Message

From: HEYDENS, WILLIAM F [FND/1000] [/O=MONSANTO/OU=NA-1000-01/CN=RECIPIENTS/CN=230737]
Sent: 9/15/1999 4:55:44 PM
To: FARMER, DONNA R [FND/1000] [REDACTED@stl.Monsanto.com]; WRATTEN, STEPHEN J [FND/1000] [REDACTED@stl.Monsanto.com]
Subject: FW:
Attachments: glyphosate150999.PDF; Tableall.PDF

FYI - in case you want to see how it ended up (hopefully, that is! - I'll strangle Kroes or Williams if they ask for any re-writes!!)

Bill

-----Original Message-----
From: Douglas Bryant [mailtoREDACTEDt@cantox.com]
Sent: Wednesday, September 15, 1999 11:12 AM
To: william.f.heydens@REDACTED
Cc: lisa.m.drake@REDACTED; Katherine H. Carr
Subject:

Dr. William F. Heydens

Bill:

Attached is the revised draft that is being sent to Dr. Gary Williams and Dr. Robert Kroes and Dr. Ian Munro today. This draft includes all the changes that were discussed today and during calls last week. Please check it over to be sure that I have been thorough.

I have asked each author to complete his review and respond so journal submission (Food and Chemical Toxicology) can be finalized for September 30, 1999. My cover letter identifies the reorganizational changes made along with alterations and edits suggested by each author.

I am sending hard copies by courier and these *.pdf files as backup.

I would like to thank you for all your effort (undoubtedly there will be more) and consideration as we have made our way to this point. Of course, I do not forget the input from the many sources that has been included in this task. I certainly hope that everyone involved, including Drs Williams and Kroes, appreciates the many contributions, hard work, and fine craftsmanship that I believe this work exhibits. No review (especially of this size and breadth) can ever be absolutely comprehensive or complete, but I believe this work satisfies the objectives set out two years ago. I look forward to pushing on to publication.

Douglas

PS Could Kathy Carr distribute the attached in Monsanto? (the files are rather large)

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

GLYPHOSATE150999.DOC

***** DRAFT *****

September 15, 1999

Abstract--Reviews on the safety of glyphosate and Roundup® herbicide that have been conducted by several regulatory agencies and scientific institutions worldwide have concluded that there is no indication of any human health concern. Nevertheless, questions regarding its safety are periodically raised. 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. No significant toxicity occurred in acute, subchronic, and chronic studies. Direct ocular exposure to the concentrated Roundup® formulation can result in transient irritation, while normal spray dilutions cause, at most, only minimal effects.

The genotoxicity data for glyphosate and Roundup® were 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 LD_{50} 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.

Abbreviations: 8! OHdG = 8-hydroxylguanine; AAPCC = American Association of Poison Control Centers; AE = acid equivalents; AI = active ingredient; AMPA = aminomethylphosphonic acid; AUC = area under the curve; CHO = Chinese hamster ovary; GLP = Good Laboratory Practices; IPA = isopropylamine; MCL = maximum contaminant level; MNPCE = micronucleated PCE; MOE = margin of exposure; MRL = Maximum Residue Levels; NCEs = normochromatic erythrocytes; NOAEL = no-observed-adverse effect levels; NOEC = no-observed-effect concentration; NTP = National Toxicology Program; PCEs = polychromatic erythrocytes; POEA = polyethoxylated tallow amine; SAP = Scientific Advisory Panel; SCE = sister chromatid exchange assay; SSB = single strand breaks; TMDI = Theoretical Maximum Daily Intake; U.S. EPA = United States Environmental Protection Agency; UDS = unscheduled DNA synthesis; WHO = World Health Organization

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 of amino acid biosynthesis 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 to innocuous 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 in agricultural, industrial, forestry, and residential weed control settings. Glyphosate-based products have been increasingly used by farmers in field preparation prior to planting, and in no-till soil conservation programs. The use of glyphosate in agriculture continues to expand particularly in applications involving plant varieties that are genetically modified to tolerate glyphosate treatment (Roundup-Ready®). Today, a variety of glyphosate-based formulations such as Roundup® are registered in more than 100 countries and are available under different brand names 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 extensively 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 [Health Canada, United States Environmental Protection Agency (U.S. EPA), and World Health Organization (WHO)] are publicly available (Health and Welfare Canada, 1986, 1992; U.S. EPA, 1993, 1997, 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. Data on Roundup® and glyphosate are constantly re-evaluated by regulatory agencies in a science-based process for many reasons including its volume of production and new uses. Nevertheless, questions regarding its safety are periodically raised.

The purpose of this review is to critically assess the current information pertaining to the safety of glyphosate and Roundup® and to produce a comprehensive 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 that have been raised by special interest groups. This review will focus on technical glyphosate acid; its major breakdown product aminomethylphosphonic acid; 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. Glyphosate-based products are currently manufactured by a variety of companies world-wide. Some sources of information including, studies produced by manufacturers of glyphosate-based products other than Monsanto, are not generally available and as such were not considered for this risk assessment. Data for such products are proprietary and not readily available, and therefore were not evaluated for inclusion in this risk assessment.

Principles of the Risk Assessment Process

The risk assessment process involves the characterization of toxicities and estimation of possible adverse outcomes from specific chemical exposures (CCME, 1996; Environment Canada, 1997; NRC, 1983; U.S. EPA, 1995, 1996a). The NRC (1983) and U.S. EPA Draft Cancer Risk Assessment Guidelines (1996b) define risk characterization as the step in the risk assessment process that integrates hazard identification, dose-response assessment, and exposure assessment, using a combination of qualitative and quantitative information. Risk assessment can provide a comprehensive estimate of the potential effect in specific, well defined and described circumstances.

Hazard identification assesses the capacity of an environmental agent to cause adverse effects in experimental systems. This is a qualitative description based on several factors such as availability of human data, data from laboratory animals, and any ancillary information (*e.g.*, structure activity analysis, genetic toxicity, pharmacokinetics) from other studies. Finally, a weight-of-evidence is prepared based on data accumulated from many sources, where a mode of action is suggested, responses in experimental animals are evaluated and the relevance of these to human outcomes is discussed (U.S. EPA, 1995).

The determination of hazard is often dependent on whether a dose-response relationship is available (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, and 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 addition 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 pK_a 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 2.13 Molar (356 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 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 effects, 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 including 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 are compared to provide an assessment of safety for humans. An outline of information presented in this assessment is shown below.

METABOLISM & PHARMACOKINETICS GLYPHOSATE, AMPA, & ROUNDUP®
Glyphosate Oral Administration in Rats
    Absorption
    Tissue Distribution
    Metabolism/Excretion
AMPA Oral Administration in rats
Glyphosate and AMPA in Non-rodents
Dermal Penetration of ROUNDUP®
 	
TOXICOLOGY STUDIES WITH GLYPHOSATE AND AMPA
Acute Toxicity and Irritation
Subchronic Toxicity
Chronic Toxicity/Carcinogenicity
Reproduction and Developmental Toxicity
 	
TOXICOLOGY STUDIES WITH POEA AND ROUNDUP®
Acute Toxicity and Irritation Studies
Subchronic Toxicity Studies
Genetic Toxicity
    Review of Studies with Glyphosate, Formulations, and AMPA
    Weight-of-Evidence Evaluation
Developmental and Reproductive Toxicity
	ORGAN-/TISSUE-SPECIFIC CONSIDERATIONS
	Salivary Gland Changes
	Potential for Endocrine Modulation
	Potential for Neurotoxicity
	Potential for Synergistic Interactions
	
	HUMAN EXPERIENCE
	Irritation Studies
	Occupational Exposure
	Ingestion
	
	HUMAN EXPOSURE
	Dietary exposure
	Occupational Application Exposure
	Non-occupational Application Exposure
	Consumption of Water
	Bystander Exposure During Application
	Possible Inadvertent Exposures
	Aggregate Exposure Estimates
	
	RISK CHARACTERIZATION
	Identification of NOAELs
	Estimation of Risks
	Chronic Exposures/Risks
	Acute Exposure/Risks

METABOLISM AND PHARMACOKINETICS GLYPHOSATE, AMPA, AND ROUNDUP®

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 or 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, or 100 ppm for 14 days; this was followed by a 10 day period during which there was no exposure to glyphosate (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 (1992), 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 19 to 23%) based on the percentage of material excreted in 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 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, in Sprague Dawley rats, showed that repetitive dosing at 10 mg/kg bw/day had no significant effect on the tissue distribution of glyphosate (Ridley and Mirly, 1988).

