggerated dosage levels. Negative results also were obtained in a dominant lethal study conducted at very high doses. While this latter test is typically used to assess genetic toxicity, substances that affect male reproductive function through endocrine modulating mechanisms can also produce effects in this type of study. To summarize, no effects were observed in two independent, multi-generation reproduction studies conducted at several doses ranging from low levels to those that exceed human glyphosate exposure by several orders of magnitude. Thus, a sufficient battery of studies has been conducted to evaluate the potential for endocrine modulation. Taken together, results from all studies demonstrate that glyphosate and AMPA are not reproductive toxicants and do not perturb the endocrine system. The U.S. EPA (1998a) reviewed these studies and also concluded that there were no effects that suggest that glyphosate produces endocrine-modulating effects.

The results of subchronic and developmental toxicity tests on POEA also showed no evidence of endocrine modulation. In addition, the metabolism of POEA would be expected to produce short chain carboxylic acids and similar derivatives, which are not considered to be endocrine modulators. The lack of any indications of hormonal activity in subchronic toxicity studies with
Roundup® herbicide supports the conclusion that POEA does not possess endocrine modulating activity.

**Summary**

The endocrine-modulating potential of glyphosate has been evaluated in a variety of studies including *in vitro* assays and standard *in vivo* toxicology studies. The *in vivo* studies comprehensively assess endocrine functions that are required for reproduction, development, and chronic health. Glyphosate produced no effects in *in vitro* assays, and there was no indication of changes in endocrine function in any of the *in vivo* studies. Results from standard studies with AMPA, Roundup® herbicide and the POEA surfactant also failed to show any effects indicative of endocrine modulation. Therefore, it is concluded that the use of Roundup® herbicide does not result in adverse effects on endocrine systems in humans and other mammals.

**Potential for Neurotoxicity**

As discussed above, glyphosate, AMPA, POEA, and Roundup® herbicide have been tested in numerous subchronic, chronic and reproductive toxicity studies. In another study, the IPA salt of glyphosate was administered to dogs for 6 months (Reyna and Thake, 1983). The design of all these studies included a number of parameters that evaluate the potential of these materials to produce neurotoxicity. Histopathologic examinations were routinely conducted on brain, spinal cord and peripheral nervous tissue such as the sciatic nerve. In addition, the animals in these studies were regularly observed for unusual clinical signs of toxicity that would indicate any
functional effect on the nervous system. The developmental toxicity studies conducted with glyphosate, AMPA, and POEA included examinations to determine if there were adverse effects in the developing nervous system. There was no evidence of neurotoxicity in any of these studies.

Roundup® was administered to beagle dogs as a single oral dose at levels of 59 and 366 mg/kg (Naylor, 1988). Animals were continuously observed for 2 to 3 hours after dosing for clinical signs of toxicity. A detailed neurological examination consisting of 12 different measurements of spinal, postural, supporting, and consensual reflexes was performed before treatment, during the post-administration observation period, and again on the following day. Reflexes appeared normal, and there were no clinical signs indicative of neuromuscular abnormalities.

Based on all this information with glyphosate, it is concluded that there was no evidence of neurotoxicity in any of the toxicology studies even at very high doses. The U.S. EPA has evaluated all the data with glyphosate and also reached this conclusion (U.S. EPA, 1998a). It was also noted by the Agency that no neuropathy or alterations were seen in the fetal nervous system in the developmental and reproductive toxicology studies.

The Potential for Synergistic Interactions

Herbicides are often applied in combination with other active ingredients and/or surfactants. This has raised the question of possible synergistic interactions (i.e. more than additive response) between these materials. It is noteworthy that studies published in the scientific literature,
including a comprehensive study of more than 400 combinations of pesticides, have shown that synergism is rare (Carpenter et al., 1961; Keplinger and Deichman, 1967; Federation of German Research Societies, 1975; Groten et al., 1997). The toxicity of glyphosate has been evaluated in combination with several surfactants and/or other herbicides in acute studies with rats and aquatic species. Based on the results of these studies, it is concluded that the simultaneous exposure of glyphosate and other materials does not produce a synergistic response.

Data that demonstrate a lack of synergism has been presented by various investigators. In a study conducted by Baba et al. (1989), oral LD50s were determined in rats for each component of Roundup® herbicide. The interactions were evaluated by a graphic plotting method and ratios were calculated using Finney’s equation. It was concluded that the interaction between glyphosate and the POEA surfactant was antagonistic rather than synergistic. Heydens and Farmer (1997) used the harmonic mean formula of Finney to compare the “expected” and “observed” LD50 and LC50 values for rats and aquatic species exposed to several combinations of glyphosate with other herbicides and/or surfactants. None of the combinations showed any evidence of synergism. Martinez and Brown (1991) studied the interaction between glyphosate and POEA administered intratracheally to rats at very high dose levels. Based on the resulting pulmonary damage and mortality data, the authors concluded that a synergistic response occurred. However, no supporting mathematical analysis or other basis for the conclusion was presented. In a similar study, Adam et al. (1997) investigated the oral and intratracheal toxicity of POEA, glyphosate and Roundup® herbicide. In contrast to the conclusions of Martinez and Brown, these authors concluded that there appeared to be no synergism with glyphosate and
POEA. In conclusion, there is no reliable evidence indicating synergistic interactions between glyphosate and other materials.

HUMAN EXPERIENCE

Irritation Studies

Dermal irritation studies with Roundup® herbicide in human volunteers have shown, at most, only mild effects. In two separate studies, exposure to Roundup® at a normal spray dilution (~0.9% glyphosate as the IPA salt, IPAG) or at a higher concentration (~4.1% IPAG) produced no skin irritation or sensitization when applied for 24 hours (Shelanski, 1973). Maibach (1986) evaluated Roundup® and commonly-used household products (Johnson & Johnson baby shampoo, Ivory dishwashing detergent, and Pinesol liquid cleaner) for acute irritation, cumulative irritation, photoirritation, as well as allergic and photoallergic activity. Mild irritation was observed in a few individuals as a result of application of concentrated product directly to skin for 24 hours; however, no dermal sensitization, photoirritation, or photosensitization was observed. The authors concluded that Roundup® herbicide and the baby shampoo had less irritant potential than either the cleaner or dishwashing detergent. There was no difference between Roundup® and the baby shampoo in terms of irritation potential.

Occupational Exposure
One controlled study that investigated the potential effects of Roundup® exposure in applicators has been reported in the scientific literature. The remaining information involves reports of effects from individuals following use of the product. These include data gathered by the State of California and three published studies.

Jauhiainen et al. (1991) evaluated the short-term effects of glyphosate exposure in agricultural herbicide applicators. Data from applicators who sprayed Roundup® was compared to results obtained from pre-exposure baseline examinations as well as to data from a group of non-exposed control workers. There were no effects on hematology, clinical chemistry, ECG, pulmonary function, blood pressure, or heart rate one week after application.

The State of California requires that physicians report all cases of known or suspected pesticide exposures presented to them by patients. If a person experiences some pain/discomfort and merely suspects that they have been exposed to a pesticide, the case will be included as a 'suspected illness' in the State’s report. This liberal reporting procedure with no verification often results in the listing of a pesticide simply because the patient recalls using or being near the material at some point in the past and does not necessarily infer a cause-and-effect relationship. Based on this information, Pease et al. (1993) reported that glyphosate-containing products were the third most common cause of skin and eye irritation among agricultural workers and ranked fifteenth for systemic and respiratory symptoms. Relative to the level of product use, however, glyphosate ranked only twelfth for the number of irritation symptoms reported.
Careful examination of the California data further indicates that the number of cases reported simply reflects greater use of the product relative to other herbicides, and shows that glyphosate has relatively low toxicity among pesticides used in the State. Despite widespread use in California among pesticide applicators and homeowners, there have been very few confirmed illnesses due to glyphosate (California EPA, 1996). In 1994 for example, glyphosate exposure was reported in only 25 cases, of which only 13 were considered “definite or probable”. Eleven of the thirteen cases involved only minor and reversible eye irritation; the other two cases were a headache and an apparent misdiagnosis of reaction to hydrocarbon solvent, which is not an ingredient in Roundup®. The California Department of Pesticide Regulation noted in its 1994 report that the majority of the people (> 80%) affected by glyphosate experienced only irritant effects and, of the 515 pesticide-related hospitalizations recorded over the 13 years on file, none was attributed to glyphosate.

Acquavella et al. (1999) evaluated ocular effects in 1,513 cases of Roundup® herbicide exposure reported to a certified regional center of the American Association of Poison Control Centers (AAPCC) from 1993 through 1997. The large majority of reported exposures were judged by specialists at the center to result in either no injury (21%) or only transient minor symptoms (70%). None of the reported exposures resulted in permanent change to the structure or function of the eye. Based on these findings, it is concluded that the potential for severe ocular effects in users of Roundup® herbicides is extremely low.

A limited number of studies have also investigated the results of occupational exposure in humans. Temple and Smith (1992) reported that accidental exposure to Roundup® herbicide can
result in eye and skin irritation. These investigators also reported other symptoms such as tachycardia, elevated blood pressure, nausea, and vomiting. However, such effects probably represent a non-specific response related to the pain associated with eye and/or skin irritation. Talbot et al. (1991) found that accidental dermal exposure to six subjects did not result in any symptoms. Jamison et al. (1986) evaluated pulmonary function in workers handling flax which was previously retted (a process which softens and separates fibers by partial rotting) either by a dew-retting process or via the application of Roundup® six weeks prior to harvest. It was reported that changes in pulmonary function were greater in the individuals exposed to pre-harvest retted flax compared to those inhaling the dew-retted vegetation. However, the levels of glyphosate still present in the flax which was sprayed 6 weeks before harvesting would be extremely low, if present at all, and could not be responsible for the altered pulmonary function observed. Rather, it is most likely that the two retting procedures produced dust particles with different physical characteristics and/or resulted in different microorganism populations in the retted vegetation.

Ingestion

Various studies reported in the literature describe the effects observed after accidental and intentional ingestion of Roundup®. Accidental exposure results in, at most, only mild effects; no deaths have been reported. However, intentional ingestion of large amounts in suicide attempts has produced severe effects including severe hypotension, renal failure, and, in some instances, death (Sawada et al., 1988; Menkes et al., 1991; Talbot et al., 1991; Tominack et al., 1991; Temple and Smith, 1992). In those cases that result in mortality, death usually occurs within a
few days of ingestion. In one study, it was estimated that the amount of concentrated Roundup® intentionally ingested in fatal cases was 184 mL (range of 85 to 200), although it was noted that ingestion of much larger amounts resulted in only mild to moderate symptoms (Talbot et al., 1991). Sawada et al. (1988) and Tominack et al. (1991) reported that average ingestions of 104 and 120 mL were not fatal while mean ingestions of 206 and 263 mL did produce death. Based on this information, it is concluded that the acute toxicity of Roundup® in humans is low and is consistent with that predicted by the results of acute toxicity studies in rats.