Biotransformation/Excretion

Orally administered glyphosate is poorly biotransformed in animals. It was shown to be rapidly 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 or 1000 mg/kg bw was 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 or 1000 mg/kg doses, respectively. The feces was the major route of glyphosate elimination at all dose levels tested; approximately 62 to 69% of the administered dose was excreted in the feces. Less than 0.3% of an administered dose was recovered as CO_2 in expired air. In rats given glyphosate at 10 or 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 mg/kg bw/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 bw/day also showed that repetitive dosing (15 days) had no significant effect on the elimination of glyphosate as compared to single dosing.

AMPA - Single Oral Dose Study in Rats

AMPA was administered *via* gavage at a dose of 6.7 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 period of experimental observation. The absorbed AMPA was not biotransformed 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 9 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 chicken at 120 ppm, residues in 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-dose (500 or 5400 $\mu g/cm^2$, 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 a 24-hour exposure period and for up to one day afterwards. 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 pK_as and therefore exist as charged molecules at the physiologic pHs found in the gut lumen. Only 15 to 36% of orally administered material given repeatedly, or as a single dose was absorbed, thereby demonstrating that glyphosate and AMPA are poorly absorbed despite the prevailing acidic conditions. As expected for substances that are not well absorbed from the alimentary tract, the feces was the major route of elimination. The relatively small amounts of absorbed glyphosate and AMPA were rapidly excreted in urine almost exclusively as unchanged parent material. This was confirmed by the determination that 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 (less than 2%) 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 the combination of poor absorption and rapid excretion of glyphosate or AMPA after oral and/or dermal contact.

TOXICOLOGY STUDIES WITH GLYPHOSATE AND AMPA

Acute Toxicity and Irritation Studies

The acute toxicity of glyphosate and AMPA has been studied in laboratory animals. Oral and dermal LD_{50} values for glyphosate in rats are greater than 5000 mg/kg bw (WHO, 1994). The oral LD_{50} for AMPA in rats is 8300 mg/kg bw (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

Mouse studies. Glyphosate was administered to B6C3F1 mice in the diet at concentrations of 0, 3125, 6250, 12500, 25000, or 50000 ppm (NTP, 1992). Decreased body weight gain was observed at the two 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 change 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 complete discussion of the significance of changes observed in the salivary glands is presented in **EVALUATION OF POTENTIAL SPECIFIC ORGAN/SYSTEM EFFECTS** (page 66). Because these salivary gland changes are considered not to be relevant to humans, the no-observed-adverse effect level (NOAEL) for glyphosate exposure in mice was based on the suppression of body weight gain, and was set at 12500 ppm (2490 mg/kg bw/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, or 50000 ppm. The only treatment-related effect was decreased cumulative body weight gain in males and females (27% and 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 for the parotid gland were observed. The NOAEL was 10000 ppm (2310 mg/kg bw/day).

Rat studies. Glyphosate was administered in the diet to F-344 rats at levels of 0, 3125, 6250, 12500, 25000, or 50000ppm 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 phophatase and alanine aminotransferase values were noted at and above dietary levels of 6250 ppm (males) and 12500 ppm (females). These increases were relatively small, not clearly related to dose, and not associated with any histological changes of toxicological significance. At necropsy, no gross lesions related to glyphosate administration were observed. Other analyses in reproductive tissues are discussed in a later section. The parotid 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. Salivary gland alteration was noted at the lowest dose tested (209 mg/kg bw/day for males and females combined), but for reasons described below, this effect can be ignored for purposes of evaluating safety in humans. The low-dose (3125 ppm or 209 mg/kg bw/day) therefore, is considered to be a 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, or 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 bw/day - males and females combined).

Dog study. Glyphosate was administered by capsule to beagle dogs at doses of 0, 20, 100, or 500 mg/kg bw/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 bw/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 mg/kg bw in rodent feeding studies and a dose of 500 mg/kg bw/day in a dog study. The primary finding was a decreased body weight gain in the rodent studies at the highest dietary concentrations tested (≥ 25000 mg/kg bw). 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 (acinar cell hypertrophy and basophilic change) was observed in some of the rodent studies; the sublingual salivary gland was not affected in any study. For reasons discussed in a later section, this 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. Overall, it can be concluded that glyphosate when administered at daily doses of up to 20000 mg/kg bw was well tolerated.

AMPA

Rat study. AMPA was administered in the diet to groups of Sprague-Dawley rats at dose levels of 0, 400, 1200, or 4800 mg/kg bw/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 bw/day based on urinary tract irritation.

Dog Study. AMPA was given to Beagle dogs *via* oral capsule at dosages of 0, 9, 26, 88, or 263 mg/kg bw/day for three months (Tompkins, 1991). There was no treatment-related effect at any dose level. Therefore, the NOAEL was ≥ 263 mg/kg bw/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 bw/day, while no effects occurred in dogs even at the highest dose tested (263 mg/kg bw/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, or 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 lesion, common to the strain of animals used, showed no correlation with dose. The NOAEL for chronic toxicity effects was 5000 ppm (885 mg/kg bw/day) based on the effects on body weight and liver histology. The incidences of renal tubular adenomas in males was 1, 0, 1, or 3 in the control, low-, mid-, and high-dose groups, respectively. No related preneoplastic lesions were observed. The incidence in high-dose males was not significantly different by pair-wise comparison to concurrent controls or by a trend test, and were within the historical control range. 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 (1994a) 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, or 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 treatment-related because: (1) it was not accompanied by an increase in hyperplasia (an expected preneoplastic 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, or 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) in females and degenerative ocular lens changes in males, 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 frequency of this lesion fell within the historical control range. At the end of the study, usually a time when the occurrence of such lesions is greatest, there was a very low incidence of inflammation in treated animals examined. Considering all these factors, it is doubtful that the inflammation is treatment-related. The rates for thyroid and pancreatic tumors incidence were slightly above background control values. The occurrence of thyroid and pancreatic tumors were judged to be sporadic, and therefore unrelated to treatment. The following reasons are given in support of this conclusion: (1) the tumors observed 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.

Based on these responses to prolonged exposure of glyphosate in rats, the 8000 ppm dose level (409 mg/kg bw/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 (1994a).

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 those were limited to the highest dietary levels tested (20000 ppm in rats or 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. For example, the weight of evidence for carcinogenic hazard potential, has been expressed by U.S. EPA using summary rankings for human and animal cancer studies. These summary rankings place the overall evidence in classification groups A through E, Group A being associated with the greatest probability of human carcinogenicity and Group E with evidence of non-carcinogenicity in humans. The U.S. EPA classified glyphosate in Category E, "Evidence of Non-carcinogenicity in Humans" (U.S. EPA, 1992a).

AMPA

Although lifetime studies were not conducted specifically with AMPA, its chronic toxicity and oncogenicity 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% AMPA (Lorenz, 1994). On this basis, it can be concluded that AMPA was present at dietary levels of 13.6, 54.4, or 136 ppm at the 2000, 8000, or 20000 ppm target concentrations for glyphosate, respectively. These dietary levels corresponded to dose levels of 0.69, 2.8, or 7.2 mg AMPA/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 AMPA is not oncogenic at dose levels up to 7.2 mg/kg bw/day, and the NOAEL for chronic effects is at least 2.8 mg/kg bw/day.

Reproduction and Developmental Toxicology Studies

Glyphosate

Reproductive toxicity. In the first of two multi-generation reproductive toxicity studies, glyphosate was administered to rats in the diet over three successive generations at dose levels of 0, 3, 10, or 30 mg/kg bw/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 such change. There were no treatment-related effects on mating, fertility or reproductive parameters. The second study, also in rats, was conducted at dietary levels of 0, 2000, 10000, or 30000 ppm for two generations (Reyna, 1990). 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 (~694 mg/kg bw/day) and 30000 ppm (~2132 mg/kg bw/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. Moreover, 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 any exposure 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; the effects included reduced ejaculate volume, sperm concentration, initial fructose levels and semen osmolality. The study also reported evidence for increased abnormal and dead sperm. There were a number of serious deficiencies in the design, conduct, and reporting of this study which make the results uninterpretable. Four rabbits per treatment group were used; this is a very low number of animals, and this limitation alone requires that the validity of this study be seriously questioned. 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 actually quantify the two dosage levels used (referred to only as $1/10^{th}$ and 100^{th} of the LD_{50}), 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 of chemical received, a meaningful assessment of these studies can not be made. 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. Additional points that seriously compromise this study include a lack of data for food consumption in control or treated animals, and failure to report variability in measurements for control and treated animals, preventing adequate statistical analysis to support conclusions of Yousef *et al.* (1995). 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 as well as 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, or 3500 mg/kg bw/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 bw/day and was accompanied by reduced fetal weights, ossification of sternebrae, and viability. The NOAEL for maternal and developmental toxicity was 1000 mg/kg bw/day.

Glyphosate was tested for developmental toxicity in rabbits following administration by oral gavage at dose levels of 0, 75, 175, or 350 mg/kg bw/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. 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 bw/day, while mortality occurred in 80% of the animals from the 500 mg/kg bw/day group. When these pilot data are included in the overall analysis, 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 or 500 mg/kg bw/day, respectively. This indicates an absence of a dose-response for treatment-related mortality below the 350 mg/kg bw/day dose. The death of the single mid-dose (175 mg/kg bw/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 bw/day in the pilot study. Therefore, the NOAEL for maternal toxicity must be represented by the 175 mg/kg bw/day dose, based on increased mortality and various clinical signs of toxicity at the next higher dose tested. The 175 mg/kg bw/day dose level was also concluded to be the NOAEL by the WHO (1994a), 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 bw/day due to the insufficient number of litters available for examination in the 350 mg/kg bw/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 2132 mg/kg bw/day), there is no evidence of 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 bw/day (sperm only) and 3393 mg/kg bw/day (sperm and estrous cycle). Since these changes 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, these findings are suspect and therefore difficult to assess. 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 bw/day in the rabbit developmental study.

AMPA

Reproduction and developmental toxicity studies. The potential for reproductive toxicity of AMPA can be assessed by examining the results from the two-generation rat reproduction study with glyphosate (Reyna, 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, or 183 ppm. Given that no effects were seen at the mid-dose level of this study, the overall NOAEL for AMPA is considered to be at least 61 ppm (~4.2 mg/kg bw/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, or 1000 mg/kg bw/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 bw/day. Therefore, the NOAEL for maternal and developmental toxicity is 400 mg/kg bw/day .

Summary. AMPA has been evaluated for potential adverse effects in reproductive and developmental studies with rats. In addition, the previously discussed reproductive tissues from the three month dog and rat toxicity studies with glyphosate, which contains AMPA (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 breakdown product, like the 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 LD_{50} values (Table 1) are greater than 5000 mg/kg bw (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 these routes of exposure.

The acute toxicity of the surfactant, POEA, is somewhat higher than for Roundup® formulation. Oral (rats) and dermal (rabbits) LD_{50} 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 LD_{50} value for POEA in rats is similar to that of Vitamin A (1960 mg/kg) and greater than that of aspirin (200 mg/kg) (NIOSH, 1987). The oral LD_{50} 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 their intended physico-chemical properties, POEA and the other surfactants in consumer products 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 confirm that Roundup® herbicide does not pose a significant eye or skin irritation hazard to humans.

Subchronic toxicity studies

POEA

Rat Study: POEA was administered to Sprague-Dawley rats in the diet for one month at concentrations of 0, 800, 2000, or 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 bw/day - males and females combined).

Dog Study: The POEA surfactant was administered in gelatin capsules to beagle dogs for 14 weeks (Filmore, 1973). Because gastrointestinal intolerance (as evidenced by emesis and diarrhea) was observed at a preliminary stage, dosages were increased during the first four weeks of the study, and then maintained at 0, 30, 60, or 90 mg/kg bw/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, or 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 8.7×10^{-6} mg/L; this is approximately 40000 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 bw/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 subchronic study with Brahman-cross heifers was carried out by administration of Roundup® *via* nasogastric tube at doses of 0, 400, 500, 630, or 790 mg/kg bw/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 bw/day, which was reduced to soft feces at the 500 mg/kg bw/day dose level. The NOAEL was 400 mg/kg bw/day. It was estimated that the cows received doses of Roundup® herbicide on the order of thirty to one hundred times greater than the dose typically applied to foliage for agricultural weed control purposes. Clearly, such exposures would never be achieved under normal agricultural use of glyphosate or Roundup®. Thus, exposure to forage sprayed at recommended use 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.

Developmental and Reproductive Toxicity

Developmental Study

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 bw/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 bw/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. There is no evidence that the surfactant or Roundup® herbicide adversely impacts reproductive function.

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, allow identification of alterations in the genome. A primary purpose of such tests is to provide information on the production of heritable changes (mutations) that could lead to further adverse biological consequences. An initial and prominent question that tests for genotoxicity is designed to answer is whether the chemical (or any derivative) interacts directly with and mutates DNA. Such interactions are known to bring about changes in gene expression or to affect other key biological processes. However, 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 be dependent on a threshold of exposure to produce an effect. The production of such indirect effects is often limited to conditions of high dose, which may be irrelevant to health risk assessment. 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" genotoxic effects. The database of results from tests related to effects on genetic material, and the production of mutational events is presented in Table 2. The following discussion details individual results, where appropriate, and then evaluates these results in a weight-of-evidence narrative that takes into account all the data available.

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 an exogenous source of metabolic activation (Li and Long, 1988; Moriya *et al.*, 1983; NTP, 1992; Wildeman and Nazar, 1982). 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 in a sensitive system 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 a surfactant. Such surfactants include POEA (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 affect 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, a single dose also 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 (*i.e.*, 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. The studies of Kier *et al.* (1997) were conducted with complete protocols to satisfy international regulatory guidelines for these assays. Accordingly, 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 their results represent a false positive result, which are known to occur sporadically in this and other genotoxicity tests (Brusick *et al.*, 1998).

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 variation 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, absence of negative controls, and high exposures that included treatment with chemical concentrations that were lethal to half the test population (LC_{50}). 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 cause structural chromosome aberrations provides relevant 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. Nevertheless, 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, results of chronic exposure studies in rats and mice demonstrate that there is no evidence of tumorigenicity for glyphosate, an important fact that should be taken into consideration 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), in contrast, 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). In other experiments reported previously by Hrelia *et al.* (1996), the fungicide failed to produce chromosomal aberrations at seventy times the dose applied by Lioi *et al.* (1998a), and failed to show other evidence of direct DNA damage in a number of tests. 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 affecting 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), without apparent explanation. 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 (Hrelia *et al.*, 1996; van de Waart, 1995) that observed no effects in either glyphosate or vinclozolin at dose levels in excess of seventy times those employed by Lioi *et al.* (1998a).