The nature of the clinical symptoms observed in cases of suicide suggests that hypovolemic shock was the cause of death (Sawada et al., 1988; Tominack et al., 1989). Because similar responses have been observed in cases involving ingestion of other surface-active agents, it has been suggested that the acute toxicity of Roundup® is likely due to the surfactant. This hypothesis is supported by results from a study in dogs that showed that the surfactant (POEA) produced a hypotensive effect, but glyphosate did not (Tai et al., 1990). Based on other data, these investigators concluded that the hypovolemic shock was due to a cardiac depressant effect of very high doses of the surfactant. Talbot et al. (1991) reported that the clinical data generated in cases of intentional ingestion did not support hypovolemia as the cause of cardiovascular shock. Other factors, such as injury to the larynx and aspiration of vomitus into the lungs, were linked to mortality and specific pathological changes observed after intoxication with Roundup® herbicide (Menkes et al., 1991; Chang et al., 1995; Hung et al., 1997).

Summary
Results from several investigations establish that the acute toxicity and irritation potential of Roundup® herbicide in humans is low. Specifically, results from controlled studies with Roundup® showed that skin irritation was similar to that of a baby shampoo and lower than that observed with a dishwashing detergent and an all-purpose cleaner; no dermal sensitization, photoirritation, or photosensitization reactions were observed. Furthermore, the incidence of occupational-related cases involving Roundup® is low given the widespread use of the product. Data from these cases indicated some potential for eye and skin irritation with the concentrated product, but exposure to dilute spray solutions rarely resulted in any significant adverse effect. Most importantly, no lasting dermal or ocular effects were noted, and significant systemic effects attributable to contact with Roundup® did not occur. Studies of Roundup® ingestions showed that death and other serious effects occurred only when large amounts were intentionally ingested for the purpose of committing suicide. These data confirmed that the acute oral toxicity in humans is low and consistent with that predicted by the results of laboratory studies in animals.

EXPOSURE ASSESSMENT

Overview and Summary

Exposure assessment is generally conducted in a tiered manner, beginning with an assessment that employs simplifying assumptions to arrive at an upper bound estimate. When that upper limit exposure level is found to provide an adequate safety margin, further refinement to identify a more accurate realistic exposure level is not generally undertaken. Generally, the first tier upper limit assessment overestimates actual exposure by 1 to 2 orders of magnitude.
Exposure of the general population to the components of Roundup® herbicide is very low and occurs almost exclusively from the diet. Two population subgroups with maximal opportunity for additional exposure can be identified for purposes of this exposure assessment. These include professional pesticide applicators, and children age 1 to 6. An upper-limit on the magnitude of potential exposure to glyphosate, AMPA, and the POEA surfactant was calculated for these applicator and child subgroups, based on the sum of highest possible exposures by dietary and other possible exposure routes. Realistic exposure for these subgroups and for the general population is expected to be a small fraction of this extreme estimate.

Applicators are directly involved during herbicide spraying operations, and can be exposed on a repeated basis. Although this exposure through occupational activities does not necessarily occur each day for a working lifetime, herbicide exposure was treated as chronic to establish an upper bound estimate. To be conservative, the applicator’s body weight was assumed to be 65.4 kg, in order to account for both male and female workers. This approach was designed to provide a maximum estimate of exposure on a mg/kg/day basis. Children age 1 to 6 years experience the highest dietary exposure because they eat more food per kilogram of body weight than other age groups. Young farm children may also contact pesticide residues in their surrounding environment and thus have more opportunity for potential incremental exposure. We therefore selected this age class as a high-end subgroup for non-occupational exposure among the general population.
Worst-case estimates of exposure to glyphosate, AMPA, and POEA were calculated for aggregated acute and chronic exposure scenarios. The chronic scenario included the aggregate exposure based on the ingestion of food commodities and drinking water containing trace residues in addition to exposures from the spraying of Roundup® by applicators. The acute exposure scenario that was developed incorporated occasional, inadvertent exposure routes (spray drifting onto bystanders, reentry into previously treated areas). The scenario also included other potential unintentional exposures that can occur on a rare basis during specific activities (e.g., consumption of wild berries and mushrooms that might be sprayed inadvertently; the activity of swimming in a pond with herbicide residues). The aggregated acute scenario included the chronic exposure sources in addition to exposure resulting from these inadvertent exposure routes.

Even though worst-case assumptions were used throughout, the calculated exposures to glyphosate, AMPA, and POEA were shown to be low (Table 5). Calculation for glyphosate, acute and chronic exposures to applicators were 0.125 and 0.0323 mg/kg/day, respectively; for young children, the values were 0.09769 and 0.052 mg/kg/day. Estimates of exposure to AMPA were also very low, ranging from 0.0048 to 0.0104 mg/kg/day. The calculated exposures for POEA ranged from 0.026 mg/kg/day for chronic exposure in children to 0.163 mg/kg/day for acute applicator exposure.

Conservative assumptions were made for each analysis for acute and chronic exposure scenarios to insure conditions for upper-limit or worst-case exposure estimates are established. For example, estimates of dietary intake used Maximum Residue Levels (MRLs), the highest legal
residue levels allowed on crops. If actual measured residue levels were used in place of the MRL values and other factors were considered (e.g. % of crop treated, reduction in residues from washing, processing, etc.), dietary exposure estimates would be substantially reduced (10 to 100-fold or more) (see Table 6). Estimates of acute drinking water exposure used the highest measured value resulting from 5 years of drinking water monitoring in the United Kingdom (1.70-37 ppb). This conservative assumption exaggerates glyphosate exposure, since 99% of the UK data did not detect glyphosate above 0.1 [symbol 109 \f"Symbol\ s 12]g/L. For applicators, the highest measured value from all monitoring work was used to estimate acute exposures. Conservative estimates were included for other sources of exposure as well. Exposure estimates using more realistic assumptions would yield substantially lower values than those determined in this assessment. However, since the worst-case analysis yielded exposure estimates that are sufficiently low, a detailed assessment using realistic assumptions was unnecessary and therefore not conducted.

Dietary Exposure to Residues in Food

Glyphosate

In order to obtain approval for the application Roundup® onto food or feed crops, residue studies are required that measured the maximum levels of glyphosate and AMPA that hypothetically occur in food using the highest and most frequent applications. These data support legally binding Maximum Residue Levels (MRLs, called “tolerances” in the U.S.) for the resulting food commodities that are established in most countries worldwide. In addition, international MRLs
are established by Codex Committee on Pesticide Residues to facilitate international trade of agricultural products.

An initial benchmark for assessment of maximum dietary exposure can be obtained by making the simplifying assumption that all food commodities contain the highest legal residue levels (MRLs). This calculation relies on the unrealistic assumptions that 100% of crop acreage is treated with Roundup® at the highest allowed rates, and that all resulting food contains the greatest permissible residues, which are not reduced through processing, washing, or cooking. When glyphosate MRLs are multiplied by average daily food consumption data and summed for all foods that can be treated, a Theoretical Maximum Daily Intake (TMDI) exposure is calculated. Of course, there are differences among countries in the magnitude of established MRLs and in food consumption estimates. The WHO considers five regional diets in the Global Environment Monitoring System - Food Contamination Monitoring and Assessment Programme (GEMS/Food) when making safety assessments for Codex MRLs (WHO, 1997). Comparison of present MRLs among different countries indicates that U.S. MRLs for glyphosate are both more numerous and of equal or greater magnitude than in most other countries. The resulting U.S. TMDI should therefore represent an upper bound exposure compared to other jurisdictions.

The TAS EXPOSURE-1® software¹ incorporates food consumption data for all U.S. crop commodities, and provides a dietary exposure estimate for the U.S. population as a whole, and

for more than 20 specific population subgroups. Using the present U.S. MRLs, the TAS model provided TMDI exposure estimates for glyphosate residues of 23.8 [symbol 10^9 \text{g/kg body weight/day} for the U.S. population and 51.9 [symbol 10^9 \text{g/kg/day} for children age 1 to 6 years. These values represent maximum daily dietary exposure for the adult worker and the child subgroups, respectively, for both the chronic and acute scenarios. These glyphosate exposure estimates include contributions from all present allowed uses, including all currently approved glyphosate-tolerant crops. These dietary exposure estimates are slightly higher than comparable estimates obtained from the WHO dietary consumption model or the German Intake Model (Kidwell et al., 1995) because of regional differences in food consumption and MRLs. Refinement of this maximum estimate could be achieved from a consideration of actual measured residue levels rather than MRLs, realistic application rates, the fraction of crops actually treated, and the effect of processing, washing, cooking, blending, etc. Thus, actual values could be incorporated to arrive at more realistic exposures (Table 6). For example, U.S. residue data from wheat treated with maximum rates of Roundup® showed the highest glyphosate residue to be 2.95 [symbol 10^9 \text{g/g}, with a mean level of 0.69 [symbol 10^9 \text{g/g}, compared to a MRL of 5 [symbol 10^9 \text{g/g} (Allin, 1989). Glyphosate-tolerant soybeans treated at maximum allowed rates and frequency contained glyphosate residues at the highest level of 5.47 [symbol 10^9 \text{g/g}, with a mean of 2.36 [symbol 10^9 \text{g/g}, compared to the MRL of 20 [symbol 10^9 \text{g/g} (Steinmetz and Goure, 1994). Clearly, only a fraction of cropped acres receive a Roundup® treatment, which can be estimated to be in the range of 10 to 50%. Because the ingredients in Roundup® are water soluble, processing, washing, and cooking are expected to further reduce residues. Therefore, considering the
combination of factors, it is expected that realistic chronic dietary exposure to glyphosate and the other ingredients in Roundup® are at least 1 to 2 orders of magnitude lower than the TMDI estimates used in this assessment. Greater accuracy in these refinements is not needed at this time for glyphosate, because even the extremely conservative TMDI assessments have shown that dietary exposures are acceptable compared to toxicological findings.

AMP A

AMP A has historically been considered a minor part of the plant residue derived from glyphosate treatment. Measured levels of AMP A in plant residue studies have averaged about 10% of the glyphosate level (U.S. EPA, 1993), and have been summed with glyphosate to arrive at total residue for MRL setting and risk assessment purposes (U.S. EPA, 1997b). Some jurisdictions have determined that AMP A is not of toxicological concern (U.S. EPA, 1993) and do not include it in MRLs any longer. Canada and the JMPR have proposed to establish a separate MRL for AMP A in cases where it is the major residue in glyphosate-tolerant crops that express an enzyme that converts glyphosate to AMP A as a mechanism of tolerance.

In order to arrive at a maximum estimate of AMP A dietary exposure, it has been assumed that AMP A represents 20% of the TMDI glyphosate exposure. This is a compromise between the bulk of the historical data that indicate that AMP A residues are 10% of glyphosate levels, and the more recent findings that specific glyphosate-tolerant crops have a higher ratio. Based on this assumption, AMP A dietary exposure was 4.8 [symbol 109 \text{g/kg body}]
weight/day for the U.S. population and 10.4 [symbol $10^9$ g/kg/day for children age 1 to 6 years.