Glyphosate alone was not active for chromosomal damage (De Marco *et al.*, 1992; *Rank et al.*, 1993). Another study has reported that Roundup® can produce chromosomal aberrations in onion root tip cells (Rank *et al.*, 1993). These investigators postulated that the toxic effect of the surfactant in Roundup® could be responsible for the effects on the plant cell 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 herbicidal activity of glyphosate, these results are considered secondary to plant toxicity, and not relevant to human health.

Of greater relevance than *in vitro* effects is evidence of *in vivo* effects. In this regard, 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 2. The micronucleus assay targets the most actively dividing cell population of the bone marrow, the polychromatic erythrocytes (PCEs). 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 high distribution of chemical into the circulatory system of the test 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 50000 ppm (11379 mg/kg bw/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.* injection 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 PCEs per animal were scored for the incidence of MNPCEs. 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, or 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 2). The herbicide Direct contains tallowamine surfactant with a longer carbon chain length than POEA, 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 evaluated 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). Roundup® (*i.p.*) reduced the frequency of PCEs in male mice compared to controls, suggesting some evidence of systemic toxicity. The results of Bolognesi *et al.* (1997) contrast with those of Kier *et al.* (1997) that reported no increased micronucleus formation (even at much higher doses than Bolognesi tested). Kier *et al.* (1997) did note 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 frequency of PCE production in control animals is unusual for 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 PCEs 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 and requiring increases in at least two. Bolognesi *et al.* (1997) did not provide micronucleus data for individual animals, as is customary, 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. These two materials 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 NCEs and PCEs as well as for the frequency of MNPCEs. The weighted mean for spontaneous MN/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 LD_{50} 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. This fact is important in the evaluation of the results of other *in vivo* and *in vitro* results.

In Vitro Sister Chromatid Exchange

Analysis of sister chromatid exchange (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 a 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 specific for mutation. The SCE assay 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).

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.

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 provided no indication of any 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).

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 exposure to 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! 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.

***In Vivo* Mutation**

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 a *S. typhimurium* mutation test performed on AMPA at concentrations of up to 5000 : g/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 : g/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) which 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 so 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.*, 1984), which are unlike adducts that would be generated from molecules like glyphosate or the surfactant. 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 300 mg/kg glyphosate or 900 mg/kg Roundup® (this corresponds to 270 mg/kg glyphosate), which are doses close to, or in excess of the *i.p.* LD_{50} for mice (WHO, 1994). DNA breaks could be detected at a brief time after initial exposure, but at 24 hours of 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! OHdG) residues in DNA of liver cells from mice treated with glyphosate, but not Roundup®. Opposite results were found for exposures to kidney cells that appeared to accumulate oxidative damage after treatment with Roundup®, but not glyphosate. Products of reactive oxygen species, including 8-OHdG, 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-OHdG residues is not by definition an indicator of chemical-DNA interaction. These products 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-OHdG in the same organs with both glyphosate and Roundup® 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®. Direct reaction with purine or pyrimidine nucleotides could lead to elimination of an altered base on exposure to alkali. Alkali sensitive sites resulting from depurination or depyrimidation events can be detected in the Comet assay, a methodology to demonstrate DNA strand breaks. Clements *et al.* (1997) used the Comet assay to examine DNA in erythrocytes from tadpoles exposed to various herbicides including Roundup®. Clements *et al.* (1997) reported evidence of a treatment-related increase in DNA breaks as measured by migration of DNA from the bulk of nuclear material 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 in a well characterized and sensitive system indicate an absence of DNA reactivity, either direct or following hepatocellular biotransformation (Williams *et al*., 1989).

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:

- Any statistically significant observations should be reproducible and biologically significant.
- A dose response relationship should exist for effects.
- The effects should be permanent and progressive, as opposed to reversing upon cessation of chemical dosing.
- The nature of DNA effects, should be characterized.
- The database should be consistent or inconsistencies adequately explained.
- The effects produced in the assay should be relevant to humans.

A central objective of the weight-of-evidence is to avoid conditions that could permit one experimental test result to be accorded greater weight 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 3. 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, 1996b, Proposed Guidelines for Carcinogen Risk Assessment) for mutagenicity risk assessment.

The key features of the weight-of-evidence scheme described in Figure 3 are its ability to accommodate results from multiple testing protocols, and its requirement 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 for similar endpoints. On the other hand, a result from a novel procedure might be acceptable because it is deemed to provide important 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 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 selected 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 the 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.

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 are 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 2) 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 reservation 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, as described, have many problems relating to the internal consistency of the data for other pesticides tested. Accordingly, these studies are not weighted equally with the assay carried out under GLP conditions (van de Waart, 1995).

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 identifiable 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! OHdG adducts in livers of mice treated with glyphosate *i.p.*, but this can not be reconciled with the fact that glyphosate is not metabolized. There has been absolutely no evidence produced to date, 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 x 150 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 of 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. Also, the negative UDS assay in hepatocytes (Li and Long, 1988) would tend to confirm that the SSB of Bolognesi *et al.* (1997) 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.

The data relating to SCE production presented by Lioi *et al.* (1998a,b) and Bolognesi *et al.* (1997) are 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, the levels observed were well within the accepted variation for the incidence of SCE in the human population.

It is concluded that on a weight-of-evidence analysis of the data for glyphosate, and for Roundup® that they are neither mutagenic nor genotoxic as a consequence of a direct chemical reaction with DNA (change by Williams was DNA binding...is this acceptable?). 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 undergoes such testing, 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 noncarcinogenic.

Summary

The potential genotoxicity of glyphosate has been 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. However 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.

The mutagenic potential of Roundup® herbicide and the POEA surfactant have been evaluated in several bacterial mutagenicity assays. While a marginal response was reported in one limited investigation, results from other complete, replicated studies conducted according to international guidelines and Good Laboratory Practices show that these materials are not mutagenic. 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 some of these studies reported positive effects, methodological limitations render the data unacceptable for regulatory purposes, and scientifically uninterpretable. For example, the positive "effects" were observed only at cytotoxic concentrations *in vitro* and at perilethal doses *in vivo* administered by an irrelevant route of exposure (*i.e.*, *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.

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 β-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 β-adrenergic agonist). Both compounds produced increased salivary gland weights. When isoproterenol was given with propranolol, a β-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 β-blocker to significantly inhibit the effects of glyphosate indicates that it does not act as a β-agonist.

Other factors were considered to help resolve questions of salivary gland effects and causality. First, if glyphosate was a β-agonist material, its effect would be to stimulate β-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 β-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 β-agonist. Furthermore, glyphosate is not structurally related to known β-agonists. It is concluded that glyphosate has no significant β-adrenergic activity, and therefore could not produce salivary gland changes *via* β-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 of observations is consistent with the hypothesis that the salivary gland change observed are a biological response to the acidic nature of glyphosate.

Regardless of the mechanism involved, there are several reasons to conclude that the salivary gland change observed is of doubtful toxicological significance. The change occurred in the absence of other significant adverse effects, indicating that the health of the animals was not adversely impacted. Furthermore, the salivary gland alteration was not associated with any adverse clinical or pathological effect even in chronic studies. Such alteration cannot be considered preneoplastic because the tumor rate was not increased in chronic bioassays. These salivary gland changes are not known to represent any pathologic condition, and have no relevance to humans. Therefore the finding is not considered to be either toxicologically significant or adverse.

Potential for Endocrine Modulation

The U.S. Environmental Protection Agency has developed a two-tiered screening and testing strategy for evaluating the endocrine modulating potential of environmental substances. Tier I screening assays include both *in vitro* and short-term *in vivo* assays designed to detect substances with the ability to interact with the endocrine system. Tier II tests include long-term *in vivo* multigeneration reproductive toxicity tests which more definitively determine and characterize any endocrine modulating effects for subsequent risk assessment. In addition to efforts within the United States, other countries, led primarily by Japan and the OECD (Office of Economic and Development) member countries, are developing similar *in vitro* and *in vivo* approaches to assess chemicals for endocrine activity.