**POEA**

Dietary exposure to POEA surfactant is not significant, since surfactants are not believed to be systemically transported in crop plants in the same manner as glyphosate and AMPA (Sherrick et al., 1986; Smith and Foy, 1966). The assumption made for purposes of this assessment was that residues would occur in proportion to glyphosate exposures, based on the relative amount of each in the formulation (2:1, glyphosate: POEA). Using this ratio, TMDI exposure for POEA residues are 11.9 and 26 [symbol $10^9$ g/kg body weight/day for the U.S. population and for children age 1 to 6 years, respectively.

**Occupational Dermal and Inhalation Exposure During Application**

The level of worker exposure to Roundup® during herbicide spraying applications has been reported in both forestry (Centre de Toxicologie du Quebec, 1988; Jauhiainen et al., 1991; Lavy et al., 1992) and agricultural (Kramer, 1978) sites. Most studies have used passive dosimetry to determine the quantity of herbicide deposited during spraying. Deposition is measured from analysis of material from gauze patches located on workers skin and clothing. These deposition results provide a basis for calculating systemic exposure using *in vivo* data for dermal penetration of glyphosate that shows 2% or less reaches systemic circulation (Wester et al., 1991). Inhalation exposure was determined by measurement of glyphosate levels in air sampled from the
workers' breathing zones. This allowed calculation of exposure estimates using hourly breathing rates (U.S. EPA, 1997c), and making the further assumption that all inhaled spray mist was bioavailable. Some studies have also utilized urine monitoring of exposed workers to quantify excreted glyphosate (Lavy et al., 1992). Workers' body burdens were calculated based on data showing that > 95% of glyphosate administered intravenously to rhesus monkeys is excreted via urine (Wester et al., 1991).

In field studies used to estimate exposure, workers generally wore protective clothing as directed according to the label, and that was considered normal for their occupation. They performed a variety of duties, including mixing and loading spray solutions, backpack, handgun, and boom spraying, weeding, and scouting fields, etc. In the studies utilizing passive dosimetry, gauze patches from both outside and inside of shirts were analyzed to determine the degree of protection provided by work clothing.

Taken together, these studies show that dermal and inhalation exposure to Roundup® during application is very low. Body burden doses of glyphosate resulting from dermal contact during application measured by passive dosimetry methods ranged from 0.003 to 4.7 $\times 10^{-9}$ g/kg body weight/work hour. Clothing reduced exposure to the arms an average of 77% (Lavy et al., 1992). Glyphosate levels in applicators' breathing air ranged from undetectable to 39 $\times 10^{-9}$ g/m$^3$ of air (Kramer, 1978), with the vast majority of quantifiable results being less than 1.3 $\times 10^{-9}$ g/m$^3$ (Jauhiainen et al., 1991). Tank filling operations created the highest dermal exposure (hands),
ranging from $4 \times 10^{-2}$ to $12 \text{g/kg body weight/filling operation}$ (Kramer, 1978), assuming that each operation lasted 10 minutes.

The results of biological monitoring showed that most of 350 urine samples analyzed from workers contained no measurable glyphosate, with detection limits ranging from 0.01 to 0.1 $\text{g/mL}$. On a few isolated occasions, urine levels of 0.025 to 0.095 $\text{g/mL}$ were found, although urine volume data were not provided to permit accurate estimation of body burden (Centre de Toxicologie du Quebec, 1988; Jauhiainen et al., 1991). The maximum body burden among workers based on urine monitoring data has been estimated at $8.0 \times 10^{-2} \text{g/kg body weight/hour worked}$, assuming that all urine without measurable glyphosate contained concentrations of one-half of the method’s detection limit (Lavy et al., 1992). The monitoring estimate based on urine herbicide levels was within the range of passive dosimetry predictions, thus lending support to the utility of passive monitoring techniques as reasonable measures of true exposure.

For the present assessment of an adult applicator working for 8 hours per day, weighing 65.4 kg and breathing 1.3 m$^3$ of air/hour during moderate outdoor exertion (U.S. EPA, 1997c), a maximum daily acute exposure to glyphosate was estimated using the highest of the above reported measurements. Dermal exposure from one 10-minute mixing and loading operation was $12 \text{g/kg body weight}$. Dermal exposure was 38 $\text{g/kg}$, and inhalation exposure was $6.2 \text{g/kg}$ during 8 hours of application. Summed together, the adult worker’s peak acute exposure during application was calculated as $56.2 \text{g/kg/day}$.
Chronic applicator exposure was estimated using average rather than peak exposure measurements. Average exposure during a 10-minute tank filling operation was 6.3 \( \times 10^9 \) g/kg body weight (Kramer, 1978). Average dermal exposure (Kramer, 1978; Lavy et al., 1992) during application was 5.1 \( \times 10^9 \) g/kg/day. Average air concentration was difficult to calculate, since many measurements were below detection limits (Jauhiainen et al., 1991). Utilizing an average air concentration of 2.87 \( \times 10^9 \) g/m³ from Kramer (1978), where the assumption was made that the air concentration associated with each undetectable result was at the detection limit, chronic inhalation exposures for the applicator were 0.46 \( \times 10^9 \) g/kg/day. Summed together, and amortizing for a five-day working week, chronic applicator exposure to glyphosate was estimated to be 8.5 \( \times 10^9 \) g/kg body weight/day.

**AMPA**

There is no application-related exposure to AMPA, since its production is dependent on environmental degradation and therefore not present in spray solutions. However, calculations were made for predicting rat NOAELs based on AMPA in technical glyphosate.

**POEA**

No data were available that directly quantify systemic exposure to POEA arising from application. Dermal deposition or inhalation of POEA would occur in proportion to glyphosate.
exposures, based on the relative amount of each in the formulation, as above. It was further assumed that dermal penetration of POEA was 10% of that deposited on skin, which is a conventional default assumption for surfactants (Martin, 1990; Lundehn et al., 1992). Based on these assumptions, utilizing the glyphosate exposure data, peak acute one-day systemic exposure to POEA was calculated to be 30 [symbol 10^9 \text{g/kg body weight (dermal during one mixing and mixing/loading operation)}], 95 [symbol 10^9 \text{g/kg (dermal during application)}], and 3.1 [symbol 10^9 \text{g/kg (inhalation)}]. Summed, the total acute daily exposure was 128 [symbol 10^9 \text{g/kg}]. Chronically, using the same assumptions and amortizing for a 5-day work week, mixing/loading contributed 11.3 [symbol 10^9 \text{g/kg/day}], dermal exposure during application contributed 9.1 [symbol 10^9 \text{g/kg/day}], and inhalation contributed 0.23 [symbol 10^9 \text{g/kg/day}]. Summed, chronic application-related exposure to POEA was estimated to be 20.6 [symbol 10^9 \text{g/kg/day}].

Non-occupational Exposure During Application

Non-occupational application-related acute exposures to Roundup® can also occur during residential applications or Roundup® to control problem weeds in the home and garden. These applications will be primarily spot treatments and edging, utilizing very small quantities on a few occasions during a year. Occupational exposure data, normalized to a kilogram of glyphosate applied basis, showed the highest exposure was 28 [symbol 10^9 \text{g/kg body weight/kg of glyphosate applied (Lavy et al., 1992)}]. It was acknowledged that homeowners may not be well trained in application techniques nor always utilize appropriate
personal protective equipment. Therefore the maximum residential exposure was estimated to be 10-fold greater than the highest measured for the forestry workers (up to 280 g/kg body weight/kg applied). If a homeowner applied an entire 10-litre container of ready-to-use Roundup® spray solution (1% glyphosate concentration), and experienced such an exaggerated exposure, the summed inhalation and dermal exposure would be 28 g/kg body weight, or about 50% of the peak acute occupational exposure. Based on this analysis, the risk assessment for adult occupational application-related exposure is sufficient to cover non-occupational homeowner exposures.

Consumption of Water

Glyphosate

Glyphosate has rarely been detected in drinking water, even though many studies have been done. This is expected because it binds tightly to soil and degrades completely into natural substances (U.S. EPA, 1993; WHO, 1994). The maximum concentration of glyphosate in well water identified in the scientific literature was 45 g/L, which was reported 21 days after the second application of Roundup® at a very high rate (4.6 kg/ha) to a gravel soil surrounding an electrical substation in Newfoundland (Smith et al., 1996). This was not a drinking water well, but it serves as an extreme worst-case upper limit for glyphosate measured under field conditions. As a result of the 0.1 g/L limit for any pesticide in drinking water in the European Union, many thousands of drinking water samples have been routinely analyzed for glyphosate and other pesticides. The best available
data on glyphosate levels in drinking water was obtained from the United Kingdom Drinking Water Inspectorate. During the years 1991 to 1996, 5,290 samples derived from surface and ground water sources were analyzed (Hydes et al., 1996; Hydes et al., 1997). All but 10 were below the contained less than 0.1 \( \text{g/L} \) limit. Among those 10 reported detections, concentrations ranged from 0.2 - 1.7 \( \text{g/L} \). Those 10 samples were all derived from surface water sources, and contained up to 0.37 \( \text{g/L} \) (Harison, 1997). These detections have not been confirmed by follow-up investigation, and it is possible that some are false positives, since follow-up investigation of other low level positive water detections have often not confirmed the initial report. As an example, one of the 10 UK detections was a sample from Llanthony, Wales that was initially reported to have 0.53 \( \text{g/L} \). Subsequent investigation of the site and repeated sampling and analysis did not reveal any amount of glyphosate in the water supply, nor could the source of the initial false finding be identified (Palmer and Holman, 1997). Even allowing for the assumption that all 10 UK detections are accurate, 99th percentile exposure to glyphosate via drinking water is below 0.1 \( \text{g/L} \).

Irrespective of measured concentrations, U.S. EPA has established a maximum contaminant level (MCL) of 700 \( \text{g/L} \) as a health-based upper legal limit for glyphosate in drinking water (U.S. EPA, 1992b). However, using the GENEEC and SCI-GROW environmental fate models, U.S. EPA more recently estimated glyphosate concentration in drinking water for the purpose of risk assessment (U.S. EPA, 1998a). These fate models were used by the U.S. EPA as coarse screening tools to provide an initial sorting of chemicals with
regard to drinking water risk. U.S. EPA concluded from the models that the average concentrations of glyphosate that could be expected in surface and ground water, respectively, were $0.063 \times 10^{-9}$ g/L and $0.0011 \times 10^{-9}$ g/L, four to five orders of magnitude below the MCL that is legally considered safe for chronic exposure.

Surface waters can be directly treated with Roundup® for the purpose of aquatic weed control, which can lead to temporary glyphosate levels in water. However, it is believed that all surface waters that would subsequently be used for drinking purposes would undergo various purifying treatments, such as standard chlorine or ozone treatments. These treatments are known to be effective at removing glyphosate and AMPA from the water (Speth, 1993).

It is difficult to identify appropriate upper limit glyphosate concentrations that can be used to characterize acute and chronic exposure from drinking water. If regulatory limits are selected, predicted exposure could vary through many orders of magnitude, depending on the jurisdictional limits used. Therefore, for this assessment, the peak acute exposure was considered to be no more than $1.7 \times 10^{-9}$ g/L, the highest reported measured value in the UK drinking water program. The same data indicated that chronic exposure could not exceed $0.1 \times 10^{-9}$ g/L, the European Union exposure limit. This value is supported by the U.S. EPA model calculations. Based on mean daily water consumption figures and body weights (U.S. EPA, 1997c) for an adult (1.4 litres and 65.4 kg) and a preschool child (0.87 litres and 13 kg), the acute exposure to glyphosate from drinking water was calculated to be $3.679 \times 10^{-23}$ (adult) and $0.11 \times 2.5 \times 10^{-23}$ (child) g/kg. The chronic
exposures, calculated in the same manner, were $2.10 \times 10^{-3}$ (adult) and $6.7 \times 10^{-3}$ (child) [symbol 109 $\frac{\text{g}}{\text{kg/day}}$].

**AMPA**

AMPA can also occur in water as a result of glyphosate degradation following Roundup® treatments, although its peak concentration is found later and at levels that are only 1 to 3% of peak glyphosate concentrations (Feng et al., 1990; Goldsborough and Beck, 1989). To be conservative and still consistent with the glyphosate assessment above, AMPA levels were assumed to be $0.1\ [\text{g/L}]$ for both the acute and chronic exposure levels. Calculations using the body weight and consumption parameters described predicted acute and chronic adult and child exposures as $2.1 \times 10^{-3}$ and $6.7 \times 10^{-3}$ [symbol 109 $\frac{\text{g}}{\text{kg body weight/day}}$], respectively. These water-derived AMPA exposures are much less than 1% of those derived from food, and are therefore essentially insignificant, eliminating a need for further refinement of the concentration information. AMPA can also be formed from degradation of phosphonate detergents and sequestering agents used in cooling water treatment (Steber and Wierich, 1987), but possible exposures derived from non-glyphosate sources was not considered here.

**POEA**

No direct analytical data were found from which exposures to POEA via drinking water could be independently estimated. Surfactants are expected to bind tightly to soil and sediment particles,
and dissipate quickly via microbial degradation (Van Ginkel et al., 1993; Giger et al., 1987). For the present assessment, the level of POEA in drinking water was assumed to be proportionate to glyphosate exposures, based on the relative amount of each in the formulation, as discussed above. Acute exposure to POEA from drinking water was calculated to be $1.84 \times 10^{-23}$ (adult) and $5.5 \times 10^{-2}$ (child) g/kg. The chronic exposures, calculated in the same manner, were $1.19 \times 10^{-3}$ (adult) and $3.34 \times 10^{-3}$ (child) g/kg/day.

**Reentry of Treated Areas**

*Glyphosate*

Exposure to glyphosate during worker reentry into agricultural fields 1, 3, and 7 days after Roundup® treatment has been measured using the passive dosimetry methods (Kramer, 1978). Two fields studied contained a mixed population of 0.5 m tall grasses and very tall (1.5 m) grassy weeds, while one was composed only of the shorter weeds. As expected, inhalation exposure during reentry was negligible because spray mist had dissipated and glyphosate is a non-volatile salt (Franz et al., 1997). Based on the measured 2% dermal penetration rate (Wester et al., 1991) acute exposures derived from these data were $3.9 \times 10^{-3}$ to 2.6 g/kg body weight/hour for an adult, with a mean value of 0.52 g/kg/hour. Exposures were 10-fold greater for reentry into tall grass compared to short, but potential for exposure decreased over time post-treatment, with values on day 7 averaging 3% of those on day 1. Adjusting for a child’s body surface area of 40% that of an adult (Richardson,
and a child’s lower body weight, exposures of a child reentering the same fields were calculated to be 0.01 to 5.2 $[\text{symbol 109} \times 12]g/kg$ body weight/hour.

One scenario to consider assumes that a 1 to 6 year old farm child could on occasion enter a recently treated field, and could remain there either playing, or helping a parent for a significant period of time. Such activity might occasionally occur for a 5-hour period on a particular day, producing a maximum exposure of 26 $[\text{symbol 109} \times 12]g$ of glyphosate/kg body weight for the child. This route of exposure for a child was considered to be an infrequent, acute event with no calculation necessary to account for chronic exposure.

The calculations above indicated that maximum female adult dermal reentry exposure rate to glyphosate on an hourly basis was 55% of peak dermal exposures experienced during application activities, and the ranges were of similar magnitude. Since acute and chronic applicator exposure levels have been established for the worker, these values, therefore, also account for any reentry exposure she may experience as part of her other activities. During any work time period, she can be making an application or reentering a recently-treated field, but not both, since Roundup®’s herbicidal effects develop too slowly to justify repeated treatment after periods of less than 2-weeks.

*AMPA*
Since reentry exposure involves transfer from treated surfaces, no AMPA would be present, because AMPA is produced by metabolic conversion in a plant or within soil microbes, and would not be found as surface residue.

**POEA**

POEA surfactant would be deposited on surfaces in a ratio that is proportional to its concentration in the formulation, and would therefore be available from surface contact. Acute exposure was calculated to be $65 \times 10^9$ g/kg body weight for the child, after adjusting for the assumed greater (10%) dermal penetration rate. Reentry exposures to POEA for the adult worker would be less than experienced by an applicator, and should be covered by the applicator-derived exposure assessment.

**Bystander Exposure During Application**

It is also possible for the farm child bystander to experience inadvertent acute dermal and inhalation exposure to Roundup® from spray drift during an application, if she is adjacent to the application area. Substantial scientific research has been devoted to measurement, estimation, and modeling of off-site spray drift (Grover *et al.*, 1991). The expected exposure is a fraction of the target treatment rate, reduced by a factor influenced by the separation distance, environmental variables, and application parameters. Aerial applications maximize drift because the droplets are released at a higher altitude. For preliminary ecological risk assessment, U.S. EPA has assumed spray drift exposures could be 5% of the aerial application rate (U.S. EPA, 1995). Off-
target deposition of glyphosate has been measured (Feng et al., 1990), and after aerial application, less than 0.1% of the on-site deposition was intercepted 8 m from the spray boundary.

For the purpose of retaining maximum conservatism, it was assumed that off-site bystander dermal and inhalation exposures could be 10% of an applicator’s on-site peak 8-hour acute exposures (calculated above). Contributions from mixing and loading operations were excluded. The summed calculated exposure estimate for the child bystander was 4.4 \( \times 10^{9} \) g of glyphosate/kg body weight/day. No adjustment was made for the child’s reduced breathing volume, body weight, or skin surface area, because this was intended as a simple upper bound estimate. No application-related bystander exposure to AMPA will occur, since it is only formed upon environmental degradation. Daily POEA acute exposure, based on relative concentrations in the formulation and calculated as 10% of peak on-site applicator exposure, was 9.8 \( \times 10^{9} \) g/kg body weight. Such bystander exposures would be infrequent, since Roundup® is only applied to a given location a few times each year, at most, and were considered only for the acute risk scenario.

Possible Inadvertent Exposures Derived from Specific Activities

In the course of this assessment, preliminary estimates were made to determine whether other possible inadvertent environmental contact might contribute significantly to incremental glyphosate exposures. Several routes of exposure were considered for glyphosate, AMPA, and POEA. These included (1) dermal contact with or accidental ingestion of treated soil; (2)
inhalation or ingestion of residential dust derived from treated soil; (3) dermal contact with waters or aquatic sediments during swimming or showering; (4) accidental ingestion of treated surface waters while swimming; and (5) ingestion of inadvertently sprayed wild foods such as berries or mushrooms. Using standard exposure parameters (U.S. EPA, 1992c, 1988, 1997c) and conservative assumptions about expected environmental concentrations and frequency of such contact, only the latter two potential incremental exposure routes were found to contribute possible exposures greater than $10^{-9}$ g/kg body weight/day. Infrequent incremental exposures below this level were judged to be insignificant compared to recurring dietary, drinking water, and application-related exposure levels.

Glyphosate formulations can be used to control surface weeds on ponds, lakes, rivers, canals, etc. according to label rates up to about 4.2 kg glyphosate per hectare, which can result in significant water concentrations immediately after treatment. These glyphosate levels in water dissipate quickly (Goldsborough and Beck, 1989), and it is unlikely that such weedy water bodies would attract swimmers or bathers. However, if such an application were made to water 0.25 m deep, the immediate resulting glyphosate concentration could be $1.68 \times 10^{-9}$ g/mL if it were mixed into the water column. It has been estimated that accidental ingestion of water during one hour of swimming could be 50 mL (U.S. EPA, 1988), so maximal incremental exposure to glyphosate was estimated to be $1.28 \times 10^{-9}$ and $6.5 \times 10^{-9}$ g/kg body weight for a swimming adult and child, respectively. Such exposures will be very rare and therefore only were considered as a possible increment to the acute exposure scenario. AMPA will not be present at significant concentrations in water shortly after treatment. POEA surfactants are not necessarily included in glyphosate formulations intended for aquatic uses. If a
surfactant were to be included in an application to aquatic systems, such a substance would be applied at doses approximately half that of glyphosate. We conclude that swimming in water from areas recently treated with Roundup® would produce an incremental oral exposure potential of 0.64 and 3.2 [symbol 109 \( \text{g/kg body weight} \)] for a swimming adult and child, respectively.

Roundup® application along roadsides or in forestry creates the potential for accidental overspray of wild foods that could later be collected for consumption. Consideration of actual use patterns, the percentage of forests or roadsides that actually receive treatment, and the resulting of phytotoxic effects on the sprayed plants suggests that inadvertent exposure will be extremely unlikely. However, since residue levels of glyphosate arising from a mock overspray of berries has been measured (Roy et al., 1989), the potential dietary exposure was quantified. Peak glyphosate residue levels in raspberries were 19.5 [symbol 109 \( \text{g/g} \)] (Roy et al., 1989), and it was estimated that maximal consumption for an individual might be 150 g for an adult and 30 g for a 1 to 6 year old child. These parameters predict an exposure of 45 [symbol 109 \( \text{g/kg body weight} \)] for both subgroups, and relies on the assumption that the surface residues were not reduced by washing before consumption. Exposure at this level is approximately equal to the total TMDI dietary estimate, suggesting it could be a significant but rare incremental contributor to acute exposure scenario. AMPA residues were also quantified in the raspberries, but were less than 1% of those for glyphosate (Roy et al., 1989), and are therefore insignificant. POEA surfactant residues were not measured, but can be assumed to be 50% of those for glyphosate, based on the relative formulation content, leading to potential incremental oral POEA exposures of 23 [symbol 109 \( \text{g/kg} \)].
Aggregate Exposure Estimates

The calculated acute and chronic exposure estimates for each population subgroup for glyphosate, AMPA, and POEA are summarized in Table 5. For glyphosate, acute exposures to applicators and children were calculated to be 0.125 and 0.0974 mg/kg/day, respectively; chronic exposures in these subgroups were 0.0323 and 0.052 mg/kg/day, respectively. Levels of exposure to AMPA were very low (~0.005 - 0.010 mg/kg/day). Estimates of exposure to POEA were 0.163 and 0.0911 mg/kg/day for the acute scenarios, while chronic exposure estimates were 4 to 5 times lower than the acute values.