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, and 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 worldwide regulatory agencies detect modulation of endocrine system activity (Carney *et al.*, 1997; Stevens *et al.*, 1997, 1998). These studies are more 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 allows detection of 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 offspring's' 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. 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 was no evidence to 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 has no potential to produce adverse effects on endocrine systems in humans nor in 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 fail to demonstrate evidence for synergism between weakly estrogenic chemicals by the absence of the production of greater response to mixtures have been presented by various investigators. In a study conducted by Baba *et al.* (1989), oral LD_{50}s were determined in rats for each component of Roundup® herbicide. The interactions were evaluated by the graphic method of Shirasu *et al.* (1978), 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" LD_{50} and LC_{50} 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 imply 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 ingestion of 104 and 120 mL were not fatal while mean ingestion 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® ingestion 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 over toxicologic findings of concern, further refinement to identify a more accurate realistic exposure level is not generally undertaken. In the majority of instances, 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 bw/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 aggregate exposure for chronic scenario was 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 scenario incorporated occasional, inadvertent exposure routes (spray drifting onto bystanders, reentry into previously treated areas). This scenario also included additional sources from 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.

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 0.126 and 0.0323 mg/kg bw/day, respectively; for young children, the values were 0.097 and 0.052 mg/kg bw/day. Estimates of exposure to AMPA were also very low, ranging from 0.0048 to 0.0104 mg/kg bw/day. The calculated exposures for POEA ranged from 0026 mg/kg bw/day for chronic exposure in children to 0.163 mg/kg bw/day for acute applicator exposure.

Conservative assumptions used in analysis of both the acute and chronic exposure scenarios insure conditions for upper-limit or worst-case exposure estimates were 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). Estimates of acute drinking water exposure used the highest measured value resulting from 5 years of drinking water monitoring in the United Kingdom (1.7 ppb). This conservative assumption exaggerates glyphosate exposure, since 99% of the UK data did not detect glyphosate above 0.1 μg/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, it is necessary to measure residues of herbicide and related products that represent the maximum levels of glyphosate and AMPA that hypothetically occur in food using the highest and most frequent herbicide applications. These data support legally binding Maximum Residue Levels (MRLs, called "tolerances" in the U.S.) that are established in most countries worldwide for the resulting food commodities. In addition, international MRLs continue to be 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® software[1] 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 μg/kg body weight/day for the U.S. population and 51.9 μg/kg bw/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

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[1] Technical Assessment Systems, Inc. (TAS). Exposure-1® software. TAS®, Inc. The Flour Mill, 1000 Potomac St. NW, Washington, D.C. 20007. United States. 1-202-337-2625. Calculations completed using 1977-78 food consumption data.

incorporated to arrive at more realistic exposures. For example, U.S. residue data from wheat treated with maximum rates of Roundup® showed the highest glyphosate residue to be 2.95 μg/g, with a mean level of 0.69 μg/g, compared to a MRL of 5 μg/g (Allin, 1989). Glyphosate-tolerant soybeans treated at maximum allowed rates and frequency contained glyphosate residues at the highest level of 5.47 μg/g, with a mean of 2.36 μg/g, compared to the MRL of 20 μg/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.

AMPA

AMPA has historically been considered a minor part of the plant residue derived from glyphosate treatment. Measured levels of AMPA 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 AMPA 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 AMPA in cases where it is the major residue in glyphosate-tolerant crops that express an enzyme that converts glyphosate to AMPA as a mechanism of tolerance.

In order to arrive at a maximum estimate of AMPA dietary exposure, it has been assumed that AMPA represents 20% of the TMDI glyphosate exposure. This is a compromise between the bulk of the historical data that indicate that AMPA residues are 10% of glyphosate levels, and the more recent findings that specific glyphosate-tolerant crops have a higher ratio. Based on this assumption, AMPA dietary exposure was 48 μg/kg body weight/day for the U.S. population and 10.4 μg/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 μg/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, 1997a), 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 47 μg/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 μg /m^3 of air (Kramer, 1978), with the vast majority of quantifiable results being less than 1.3 μg/m^3 (Jauhiainen *et al.*, 1991). Tank filling operations created the highest dermal exposure (hands), ranging from 4 x 10^{-2} to 12 μg/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 μg/mL. On a few isolated occasions, urine levels of 0.025 to 0.095 μg/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 x 10^{-2} μg/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, 1997a), 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 μg/kg body weight. Dermal exposure was 38 μg/kg bw, and inhalation exposure was 6.2 μg/kg bw during 8 hours of application. Summed together, the adult worker's peak acute exposure during application was calculated as 56.2 μg/kg bw/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 μg/kg body weight (Kramer, 1978). Average dermal exposure (Kramer, 1978; Lavy *et al.*, 1992) during application was 5.1 μg/kg bw/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 $\mu g/m^3$ 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 μg/kg bw/day. Summed together, and amortizing for a five-day working week, chronic applicator exposure to glyphosate was estimated to be 8.5 μg/kg body weight/day.

AMPA

There is no application-related exposure to AMPA, since its presence 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 μg/kg body weight (dermal during one mixing and mixing/loading operation), 95 μg/kg bw (dermal during application), and 3.1 μg/kg bw (inhalation). Summed, the total acute daily exposure was 128 μg/kg body weight. Chronically, using the same assumptions and amortizing for a 5-day work week, mixing/loading contributed 11.3 μg/kg bw/day, dermal exposure during application contributed 9.1 μg/kg bw/day, and inhalation contributed 0.23 μg/kg bw/day. Summed, chronic application-related exposure to POEA was estimated to be 20.6 μg/kg body weight/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 μg of glyphosate/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 μg/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 μg/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 μg/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 μg/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, 5290 samples derived from surface and ground water sources were analyzed (Hydes *et al.*, 1996; Hydes *et al.*, 1997). All but 10 were below the 0.1 μg/L limit. Among those 10 reported detections, concentrations ranged from 0.2 to 1.7 μg/L. The exceedences detected 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 μg glyphosate /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 μg/L.

Irrespective of measured concentrations, U.S. EPA has established a maximum contaminant level (MCL) of 700 μg/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, 1998). 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 μg/L and 0.0011 μg/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 μg/L, the highest reported measured value in the UK drinking water program. The same data indicated that chronic exposure could not exceed 0.1 μg/L, the European Union exposure limit. This value is supported by the U.S. EPA model calculations. Based on figures for mean daily water consumption, and body weights (U.S. EPA, 1997a) 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 3.6×10^{-2} (adult) and 0.11 (child) μg/kg body weight. The chronic exposures, calculated in the same manner, were 2.1×10^{-3} (adult) and 6.7×10^{-3} (child) μg/kg bw/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 μg/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 2.1×10^{-3} and 6.7×10^{-3} μg/kg bw/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 1.8×10^{-2} (adult) and 5.5×10^{-2} (child) μg/kg bw. The chronic exposures, calculated in the same manner, were 1.1×10^{-3} (adult) and 3.3×10^{-3} (child) μg/kg bw/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 x 10^{-3} to 2.6 µg/kg body weight/hour for an adult, with a mean value of 0.52 µg/kg bw/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, 1997; U.S. EPA, 1997a) and a child's lower body weight, exposures of a child reentering the same fields were calculated to be 0.01 to 5.2 µg/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 µg 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 a woman may experience as part of her other activities. During any work time period, a woman 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 μg/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, 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 μg 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 μg/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, 1992b, 1988, 1997a) 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 1 μg/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 μg/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 and 6.5 μg/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 μg/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 µg/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 µg/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 µg/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 0.097 mg/kg bw/day, respectively; chronic exposures in these subgroups were 0.0323 and 0.052 mg/kg bw/day, respectively. Levels of exposure to AMPA were very low (~0.005 - 0.010 mg/kg bw/day). Estimates of exposure to POEA were 0.163 and 0.0911 mg/kg bw/day for the acute scenarios, while chronic exposure estimates were 4 to 5 times lower that the acute values.