RISK CHARACTERIZATION

Introduction

Risk characterization involves a determination of the likelihood that an adverse effect will result from exposure to a given substance. The method used to characterize risk in this assessment was the MOE analysis, in which dose levels from animal toxicity tests were compared to conservative, upper-limit estimates of human exposure. To evaluate the risks resulting from chronic exposure, estimates of human exposure were compared to the lowest dose that produced no adverse effects in repeat-dose studies with animals. For acute effects, human exposure estimates were compared to oral LD50 values in rats.
Identification of NOAELs

The toxicity of glyphosate and AMPA have been investigated in a comprehensive battery of studies. In addition, POEA has been tested in acute, subchronic, genetic, and developmental toxicity studies. A summary of the no-effect levels identified in the various studies conducted with these materials are provided below and in Tables 6, 7, and 8. The no-effect levels selected for risk characterization are discussed below.

Glyphosate

The lowest no-effect level for purposes of risk characterization for adults is the NOAEL of 175 mg/kg/day; this value is based on the occurrence of maternal toxicity at the highest dose tested (350 mg/kg/day) in the rabbit developmental toxicity study. The NOAELs in the chronic rodent or dog studies, multi-generation reproduction studies and the rat developmental toxicity study ranged from approximately 400 to 1000 mg/kg/day.

Calculation of an MOE based on the endpoint of maternal toxicity is biologically irrelevant for the young (1 to 6 years). Nevertheless, such an analysis was conducted by the U.S. EPA and is included here to demonstrate that even use of an unrealistic assumption provides an acceptable margin of exposure. The NOAEL of 209 mg/kg/day from the second subchronic rat study (NTP, 1992) was also used to calculate the MOE for children because this value was the next higher no-effect level and was based on a more relevant toxicological endpoint.
**AMPA**

Some regulatory agencies have determined that AMPA is not of toxicological concern and do not include it in assessments of risk. Other agencies have summed AMPA with glyphosate to arrive at total exposure for risk assessment purposes. Nevertheless, a separate MOE analysis was conducted here to characterize the risks associated with AMPA exposure. The NOAEL of 400 mg/kg/day in the subchronic rat study is considered to be the most appropriate value for use in this risk assessment. As noted previously, AMPA was also assessed as a component of the test material used in the glyphosate reproduction and chronic/oncogenicity studies. The lowest NOAEL established in these studies was 2.8 mg/kg/day for chronic effects. This value was also used in the MOE analysis to provide a very conservative estimate of the overall no-effect level for this material.

**POEA**

The lowest NOAEL of 15 mg/kg/day was selected as a reference point for risk assessment purposes; this value was based on maternal toxicity in the rat developmental toxicity study. As noted above with glyphosate, calculation of an MOE for children based on a NOAEL for maternal toxicity is not biologically relevant. Therefore, the MOE was also calculated using the NOEL of 2536 mg/kg/day from the subchronic rat study.
Estimation of Risks to Humans

The potential risks to humans resulting from exposure to glyphosate, AMPA, and POEA were determined for pesticide applicators and farm children age 1 to 6. Applicators were selected because they have the highest potential for exposure among adult sub-populations. The children were selected because they receive the highest dietary intake of all sub-populations on a mg/kg/day basis and are considered to represent a sensitive sub-population. Chronic risks were evaluated using a MOE analysis in which MOE values for each of the three substances were calculated by dividing the applicable NOAEL by the estimates of maximum chronic human exposure (Table 9). To assess acute risks, oral LD50s values in rats were divided by estimates of maximum acute human exposure. All MOE values were rounded to three significant figures.

Determination of an acceptable MOE relies on the judgment of the regulatory authority and varies with such factors as nature/severity of the toxicological endpoint observed, completeness of the database, and size of the exposed population. For compounds which have a substantial toxicological database, MOE values 100 or more are generally considered to indicate that the potential for causing adverse health effects is negligible.

Glyphosate

Chronic exposure. In children, the exposure resulting from ingestion of glyphosate residues in food and water was calculated to be 0.052 mg/kg/day. Exposure to professional applicators, which included exposure resulting from the spraying operation along with dietary intake, was estimated to be 0.0323 mg/kg/day. Comparison of these values to the NOAEL of 175 mg/kg/day...
based on maternal toxicity in the rabbit developmental toxicity study produced MOEs of 3370 and 5420 in children and adults, respectively. Using the more biologically relevant NOAEL of 209 mg/kg/day from the subchronic rat study, the MOE for children was 4020.

**Acute exposure.** Total acute exposure for children living on a farm was estimated by adding several potential exposures (reentry, bystander, consumption of sprayed wild foods, swimming in a pond) to that resulting from normal dietary intake as described above. The resulting exposure value was 0.09764 mg/kg/day. For applicators, the corresponding aggregate acute exposure value was calculated to be 0.125 mg/kg/day. The acute exposure calculation utilized peak dermal and inhalation measurements (instead of the mean value used for chronic exposure calculations) and included significant exposure from the consumption of sprayed wild foods. The oral LD50 of glyphosate is greater than 5000 mg/kg. The acute exposure values for both children and adult applicators are approximately 40,000 to 50,000 times lower than this value, indicating an extremely low potential for acute toxicity.

**AMPA**

**Chronic exposure.** The only significant source of AMPA exposure could occur from ingestion of treated crops in which the metabolite has been formed. Herbicide application does not result in exposure to AMPA, and the metabolite does not occur to an appreciable degree in water. The chronic exposure estimates for AMPA were calculated to be 0.0104 mg/kg/day for children and 0.0048 mg/kg/day for adults. MOEs were calculated using the definitive NOAEL of 400 mg/kg/day from the subchronic rat study and the lowest estimated NOAEL (> 2.8 mg/kg/day)
derived from long term studies with glyphosate. The corresponding MOEs are > 269 to 38,500 for children and > 583 to 83,300 for adult applicators.

**Acute exposure.** Individuals are not exposed to AMPA as bystanders or via reentry into sprayed areas, and levels of the metabolite in water are negligible. Therefore, acute exposure estimates are identical to chronic scenarios and were calculated to be 0.0104 mg/kg/day for children and 0.0048 mg/kg/day for adults. Based on the oral LD50 value of 8300 mg/kg, acute MOEs for children and adults are 798,000 and 1,730,000, respectively.

**POEA**

**Chronic exposure.** Aggregate exposure was calculated to be 0.026 mg/kg/day in children and 0.0325 mg/kg/day in adult applicators. The ingestion of food residues accounted for virtually all of the exposure in children, while dermal/inhalation exposure resulting from the spraying operation was the predominant pathway contributing to applicator exposure. Based on the NOAEL of 15 mg/kg/day for maternal toxicity in the rat developmental study, MOEs were determined to be 577 and 461 in children and adults, respectively. When the more biologically relevant NOAEL of 36.25 mg/kg/day from the subchronic rat study was used, the resulting MOE for children was calculated to be 1380.964.

**Acute exposure.** Estimates of aggregated acute exposure in adult applicators (0.163 mg/kg/day) and children (0.0911 mg/kg/day) were substantially higher than those for chronic exposure. In children, this increase was primarily due to contributions from reentry exposure.
and, to a lesser degree, the ingestion of wild foods. The acute oral LD50 of POEA is approximately 1200 mg/kg. The estimated acute exposure values are 736\(\frac{0}{2}\) to 13,204\(\frac{77}{2}\) times lower than this value.

**Overall Conclusions and Summary Statement**

This assessment was conducted for adult applicators and children (age 1 to 6) because they have the highest potential exposures. MOEs for worst-case chronic exposure to glyphosate, AMPA, and POEA ranged from 337065 to 542048, > 2697 to 83,30088,995, and 461 to 1380964, respectively. Based on these values, it is concluded that the potential for adverse effects resulting from chronic exposure to these substances is negligible. Acute exposures to glyphosate, AMPA, and POEA were estimated to be 736\(\frac{0}{2}\) - 1,730,000\(\frac{29,467}{6}\) times lower than the corresponding LD50 values, thereby demonstrating that potential acute exposure is not a health concern.

Estimates of exposure and thus, risk, to sub-populations other than those considered here would be significantly lower. Likewise, evaluations using more realistic estimates of exposure would demonstrate that the risks of adverse health effects are even lower that those calculated here. It is concluded that, under present and expected conditions of use, Roundup\textsuperscript{®} herbicide does not pose a health risk to humans.

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[page ]


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[page]
## Table 1  Acute Toxicity and Irritation of Roundup® Herbicides and POEA Surfactant

<table>
<thead>
<tr>
<th>Test Material</th>
<th>Oral LD50 (mg/kg)</th>
<th>Dermal LD50 (mg/kg)</th>
<th>Inhalation (mg/L)</th>
<th>Eye Irritation</th>
<th>Skin Irritation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roundup®</td>
<td>&gt;5000</td>
<td>&gt;5000</td>
<td>3.18</td>
<td>Severe</td>
<td>Slight</td>
</tr>
<tr>
<td>(41% IPAG)</td>
<td>(IV)</td>
<td>(IV)</td>
<td>(IV)</td>
<td>(I)</td>
<td>(IV)</td>
</tr>
<tr>
<td>POEA</td>
<td>1200</td>
<td>&gt;1260</td>
<td>---</td>
<td>Corrosive</td>
<td>Severe</td>
</tr>
<tr>
<td>Roundup® L &amp; G (18% IPAG)</td>
<td>&gt;5000</td>
<td>&gt;5000</td>
<td>&gt;5.7</td>
<td>Moderate</td>
<td>Very- Slightly None</td>
</tr>
<tr>
<td>Ready-to-Use (1% IPAG)</td>
<td>(IV)</td>
<td>(IV)</td>
<td>(IV)</td>
<td>(III)</td>
<td>(IV)</td>
</tr>
</tbody>
</table>

* IPAG - isopropylamine salt of glyphosate.

b Roman numerals in parentheses denote EPA categorized, where IV is the least toxic or irritating and I is the most toxic or irritating.

References - Roundup: oral and dermal LD50 (WHO, 1994), inhalation (Velasquez, 1983a), eye irritation (Bilas, 1990), skin irritation (Blaszcak, 1988); POEA: all studies (Birch, 1977); Roundup TA: oral, dermal, eye and skin (Auletta, 1985a-d), inhalation (Becchi, 1987); Roundup L&G Ready-to-Use: oral, dermal, eye, and skin (Blaszcak, 1987a,b,d,e), inhalation (Dudek, 1987)
<table>
<thead>
<tr>
<th>Test Organism</th>
<th>Endpoint</th>
<th>Compound (Purity)</th>
<th>Dose LED/HID $^a$</th>
<th>Without S9</th>
<th>With S9</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. typhimurium</em> TA98, TA100</td>
<td>Reverse mutation</td>
<td>Glyphosate (not specified)</td>
<td>0.025 mg/plate</td>
<td>!</td>
<td>! (S9 plant)</td>
<td>Wildeman and Nazar (1982)</td>
</tr>
<tr>
<td><em>S. typhimurium</em> TA98, TA100, TA1535, TA1537, TA1538</td>
<td>Reverse mutation</td>
<td>Glyphosate (not specified)</td>
<td>5 mg/plate</td>
<td>[symbol 33 ^f &quot;WP MathA&quot; &amp; 10]</td>
<td>[symbol 33 ^f &quot;WP MathA&quot; &amp; 10]</td>
<td>Moriya <em>et al.</em> (1983)</td>
</tr>
<tr>
<td><em>S. typhimurium</em> TA98, TA100, TA1535, TA1537, TA1538</td>
<td>Reverse mutation</td>
<td>Glyphosate (98%)</td>
<td>5 mg/plate</td>
<td>[symbol 33 ^f &quot;WP MathA&quot; &amp; 10]</td>
<td>[symbol 33 ^f &quot;WP MathA&quot; &amp; 10]</td>
<td>Li and Long (1988)</td>
</tr>
<tr>
<td><em>S. typhimurium</em> TA97, TA98, TA100, TA1535</td>
<td>Reverse mutation</td>
<td>Glyphosate (99%)</td>
<td>10 mg/plate</td>
<td>[symbol 33 ^f &quot;WP MathA&quot; &amp; 10]</td>
<td>[symbol 33 ^f &quot;WP MathA&quot; &amp; 10]</td>
<td>NTP, 1992</td>
</tr>
<tr>
<td><em>S. typhimurium</em> TA98, TA100, TA1535, TA1537, TA1538, TA1978</td>
<td>Reverse mutation</td>
<td>Roundup® (glyphosate 48%, isopropylamine salt, 36%)</td>
<td>5 mg/plate</td>
<td>!</td>
<td>!</td>
<td>Njagi and Gopalan (1980)</td>
</tr>
<tr>
<td><em>S. typhimurium</em> TA98</td>
<td>Reverse mutation</td>
<td>Roundup® (glyphosate 48%, 7.7% MON 0841&amp;POEA)</td>
<td>1.44 mg/plate</td>
<td>[symbol 33 ^f &quot;WP MathA&quot; &amp; 10]</td>
<td>[symbol 33 ^f &quot;WP MathA&quot; &amp; 10]</td>
<td>Rank <em>et al.</em> (1993)</td>
</tr>
<tr>
<td>Test Organism</td>
<td>Endpoint</td>
<td>Compound (Purity)</td>
<td>Dose LED/HID</td>
<td>EVALUATION</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>---------------</td>
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<td></td>
</tr>
<tr>
<td>S. typhimurium TA100</td>
<td>Reverse mutation</td>
<td>Roundup® (glyphosate 48%; MON 61416 &amp; POEA)</td>
<td>0.72 mg/plate</td>
<td>[symbol 33 ( \leq ) &quot;WP MathA&quot; ( \leq 10 )]</td>
<td>+</td>
<td>Rank et al. (1993)</td>
</tr>
<tr>
<td>S. typhimurium TA98, TA100, A1535, TA1537, TA1538</td>
<td>Reverse mutation</td>
<td>Roundup® (glyphosate 30.4%; 15% MON-0818 POEA)</td>
<td>0.5 mg/plate</td>
<td>[symbol 33 ( \leq ) &quot;WP MathA&quot; ( \leq 10 )]</td>
<td>+</td>
<td>Monsanto (1992); Kier et al. (1997)</td>
</tr>
<tr>
<td>S. typhimurium TA98, TA100, A1535, TA1537, TA1538</td>
<td>Reverse mutation</td>
<td>Rodeo® (glyphosate as isopropylamine salt, 54% isopropylamine)</td>
<td>5 mg/plate</td>
<td>[symbol 33 ( \leq ) &quot;WP MathA&quot; ( \leq 10 )]</td>
<td>+</td>
<td>Monsanto (1992); Kier et al. (1997)</td>
</tr>
<tr>
<td>S. typhimurium TA98, TA100, A1535, TA1537, TA1538</td>
<td>Reverse mutation</td>
<td>Direct® (glyphosate as ammonium salt; 72% Echelon + T25 surfactant)</td>
<td>0.5 mg/plate</td>
<td>[symbol 33 ( \leq ) &quot;WP MathA&quot; ( \leq 10 )]</td>
<td>+</td>
<td>Monsanto (1992); Kier et al. (1997)</td>
</tr>
<tr>
<td>E. coli WP2/uvr</td>
<td>Reverse-mutation</td>
<td>Glyphosate (not-specified)</td>
<td>2.5 mg/plate</td>
<td>[symbol 33 ( \leq ) &quot;WP MathA&quot; ( \leq 10 )]</td>
<td></td>
<td>Shimizu et al. (1982)</td>
</tr>
<tr>
<td>Test Organism</td>
<td>Endpoint</td>
<td>Compound (Purity)</td>
<td>Dose LED/HID[^a]</td>
<td>Without S9</td>
<td>With S9</td>
<td>Reference</td>
</tr>
<tr>
<td>-------------------------------</td>
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<tr>
<td><em>E. coli</em> WP2 hcr</td>
<td>Reverse mutation</td>
<td>Glyphosate (not specified)</td>
<td>5 mg/plate</td>
<td>[symbol ^33 (\text{WP MathA} \leq 10)]</td>
<td></td>
<td>Moriya et al. (1983)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5 mg/plate with S9, 1 mg/plate without S9</td>
<td>[symbol ^33 (\text{WP MathA} \leq 10)]</td>
<td></td>
<td>Li and Long (1988)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glyphosate (98%)</td>
<td>22.5 mg/mL</td>
<td>[symbol ^33 (\text{WP MathA} \leq 10)]</td>
<td></td>
<td>Li and Long (1988)</td>
</tr>
<tr>
<td><em>D. melanogaster</em></td>
<td>Sex-linked recessive lethals</td>
<td>Roundup® (glyphosate 41%-70% MON-0818POE-A) (chronic to pupation)</td>
<td>1 mg/L (1ppm)</td>
<td>+</td>
<td>0</td>
<td>Kale et al. (1995)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Roundup® (not specified)</td>
<td>10</td>
<td>[symbol ^33 (\text{WP MathA} \leq 10)]</td>
<td></td>
<td>Gopalan and Njagi (1981)</td>
</tr>
</tbody>
</table>
**Table 2**  Summary of Results on the Genotoxicity of Glyphosate, Roundup®, and Other Glyphosate Formulations

<table>
<thead>
<tr>
<th>Test Organism</th>
<th>Endpoint</th>
<th>Compound (Purity)</th>
<th>Dose LED/HID[^a]</th>
<th>Without S9</th>
<th>With S9</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Allium cepa</em> (onion root tip)</td>
<td>Chromosomal aberrations</td>
<td>Glyphosate (isopropylamine salt)</td>
<td>2.88 mg/L</td>
<td>0</td>
<td>0</td>
<td>Rank <em>et al.</em> (1993)</td>
</tr>
<tr>
<td><em>Allium cepa</em> (onion root tip)</td>
<td>Chromosomal aberrations</td>
<td>Roundup® (glyphosate 48%; 75% MON-6094 POEA)</td>
<td>1.44 mg/L</td>
<td>+</td>
<td>0</td>
<td>Rank <em>et al.</em> (1993)</td>
</tr>
<tr>
<td><em>V. faba</em> (root tips)</td>
<td>Chromosomal aberrations</td>
<td>Solado (glyphosate 21%)</td>
<td>1.4 mg/mL</td>
<td>+</td>
<td>0</td>
<td>De Marcella <em>et al.</em> (1992)</td>
</tr>
<tr>
<td>Peripheral lymphocytes (human) in vitro</td>
<td>Chromosomal aberrations</td>
<td>Glyphosate (&gt;98%)</td>
<td>0.56 mg/mL without S9</td>
<td>[symbol 33 ( WP MathA ) ( \leq 10 )]</td>
<td>0</td>
<td>Nøtbo <em>van de Waart</em> (1995a)</td>
</tr>
<tr>
<td>Peripheral lymphocytes (human) in vitro</td>
<td>Chromosomal aberrations</td>
<td>Glyphosate (&gt;98%)</td>
<td>1.4 mg/L</td>
<td>+</td>
<td>0</td>
<td>Lioi <em>et al.</em> (1998a)</td>
</tr>
<tr>
<td>Peripheral lymphocytes (bovine) in vitro</td>
<td>Chromosomal aberrations</td>
<td>Glyphosate (&gt;98%)</td>
<td>2.9 mg/L</td>
<td>+</td>
<td>0</td>
<td>Lioi <em>et al.</em> (1998b)</td>
</tr>
<tr>
<td>Rat bone marrow (<em>in vivo</em>) 6, 12, 24 h</td>
<td>Chromosomal aberration</td>
<td>Glyphosate (98%)</td>
<td>1.0g/kg</td>
<td></td>
<td>0</td>
<td>Li and Long (1988)</td>
</tr>
</tbody>
</table>

**CHROMOSOMAL ABERRATION**
<table>
<thead>
<tr>
<th>Test Organism</th>
<th>Endpoint</th>
<th>Compound (Purity)</th>
<th>Dose LED/HID</th>
<th>Without S9</th>
<th>With S9</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral blood (human) in vitro</td>
<td>SCE</td>
<td>Roundup® (not specified)</td>
<td>2.5 mg/mL</td>
<td>+</td>
<td>0</td>
<td>Vigfusson and Vyse (1980)</td>
</tr>
<tr>
<td>Peripheral blood (human) in vitro</td>
<td>SCE</td>
<td>Glyphosate (99.9%)</td>
<td>1.0 mg/mL</td>
<td>+</td>
<td>0</td>
<td>Bolognesi et al. (1997)</td>
</tr>
<tr>
<td>Peripheral blood (human) in vitro</td>
<td>SCE</td>
<td>Roundup® (glyphosate 30.4:15% MON-818 _ surfactant)</td>
<td>0.1 mg/mL</td>
<td>+</td>
<td>0</td>
<td>Bolognesi et al. (1997)</td>
</tr>
<tr>
<td>Peripheral blood (human) in vitro</td>
<td>SCE</td>
<td>Glyphosate (&gt;98%)</td>
<td>2.91.4 mg/L</td>
<td>+</td>
<td>0</td>
<td>Lioi et al. (1998a)</td>
</tr>
<tr>
<td>Peripheral lymphocytes (bovine) in vitro</td>
<td>SCE</td>
<td>Glyphosate (&gt;98%)</td>
<td>2.9 mg/L</td>
<td>+</td>
<td>0</td>
<td>Lioi et al. (1998b)</td>
</tr>
<tr>
<td>V. faba (root tips)</td>
<td>Micronucleus test</td>
<td>Solado (glyphosate 21%)</td>
<td>1.4 mg/kg, soil</td>
<td></td>
<td>0</td>
<td>De Marco et al. (1992)</td>
</tr>
<tr>
<td>Mouse bone marrow (in vitro), dietary for 13 weeks</td>
<td>Micronucleus test</td>
<td>Glyphosate (99%)</td>
<td>11.379 mg/kg/day</td>
<td></td>
<td>0</td>
<td>NTP, 1992</td>
</tr>
</tbody>
</table>
Table 2  Summary of Results on the Genotoxicity of Glyphosate, Roundup®, and Other Glyphosate Formulations