RISK CHARACTERIZATION

Introduction

Risk characterization involves a determination of the likelihood that an adverse health effect will result from exposure to a given substance. The method used in this assessment to characterize risk was the Margin of Exposure (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 LD_{50} values in rats. The MOE is the defined as the quotient of the NOAEL divided by the aggregate human exposure calculated from total daily intake from all sources.

The introduction of safety factors is a concept that has had wide acceptance in the scientific and regulatory communities around the world. The Joint European Committee on Food Additives (JECFA) proposed principles for determining a margin of safety (MOS), and has developed a methodology to establish an acceptable value for a factor that would directly link animal toxicological data to human health and safety (FAO/WHO, 1958). For purposes of extrapolation of data from animals to man, the figure is based on an established dosage level that causes no demonstrable effects in the animals. The MOS allows for any species differences in susceptibility, the numerical differences between the test animals and the exposed human population, the greater variety of complicating disease processes in the human population, the difficulty of estimating the human intake, and the possibility of synergistic action.

JECFA stated that the 100-fold margin of safety applied to the maximum ineffective dose (expressed in mg/kg body weight per day) was believed to be an adequate factor (FAO/WHO, 1958). The value of 100 has been regarded as comprising two factors of ten to allow for interspecies and inter-individual (intra-species) variation (WHO, 1994b).

The validity and size of safety/uncertainty factors, and their application across many substances including pesticides has undergone periodic reevaluation (Renwick and Lazarus, 1998). By and large the allocation of appropriate safety factors is considered on a case-by-case basis, relying on analysis of the total weight of evidence including a consideration of data gaps (WHO, 1990). WHO Scientific Groups have confirmed a 100-fold safety factor as an adequate and useful guide, particularly when there are few toxicological data gaps (WHO, 1967; WHO, 1994b).

The National Research Council report on Pesticides in the Diets of Infants and Children (NRC, 1993) indicated that the current 10-fold intraspecies factor adequately protects for socioeconomic, nutritional, and health status factors that influence the vulnerability of children to environmental toxicants. The NRC report (NRC, 1993) also indicated the possible requirement for an additional 10-fold uncertainty factor to be applied to the ADI for pesticide residues in food to protect infants in the absence of specific data on developmental toxicity. The Environmental Protection Agency sometimes applies a three to tenfold margin of safety for infants and children in the case of threshold effects. This additional factor would account for pre- and post-natal toxicity and is applied when existing data indicates a possible increased sensitivity to infants or to children, or when the database if effects is incomplete (U.S. EPA, 1998a).

Recently the U.S. EPA conducted a review of the risks associated with aggregate exposures to glyphosate residues from all sources (U.S. EPA, 1998a). Using a margin of exposure analysis, it was concluded that "reliable data support the use of the standard 100-fold uncertainty factor for glyphosate, and that an additional ten-fold uncertainty factor is not needed to protect the safety of infants and children." There was no suggestion of increased severity of effect in infants or children, or of increased potency or unusual toxic properties of glyphosate in infants and children. Therefore, in the view of U.S. EPA, there are no concerns regarding the adequacy of the standard MOE/safety factor of 100 fold (U.S. EPA, 1998a).

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 bw/day; this value is based on the occurrence of maternal toxicity at the highest dose tested (350 mg/kg bw/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 bw/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 bw/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 bw/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 bw/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 bw/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 36 mg/kg bw/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 bw/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 LD_{50}s 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 bw/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 bw/day. Comparison of these values to the NOAEL of 175 mg/kg bw/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 bw/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 0.097 mg/kg bw/day. For applicators, the corresponding aggregate acute exposure value was calculated to be 0.125 mg/kg bw/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 LD_{50} of glyphosate is greater than 5000 mg/kg. The acute exposure values for both children and adult applicators are approximately 40000 to 50000 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 plant/bacterial 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 bw/day for children and 0.0048 mg/kg bw/day for adults. MOEs were calculated using the definitive NOAEL of 400 mg/kg bw/day from the subchronic rat study and the lowest estimated NOAEL (> 2.8 mg/kg bw/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 bw/day for children and 0.0048 mg/kg bw/day for adults. Based on the oral LD_{50} 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 bw/day in children and 0.0325 mg/kg bw/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 bw/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 mg/kg bw/day from the subchronic rat study was used, the resulting MOE for children was calculated to be 1380.

Acute exposure. Estimates of aggregated acute exposure in adult applicators (0.163 mg/kg bw/day) and children (0.0911 mg/kg bw/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 LD_{50} of POEA is approximately 1200 mg/kg. The estimated acute exposure values are 7,360 to 13,200 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. Estimates of exposure described for these two sub-populations, and used in these risk calculations are considered excessive compared for those likely to result from the use of Roundup® herbicide. Margin of Exposure (MOE) analyses compares the lowest NOAELs determined from animal studies to anticipated levels of human exposure. MOEs of greater than 100 are considered by authoritative bodies to indicate confidence that no adverse health effects would occur (WHO, 1990).

The MOEs for worst-case chronic exposure to glyphosate ranged from 3,370 to 5,420; the MOEs for AMPA ranged from greater than 269 to 83,300; and for POEA the MOEs ranged 461 to 1,380. Based on these values, it is concluded that these substances do not have the potential to produce adverse effects in humans. Acute exposures to glyphosate, AMPA, and POEA were estimated to be 7,360 - 1,730,000 times lower than the corresponding LD_{50} values, thereby demonstrating that potential acute exposure is not a health concern. Finally, under the intended conditions of herbicide use, Roundup® risks to sub-populations other than those considered here would be significantly lower. It is concluded that, under present and expected conditions of new use, there is no potential for Roundup® herbicide to pose a health risk to humans.

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Table 1 Acute Toxicity and Irritation of Roundup® Herbicides and POEA Surfactant

Test Material	Oral LD50 (mg/kg)	Dermal LD50 (mg/kg)	Inhalation (mg/L)	Eye Irritation	Skin Irritation
Roundup®	>5000	>5000	3.18	Severe	Slight
(41% IPAG)[a]	(IV)[b]	(IV)	(IV)	(I)	(IV)
POEA	1200	>1260	---	Corrosive	Severe
Roundup® T/O	>5000	>5000	>5.7	Moderate	Essentially None
(18% IPAG)	(IV)	(IV)	(IV)	(III)	(IV)
Roundup® L & G	>5000	>5000	>8.9	Slight	Essentially None
Ready-to-Use (1% IPAG)	(IV)	(IV)	(IV)	(IV)	(IV)

[a] 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 (Blaszcak, 1990), skin irritation (Blaszcak, 1988); POEA: all studies (Birch, 1977); Roundup T/O: oral, dermal, eye and skin (Auletta, 1985a-d), inhalation (Bechtel, 1987); Roundup L&G Ready-to-Use: oral, dermal, eye, and skin (Blaszcak, 1987a,b,d,e), inhalation (Dudek, 1987)

Table 2 Summary of Results on the Genotoxicity of Glyphosate, Roundup®, and Other Glyphosate Formulations