<table>
<thead>
<tr>
<th>Test Organism</th>
<th>Endpoint</th>
<th>Compound (Purity)</th>
<th>Dose LED/HID \textsuperscript{a}</th>
<th>Without S9</th>
<th>With S9</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse bone marrow (\textit{in vivo}) i.p. injection, 24h</td>
<td>Micronucleus test</td>
<td>Glyphosate (not specified)</td>
<td>200 mg/kg</td>
<td>[symbol 33 \textsuperscript{b} \text{&quot;WP MathA&quot; \textbackslash s 10}]</td>
<td>0</td>
<td>Rank et al. (1993)</td>
</tr>
<tr>
<td>Mouse bone marrow (\textit{in vivo}) i.p. injection, 24h</td>
<td>Micronucleus test</td>
<td>Roundup® (glyphosate 48%, 7.76% MON-0818POEA)</td>
<td>200 mg/kg</td>
<td>[symbol 33 \textsuperscript{b} \text{&quot;WP MathA&quot; \textbackslash s 10}]</td>
<td>0</td>
<td>Rank et al. (1993)</td>
</tr>
<tr>
<td>Mouse bone marrow (\textit{in vivo}) i.p. injection</td>
<td>Micronucleus test</td>
<td>Glyphosate (99.9%)</td>
<td>300 mg/kg</td>
<td>+</td>
<td>0</td>
<td>Bolognesi et al. (1997)</td>
</tr>
<tr>
<td>Mouse bone marrow (\textit{in vivo}) i.p. injection</td>
<td>Micronucleus test</td>
<td>Roundup® (glyphosate 30.4%, 15% MON-0818POEA)</td>
<td>135 mg/kg</td>
<td>+</td>
<td>0</td>
<td>Bolognesi et al. (1997)</td>
</tr>
<tr>
<td>Mouse bone marrow (\textit{in vivo}) i.p. injection</td>
<td>Micronucleus test</td>
<td>Roundup® (glyphosate 30.4%, 15% MON-0818POEA)</td>
<td>555 mg/kg</td>
<td>[symbol 33 \textsuperscript{b} \text{&quot;WP MathA&quot; \textbackslash s 10}]</td>
<td>0</td>
<td>Monsanto (1992); Kier et al. (1997)</td>
</tr>
<tr>
<td>Mouse bone marrow (\textit{in vivo}) i.p. injection</td>
<td>Micronucleus test</td>
<td>Rodeo® (glyphosate IPA 54%, water)</td>
<td>3400 mg/kg</td>
<td>[symbol 33 \textsuperscript{b} \text{&quot;WP MathA&quot; \textbackslash s 10}]</td>
<td>0</td>
<td>Monsanto (1992); Kier et al. (1997)</td>
</tr>
</tbody>
</table>
## Table 2  Summary of Results on the Genotoxicity of Glyphosate, Roundup®, and Other Glyphosate Formulations

<table>
<thead>
<tr>
<th>Test Organism</th>
<th>Endpoint</th>
<th>Compound (Purity)</th>
<th>Dose LED/HID a</th>
<th>EVALUATION b</th>
<th>Without S9</th>
<th>With S9</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse bone marrow (in vivo) gavage</td>
<td>Micronucleus test</td>
<td>Direct (glyphosate 72% NH₄ salt)</td>
<td>365 mg/kg</td>
<td></td>
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<td>Monsanto (1992); Kier et al. (1997)</td>
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<tr>
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<td>Dominant lethal</td>
<td>Glyphosate (98.7%)</td>
<td>2060 mg/kg</td>
<td></td>
<td>1</td>
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<td>Wrenn (1980)</td>
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<tr>
<td>B. subtilis H17, rec+, M45, rec-</td>
<td>rec-assay</td>
<td>Glyphosate (98%)</td>
<td>2 mg/disk</td>
<td></td>
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<td>[symbol 33 ( \leq 10 )] WP MathA</td>
<td>Li and Long (1988)</td>
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<tr>
<td>Rat hepatocytes (exposed in vitro)</td>
<td>UDS</td>
<td>Glyphosate (98%)</td>
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<td>[symbol 33 ( \leq 10 )] WP MathA</td>
<td>[symbol 33 ( \leq 10 )] WP MathA</td>
<td>Li and Long (1988)</td>
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<tr>
<td>Mouse i.p. exposure (in vivo)</td>
<td>DNA adducts</td>
<td>Glyphosate (isopropylamine salt)</td>
<td>270 mg/kg</td>
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<td>Peluso et al. (1998)</td>
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<tr>
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<td>[symbol 33 ( \leq 10 )] WP MathA</td>
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<tr>
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<td>DNA single-strand breaks</td>
<td>Glyphosate (99.9%)</td>
<td>300 mg/kg</td>
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<td>+</td>
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<td>Bolognesi et al. (1997)</td>
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<tr>
<td>Test Organism</td>
<td>Endpoint</td>
<td>Compound (Purity)</td>
<td>Dose LED/HID&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Without S9</td>
<td>With S9</td>
<td>Reference</td>
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<tr>
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<td>-------------------------------</td>
<td>----------------------------------------</td>
<td>---------------------------</td>
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<tr>
<td>Mouse i.p. exposure (in vivo)</td>
<td>alkaline elution of extracted DNA</td>
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<td>+</td>
<td>0</td>
<td>Bolognesi et al. (1997)</td>
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<tr>
<td><em>R. catesbeiana</em> (tadpole)</td>
<td>DNA single-strand breaks: Comet assay</td>
<td>Roundup® (glyphosate 30.4%; 15% MON0888, POHA)</td>
<td>6.75 mg/L</td>
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<td>8-OHdG</td>
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<td>300 mg/kg</td>
<td>+/[-[symbol of 33 (WP MathA) (s 10)]</td>
<td>0</td>
<td>Bolognesi et al. (1997)</td>
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<sup>a</sup> Lowest Effective Dose/ Highest Ineffective Dose  
<sup>b</sup> + = positive, - = negative, 0 = not tested
### Table 3  Summary Incidence of Microscopic Findings in a 2-Year Rat Study with Glyphosate

<table>
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<tr>
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<td>0 (60)</td>
<td>0 (60)</td>
<td>0 (60)</td>
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<td>1 (60)</td>
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<td><strong>Testis (es)</strong></td>
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<td>16 (60)</td>
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<td>1 (60)</td>
<td>0 (60)</td>
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<td><strong>Uterus</strong></td>
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<td>3 (60)</td>
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<tr>
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<td>0 f (60)</td>
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<tr>
<td><strong>Mammary gland</strong></td>
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<tr>
<td>adenoma/adenofibroma/fibroma</td>
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<td>1 m (41)</td>
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<td>3 m (31)</td>
<td>2 m (41)</td>
<td>2 m (37)</td>
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<td>8 f (58)</td>
<td>14 f (54)</td>
<td>4 f (59)</td>
<td>9 f (57)</td>
</tr>
<tr>
<td>prominent secretory activity</td>
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<td>16 f (58)</td>
<td>19 f (54)</td>
<td>13 f (59)</td>
<td>22 f (57)</td>
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</table>
Table 3  Summary Incidence of Microscopic Findings in a 2-Year Rat Study with Glyphosate\textsuperscript{a}

<table>
<thead>
<tr>
<th>Dose levels (ppm)</th>
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</tr>
</thead>
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<td>0 m (41)</td>
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<td>13 f (58)</td>
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<td>9 f (57)</td>
</tr>
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<td>adenoacanthoma</td>
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<td>0 m (41)</td>
<td>1 m (37)</td>
</tr>
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<td>0 f (59)</td>
<td>1 f (57)</td>
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<td>0 m (31)</td>
<td>0 m (41)</td>
<td>0 m (37)</td>
</tr>
<tr>
<td></td>
<td>0 f (58)</td>
<td>1 f (54)</td>
<td>0 f (59)</td>
<td>1 f (57)</td>
</tr>
<tr>
<td>fibrosis</td>
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<td>0 f (59)</td>
<td>0 f (57)</td>
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Thyroid

<table>
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<td>C cell adenoma</td>
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<td>2 f (60)</td>
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<td>C cell hyperplasia</td>
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<td>5 f (60)</td>
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\textsuperscript{a} Data from Stont and Ruecker, 1990

\textsuperscript{b} All deaths reported. Incidence (total number of animals examined).

\textit{m = males, f = females}
<table>
<thead>
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<td><strong>Generation</strong></td>
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<td>F1A</td>
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<td>Total paired females</td>
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<td>30</td>
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<tr>
<td>Females with confirmed copulation/total paired</td>
<td>96.7%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Pregnant/total paired</td>
<td>80.0%</td>
<td>93.3%</td>
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<tr>
<td>Pregnant/confirmed copulation</td>
<td>82.8%</td>
<td>93.3%</td>
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<tr>
<td>Males with confirmed copulation/total paired</td>
<td>86.7%</td>
<td>93.3%</td>
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<tr>
<td>Males impregnating females/total paired</td>
<td>70%</td>
<td>90.0%</td>
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<tr>
<td>Males impregnating females/confirmed copulation</td>
<td>80.8%</td>
<td>96.4%</td>
</tr>
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<td>Precoital length for pregnant animals (days)</td>
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<td>Gestational length (days)</td>
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<tr>
<td>vacuolation, duct epithelium</td>
<td>1 (30)**</td>
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<tr>
<td>inflammation, mononuclear, interstitial</td>
<td>1(30)</td>
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<tr>
<td>chronic inflammation, fibrosis</td>
<td>1 (29)</td>
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Table 4  Summary of Reproductive and Microscopic Findings in a 2-Generation Rat Reproduction Study with Glyphosate