Test Organism	Endpoint	Compound (Purity)	Dose LED/HID [a]	EVALUATION [b] Without S9	With S9	Reference
GENE MUTATION						
S. typhimurium TA98, TA100	Reverse mutation	Glyphosate (not specified)	0.025 mg/plate	!	! (S9 plant)	Wildeman and Nazar (1982)
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	Reverse mutation	Glyphosate (not specified)	5 mg/plate	!	!	Moriya *et al.* (1983)
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	Reverse mutation	Glyphosate (98%)	5 mg/plate	!	!	Li and Long (1988)
S. typhimurium TA97, TA98, TA100, TA1535	Reverse mutation	Glyphosate (99%)	10 mg/plate	!	!	NTP, 1992
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538, TA1978	Reverse mutation	Roundup® (glyphosate as isopropylamine salt, 36%)	5 mg/plate	!	!	Njagi and Gopalan (1980)
S. typhimurium TA98	Reverse mutation	Roundup® (glyphosate 48%: POEA)	1.44 mg/plate	!	!	Rank *et al.* (1993)
S. typhimurium TA100	Reverse mutation	Roundup® (glyphosate 48%: POEA)	0.72 mg/plate	!	+	Rank *et al.* (1993)
S. typhimurium TA98, TA100, A1535, TA1537	Reverse mutation	Roundup® (glyphosate 30.4%: 15% POEA)	0.5 mg/plate	!	!	Kier *et al.* (1997)
S. typhimurium TA98, TA100, A1535, TA1537	Reverse mutation	Rodeo® (glyphosate as isopropylamine salt, 54%)	5 mg/plate	!	!	Kier *et al.* (1997)
S. typhimurium TA98, TA100, A1535, TA1537	Reverse mutation	Direct® (glyphosate as ammonium salt 72%: surfactant)	0.5 mg/plate	!	!	Kier *et al.* (1997)

Table 2 Summary of Results on the Genotoxicity of Glyphosate, Roundup®, and Other Glyphosate Formulations

Test Organism	Endpoint	Compound (Purity)	Dose LED/HID [a]	EVALUATION [b] Without S9	With S9	Reference
E. coli WP2 *hcr*	Reverse mutation	Glyphosate (not specified)	5 mg/plate	!	!	Moriya *et al.* (1983)
E. coli WP2 *hcr*	Reverse mutation	Glyphosate (98%)	5 mg/plate with S9, 1 mg/plate without S9	!	!	Li and Long (1988)
CHO cells (HGPRT)	Reverse mutation	Glyphosate (98%)	22.5 mg/mL	!	!	Li and Long (1988)
D. melanogaster	Sex-linked recessive lethals	Roundup® (glyphosate 41%:POEA) (chronic to pupation)	1 mg/L (1ppm)	+	0	Kale *et al.* (1995)
D. melanogaster	Sex-linked recessive lethals	Roundup® (not specified)		!	0	Gopalan and Njagi (1981)
		CHROMOSOMAL ABERRATION				
Allium cepa (onion root tip)	Chromosomal aberrations	Glyphosate (isopropylamine salt)	2.88 mg/L	!	0	Rank *et al.* (1993)
Allium cepa (onion root tip)	Chromosomal aberrations	Roundup® (glyphosate 48%: POEA)	1.44 mg/L	+	0	Rank *et al.* (1993)
Peripheral lymphocytes (human) *in vitro*	Chromosomal aberrations	Glyphosate (>98%)	0.56 mg/mL with S9, 0.33 mg/mL without S9	!	!	van de Waart (1995)
Peripheral lymphocytes (human) *in vitro*	Chromosomal aberrations	Glyphosate (>98%)	1.4 mg/L	+	0	Lioi *et al.* (1998a)

Table 2 Summary of Results on the Genotoxicity of Glyphosate, Roundup®, and Other Glyphosate Formulations

Test Organism	Endpoint	Compound (Purity)	Dose LED/HID [a]	EVALUATION [b] Without S9	With S9	Reference
Peripheral lymphocytes (bovine) *in vitro*	Chromosomal aberrations	Glyphosate (>98%)	2.9 mg/L	+	0	Lioi *et al.* (1998b)
Rat bone marrow (*in vivo*) 6, 12, 24 h	Chromosomal aberration	Glyphosate (98%)	1.0g/kg	!	0	Li and Long (1988)
Peripheral blood (human) *in vitro*	SCE	Roundup® (not specified)	2.5 mg/mL	+/!	0	Vigfusson and Vyse (1980)
Peripheral blood (human) *in vitro*	SCE	Glyphosate (99.9%)	1.0 mg/mL	+	0	Bolognesi *et al.* (1997)
Peripheral blood (human) *in vitro*	SCE	Roundup® (glyphosate 30.4%:15% surfactant)	0.1 mg/mL	+	0	Bolognesi *et al.* (1997)
Peripheral blood (human) *in vitro*	SCE	Glyphosate (>98%)	1.4 mg/L	+/!	0	Lioi *et al.* (1998a)
Peripheral lymphocyttes (bovine) *in vitro*	SCE	Glyphosate (>98%)	2.9 mg/L	+/!	0	Lioi *et al.* (1998b)
V. faba (root tips)	Micronucleus test	Solado (glyphosate 21%)	1.4 mg/g soil	!	0	De Marco *et al.* (1992)
Mouse bone marrow (*in vivo*), dietary for 13 weeks	Micronucleus test	Glyphosate (99%)	11,379 mg/kg/day	!	0	NTP, 1992
Mouse bone marrow (*in vivo*) i.p. injection, 24h, 48h	Micronucleus test	Glyphosate (not specified)	200 mg/kg	!	0	Rank *et al.* (1993)
Mouse bone marrow (*in vivo*) i.p. injection, 24h	Micronucleus test	Roundup® (glyphosate 48%: POEA)	200 mg/kg	!	0	Rank *et al.* (1993)
Mouse bone marrow (*in vivo*) i.p. injection	Micronucleus test	Glyphosate (99.9%)	300 mg/kg	+	0	Bolognesi *et al.* (1997)

Table 2 Summary of Results on the Genotoxicity of Glyphosate, Roundup®, and Other Glyphosate Formulations

Test Organism	Endpoint	Compound (Purity)	Dose LED/HID [a]	EVALUATION [b] Without S9	With S9	Reference
Mouse bone marrow (*in vivo*) i.p. injection	Micronucleus test	Roundup® (glyphosate 30.4%:15% surfactant)	135 mg/kg	+	0	Bolognesi *et al.* (1997)
Mouse bone marrow (*in vivo*) i.p. injection	Micronucleus test	Roundup® (glyphosate 30.4%:15% POEA)	555 mg/kg	!	0	Kier *et al.* (1997)
Mouse bone marrow (*in vivo*) i.p. injection	Micronucleus test	Rodeo® (glyphosate IPA 54%:water)	3400 mg/kg	!	0	Kier *et al.* (1997)
Mouse bone marrow (*in vivo*) i.p. injection	Micronucleus test	Direct® (glyphosate 72%as NH_4salt:surfactant)	365 mg/kg	!	0	Kier *et al.* (1997)
Mouse (*in vivo*) gavage	Dominant lethal	Glyphosate (98.7%)	2000 mg/kg	!		Wrenn (1980)
DNA DAMAGE/REACTIVITY						
B. subtilis H17, rec+; M45, rec-	*rec*-assay	Glyphosate (98%)	2 mg/disk	!	!	Li and Long (1988)
Rat hepatocytes (exposed *in vitro*)	UDS	Glyphosate (98%)	0.125 mg/mL	!	!	Li and Long (1988)
Mouse i.p. exposure (*in vivo*)	DNA adducts	Glyphosate (isopropylamine salt)	270 mg/kg	!	0	Peluso *et al.* (1998)
Mouse i.p. exposure (*in vivo*)	DNA adducts	Roundup® (30.4% glyphosate isopropylamine salt): 15% surfactant	400 mg/kg	+	0	Peluso *et al.* (1998)
Mouse i.p. exposure (*in vivo*) alkaline elution of extracted DNA	DNA single-strand breaks	Glyphosate (99.9%)	300 mg/kg	+	0	Bolognesi *et al.* (1997)

Table 2 Summary of Results on the Genotoxicity of Glyphosate, Roundup®, and Other Glyphosate Formulations