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<thead>
<tr>
<th>Dose Levels (ppm)</th>
<th>0</th>
<th>30,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Generation</td>
<td>FO F1A</td>
<td>F1A-Remate FO F1A F1A-Remate</td>
</tr>
<tr>
<td>periepididymal adipose tissue, inflammation, granulomatous</td>
<td>1 (29)</td>
<td></td>
</tr>
<tr>
<td>hypospermia, unilateral</td>
<td>1 (29)</td>
<td></td>
</tr>
<tr>
<td>Testis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hypoplasia/atrophy seminiferous tubule, bilateral</td>
<td>2 (30) 1 (30) 1 (30)</td>
<td></td>
</tr>
<tr>
<td>Degeneration seminiferous tubules, unilateral</td>
<td>1 (30) 1 (29)</td>
<td></td>
</tr>
<tr>
<td>hemorrhage</td>
<td>1 (30)</td>
<td>1 (29)</td>
</tr>
<tr>
<td>granuloma, spermatic</td>
<td>1 (29)</td>
<td></td>
</tr>
<tr>
<td>Ovary (ies)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cyst (s)</td>
<td>3 (30)</td>
<td>1 (30)</td>
</tr>
<tr>
<td>inactive</td>
<td>1 (30)</td>
<td></td>
</tr>
<tr>
<td>Uterus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>remnant, implantation site</td>
<td>10 (29) 11 (29) 7 (29) 13 (29))</td>
<td></td>
</tr>
<tr>
<td>mesometrium, calcified implantation remnant</td>
<td>1(29)</td>
<td></td>
</tr>
<tr>
<td>dilation of uterine lumen (hydrometra)</td>
<td>5 (29) 5 (29) 9(29) 7 (29)</td>
<td></td>
</tr>
<tr>
<td>pigment deposition</td>
<td>3 (29) 7(29)</td>
<td></td>
</tr>
<tr>
<td>mononuclear infiltrate endometrium</td>
<td>1 (29) 1 (29)</td>
<td></td>
</tr>
<tr>
<td>vascular necrosis mesometrium</td>
<td>1 (29)</td>
<td></td>
</tr>
<tr>
<td>Vagina</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mononuclear cell infiltrate</td>
<td>1 (29)</td>
<td></td>
</tr>
<tr>
<td>Prostate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>chronic inflammation</td>
<td>14 (30) 4 (29) 12 (30) 12 (29)</td>
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</tr>
<tr>
<td>mononuclear cell infiltrate</td>
<td>1 (29) 1 (29)</td>
<td></td>
</tr>
<tr>
<td>edema</td>
<td>2 (29)</td>
<td>0 (29)</td>
</tr>
<tr>
<td>Seminal vesicle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mononuclear cell infiltrate</td>
<td>1 (29) 1 (29)</td>
<td></td>
</tr>
<tr>
<td>Dose Levels (ppm)</td>
<td>0</td>
<td>30,000</td>
</tr>
<tr>
<td>------------------</td>
<td>---</td>
<td>--------</td>
</tr>
<tr>
<td><strong>Generation</strong></td>
<td>FO</td>
<td>F1A</td>
</tr>
<tr>
<td><strong>Pituitary</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cyst (s)</td>
<td>2 m (30)</td>
<td></td>
</tr>
<tr>
<td>adenoma - pars distalis</td>
<td>1 f (30)</td>
<td></td>
</tr>
<tr>
<td><strong>Mammary gland</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>galactocele</td>
<td>1 f (28)</td>
<td></td>
</tr>
<tr>
<td>mononuclear cell, infiltrate</td>
<td>1 m (25)</td>
<td></td>
</tr>
</tbody>
</table>

* Data from Reyna, 1990

** Incidence (total number of animals examined)

Significantly different from control, **p < 0.01

m = males
f = females
Table 5 Worst-Case Daily Exposure Estimates for Glyphosate, AMPA, and POEA (µg/kg/day)

<table>
<thead>
<tr>
<th>Nature/Source of Exposure</th>
<th>Glyphosate</th>
<th>AMPA</th>
<th>POEA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female adult applicator</td>
<td>1-6 year female child</td>
<td></td>
</tr>
<tr>
<td></td>
<td>acute</td>
<td>chronic</td>
<td>acute</td>
</tr>
<tr>
<td>Routine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Dietary</td>
<td>23.8</td>
<td>23.8</td>
<td>51.9</td>
</tr>
<tr>
<td>- Application</td>
<td>56.2</td>
<td>8.5</td>
<td>--</td>
</tr>
<tr>
<td>Occasional</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Drinking Water</td>
<td>3.6x10^-2</td>
<td>2.1x10^-3</td>
<td>0.11</td>
</tr>
<tr>
<td>- Reentry</td>
<td>--</td>
<td>--</td>
<td>26</td>
</tr>
<tr>
<td>- Bystander</td>
<td>--</td>
<td>--</td>
<td>4.4</td>
</tr>
<tr>
<td>Infrequent/ rare</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Swimming</td>
<td>1.28</td>
<td>--</td>
<td>6.5</td>
</tr>
<tr>
<td>- Wild Foods</td>
<td>45</td>
<td>--</td>
<td>45</td>
</tr>
<tr>
<td>Aggregate*</td>
<td>125</td>
<td>32.3</td>
<td>97</td>
</tr>
</tbody>
</table>

* Aggregate exposure is the sum of dietary, drinking water, and application derived contributions, plus 45 µg glyphosate/kg/day or either 23 (adults) or 65 (children) µg POEA/kg/day acute exposure to account for all incidental exposures related to occasional behaviours. For AMPA, aggregate exposure is the sum of dietary and drinking water contributions, since no other routes provided significant incremental contributions.
<table>
<thead>
<tr>
<th></th>
<th>Typical low-end exposure</th>
<th>Typical high-end exposure</th>
<th>Worst-case exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult Applicator</td>
<td>5.6</td>
<td>10.4</td>
<td>25.8</td>
</tr>
<tr>
<td>Child Age 1 to 6</td>
<td>14.0</td>
<td>24.0</td>
<td>51.9</td>
</tr>
<tr>
<td>Type of Study and Species Tested</td>
<td>NOAEL (mg/kg/day)</td>
<td>Comments</td>
<td>Study Reference</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>-------------------</td>
<td>----------</td>
<td>----------------</td>
</tr>
<tr>
<td><strong>Subchronic toxicity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse, 90-day</td>
<td>2310</td>
<td>based on decreased b.w. gain and changes in organ weights</td>
<td>Tierney, Bio/Dynamics, 1979</td>
</tr>
<tr>
<td>Dog, 90-day</td>
<td>630</td>
<td>based on salivary gland lesions</td>
<td>NTPP, 1992</td>
</tr>
<tr>
<td>Rat, 90-day</td>
<td>1445</td>
<td>no adverse effects at HDT&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Mensan, 1987</td>
</tr>
<tr>
<td>Rat, 90-day</td>
<td>209</td>
<td>salivary gland changes at the lowest dose tested not considered toxicologically significant</td>
<td>NTP, 1992</td>
</tr>
<tr>
<td>Dog, 12-month</td>
<td>500</td>
<td>no adverse effects at HDT</td>
<td>Reyna and Rucker, Mensan, 1985</td>
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<tr>
<td><strong>Chronic toxicity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse, 24-month</td>
<td>885</td>
<td>based on liver effects</td>
<td>Koveszich, Bio/Dynamics, 1983</td>
</tr>
<tr>
<td>Rat, 26-month</td>
<td>30</td>
<td>no adverse effects at HDT</td>
<td>Lankos, Bio/Dynamics, 1981</td>
</tr>
<tr>
<td>Rat, 24-month</td>
<td>409</td>
<td>based on decreased b.w. gain and ocular lesion</td>
<td>Mensan, 1990</td>
</tr>
<tr>
<td><strong>Developmental toxicity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>1000</td>
<td>based on maternal and fetal effects</td>
<td>Tasker, Bio/Dynamics, 1980b</td>
</tr>
<tr>
<td>Rabbit</td>
<td>175</td>
<td>based on maternal toxicity</td>
<td>Tasker, IRDC, 1980b</td>
</tr>
<tr>
<td><strong>Reproductive toxicity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>30</td>
<td>no adverse effects at HDT</td>
<td>Schroeder, IRDC, 1981</td>
</tr>
<tr>
<td>Rat</td>
<td>69</td>
<td>based on systemic toxicity; no reproductive effect</td>
<td>Mensan, Reyna, 1990</td>
</tr>
</tbody>
</table>

<sup>a</sup> b.w. = body weight  
<sup>b</sup> HDT = highest dose tested
<table>
<thead>
<tr>
<th>Table 87</th>
<th>AMPA NOAELs for Toxicological Endpoints</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of Study and Species Tested</td>
<td>NOAEL (mg/kg/day)</td>
</tr>
<tr>
<td>Subchronic toxicity</td>
<td></td>
</tr>
<tr>
<td>Rat, 90-day</td>
<td>400</td>
</tr>
<tr>
<td>Dog, 90-day</td>
<td>263</td>
</tr>
<tr>
<td>Chronic toxicity</td>
<td>&gt;2.8</td>
</tr>
<tr>
<td>Rat, 24 month</td>
<td></td>
</tr>
<tr>
<td>Developmental toxicity</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>400</td>
</tr>
<tr>
<td>Reproductive toxicity</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>&gt;423.4</td>
</tr>
</tbody>
</table>

* b.w. = body weight
<table>
<thead>
<tr>
<th>Table 98</th>
<th>POEA NOAELs for Toxicological Endpoints</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type of Study and Species Tested</strong></td>
<td><strong>NOAEL (mg/kg/day)</strong></td>
</tr>
<tr>
<td>Subchronic Toxicity</td>
<td></td>
</tr>
<tr>
<td>Rat, 1-month</td>
<td>527</td>
</tr>
<tr>
<td>Rat, 3-month</td>
<td>3536</td>
</tr>
<tr>
<td>Dog, 14-week</td>
<td>$\leq$30</td>
</tr>
<tr>
<td>Developmental Toxicity</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>15</td>
</tr>
</tbody>
</table>

* b.w. = body weight
<table>
<thead>
<tr>
<th>Chemical</th>
<th>NOAEL (mg/kg/day)</th>
<th>Basis of NOAEL</th>
<th>Worst-case chronic exposure (mg/kg/day)</th>
<th>Margin of exposure* Adults</th>
<th>Margin of exposure* Children</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Adults</td>
<td>Children</td>
<td>Adults</td>
</tr>
<tr>
<td>Glyphosate</td>
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<td>Maternal toxicity in developmental toxicity study</td>
<td>0.0323</td>
<td>0.052</td>
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<td></td>
<td>209</td>
<td>90-day rat study</td>
<td>...</td>
<td>...</td>
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<tr>
<td>AMPA</td>
<td>400</td>
<td>90-day rat and developmental toxicity studies</td>
<td>0.0048</td>
<td>0.0104</td>
<td>83,300</td>
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<tr>
<td></td>
<td>&gt;2.8</td>
<td>Based on AMPA content in glyphosate used for chronic rat study</td>
<td>&gt;583</td>
<td>&gt;269</td>
<td>&gt;583</td>
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<tr>
<td>POEA</td>
<td>15</td>
<td>Maternal toxicity in developmental toxicity study</td>
<td>0.0325</td>
<td>0.026</td>
<td>461</td>
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<td>2536</td>
<td>90-day rat study</td>
<td>...</td>
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</tbody>
</table>

* All MOE values rounded to 3 significant figures.
Figure 1: Mechanism of action for glyphosate in plants. Glyphosate inhibits synthesis of essential aromatic amino acids by competitive inhibition of the enzyme enolpyruvylshikimate phosphate synthase (EPSPS).
Figure 2: A simplified pathway for degradation of glyphosate in the terrestrial environment. Adapted from R. Wiersema, M. Burns, and D. Hershberger. University of Minnesota, 1997.