				EVALUATION [b]		
Test Organism	**Endpoint**	**Compound (Purity)**	**Dose LED/HID [a]**	**Without S9**	**With S9**	**Reference**
Mouse i.p. exposure (*in vivo*) alkaline elution of extracted DNA	DNA single-strand breaks	Roundup® (glyphosate 30.4%: 15% surfactant)	270 mg/kg	+	0	Bolognesi *et al.* (1997)
R. catesbeiana (tadpole)	DNA single-strand breaks: Comet assay	Roundup® (glyphosate 30.4%:15% POEA)	6.75 mg/L	+		Clements *et al*., (1997)
Mouse i.p exposure (*in vivo*)	8-OHdG	Glyphosate (99.9%)	300 mg/kg	+/!	0	Bolognesi *et al.* (1997)

[a] Lowest Effective Dose/Highest Ineffective Dose
[b] + = postive, ! = negative, 0 = not tested

Table 3 Summary Incidence of Microscopic Findings in a 2-Year Rat Study with Glyphosate[a]

Dose levels (ppm)	0	2000	8000	20,000
Epididymis (ides)				
decrease/absence of sperm	12 (60)[b]	14 (60	17 (60)	19 (60)
granuloma, sperm	1 (60)	0 (60)	1 (60)	0 (60)
atrophy	1 (60)	0 (60)	0 (60)	0 (60)
hyperplasia, ductal epithelium	0 (60)	0 (60)	1 (60)	1 (60)
Testis (es)				
degeneration/atropy, seminiferous tubules, bilateral	14 (60)	16 (60)	14 (60)	22 (60)
arteritis/periarteritis	17 (60)	12 (60)	18 (60)	21 (60)
hyperplasia, interstitial cells	1 (60)	1 (60)	0 (60)	1 (60)
spermatocoele	1 (60)	0 (60)	0 (60)	0 (60)
interstitial cell tumor	2 (60)	0 (60)	3 (60)	2 (60)
granuloma, spermatic	0 (60)	1 (60)	0 (60)	1 (60)
degeneration/atropy, seminiferous tubules	6 (60)	8 (60)	8 (60)	8 (60)
Ovaries				
cyst (s), follicular	13 (60)	7 (60)	8 (60)	9 (59)
cyst (s), paraovarian bursa	0 (60)	1 (60)	1 (60)	1 (59)
granulosa cell tumor	0 (60)	2 (60)	1 (60)	0 (59)
lymphoma infiltrate	0 (60)	0 (60)	0 (60)	1 (59)
theca cell tumor	1 (60)	0 (60)	0 (60)	0 (59)
arteritis/periarteritis	0 (60)	0 (60)	1 (60)	0 (59)
metastatic cortical carcinoma, adrenal	0 (60)	0 (60)	0 (60)	1 (59)
Uterus				
dilatation, endometrial glands	7 (60)	6 (60)	5 (60)	3 (59)
squamous metaplasia, endometrial glands	6 (60)	2 (60)	1 (60)	2 (59)
inflammation, endometreum	0 (60)	1 (60)	2 (60)	2 (59)
dilation of uterine lumen (hydrometra)	7 (60)	9 (60)	16 (60)	8 (59)
hyperplasia, endometrial glands	0 (60)	0 (60)	2 (60)	3 (59)

Table 3 Summary Incidence of Microscopic Findings in a 2-Year Rat Study with Glyphosate[a]

Dose levels (ppm)	0	2000	8000	20,000
hypertrophy/hyperplasia, endometrial stroma	1 (60)	0 (60)	0 (60)	1 (59)
Prostate				
infiltrate, mononuclear/lymphocytic, interstitial	3 (60)	0 (60)	1 (60)	1 (60)
inflammation	11 (60)	14 (60)	16 (60)	16 (60)
hyperplasia, acinar epithelium	2 (60)	4 (60)	1 (60)	4 (60)
adenocarcinoma	1 (60)	0 (60)	0 (60)	0 (60)
atrophy	1 (60)	2 (60)	0 (60)	2 (60)
mucoid epithelial metaplasia	0 (60)	1 (60)	1 (60)	1 (60)
cyst	0 (60)	0 (60)	1 (60)	0 (60)
Seminal vesicle (s)				
inflammation	2 (60)	3 (60)	3 (60)	3 (60)
atrophy	11 (60)	5 (60)	12 (60)	13 (60)
distended with secretion	2 (60)	0 (60)	0 (60)	0 (60)
inflammation, coagulation gland	1 (60)	5 (60)	1 (60)	2 (60)
secretion decreased	0 (60)	2 (60)	0 (60)	1 (60)
hyperplasia, epithelium	0 (60)	1 (60)	1 (60)	0 (60)
Pituitary				
adenoma, pars distalis	34 m (60) 45 f (60)	32 m (58) 48 f (60)	34 m (58) 46 f (60)	31 m (59) 34 f (59)
hyperplasia, parts distalis	10 m (60) 6 f (60)	10 m (58) 7 f (60)	9 m (58) 7 f (60)	10 m (59) 8 f (59)
vacuolation, pituicytes	0 m (60) 0 f (60)	0 m (58) 0 f (60)	0 m (58) 2 f (60)	1 m (59) 1 f (59)
Mammary gland				
adenoma/adenofibroma/fibroma	0 m (43) 25 f (58)	1 m (31) 24 f (54)	1 m (41) 27 f (59)	1 m (37) 28 f (57)
galactocele (s)	3 m (43) 8 f (58)	3 m (31) 14 f (54)	2 m (41) 4 f (59)	2 m (37) 9 f (57)
prominent secretory activity	6 m (43) 29 f (58)	8 m (31) 26 f (54)	11 m (41) 28 f (59)	5 m (37) 28 f (57)

Table 3 Summary Incidence of Microscopic Findings in a 2-Year Rat Study with Glyphosate[a]

Dose levels (ppm)	0	2000	8000	20,000
hyperplasia	0 m (43) 16 f (58)	2 m (31) 19 f (54)	2 m (41) 13 f (59)	0 m (37) 22 f (57)
carcinoma/adenomacarcinoma	1 m (43) 13 f (58)	0 m (31) 10 f (54)	0 m (41) 14 f (59)	0 m (37) 9 f (57)
adenoacanthoma	0 m (43)	0 m (31)	0 m (41)	1 m (37)
inflammation, granulomatous	0 f (58)	1 f (54)	0 f (59)	1 f (57)
inflammation, chronic	1 m (43) 0 f (58)	0 m (31) 1 f (54)	0 m (41) 0 f (59)	0 m (37) 1 f (57)
fibrosis	0 f (58)	1 f (54)	0 f (59)	0 f (57)
carcinosarcoma	1 f (58)	0 f (54)	0 f (59)	1 f (57)
Thyroid				
hypetplasia/cystic hyperplasia, follicular epithelium	4 m (60) 1 f (60)	2 m (58) 1 f (60)	1 m (58) 0 f (60)	2 m (60) 3 f (60)
C cell adenoma	2 m (60) 2 f (60)	4 m (58) 2 f (60)	8 m (58) 6 f (60)	7 m (60) 6 f (60)
C cell hyperplasia	5 m (60) 10 f (60)	1 m (58) 5 f (60)	6 m (58) 9 f (60)	5 m (60) 5 f (60)
follicular cyst (s)	2 m (60) 2 f (60)	1 m (58) 1 f (60)	3 m (58) 0 f (60)	3 m (60) 1 f (60)
C cell carcinoma	0 m (60) 0 f (60)	2 m (58) 0 f (60)	0 m (58) 1 f (60)	1 m (60) 0 f (60)

[a] Data from Stout and Ruecker, 1990

[b] All deaths reported. Incidence (total number of animals examined).

m = males f = females

Table 4 Summary of Reproductive and Microscopic Findings in a 2-Generation Rat Reproduction Study with Glyphosate [a]

Dose Levels (ppm)	0			30,000		
Generation	**FO**	**F1A**	**F1A-Remate**	**FO**	**F1A**	**F1A-Remate**
Total paired females	30	30	30	30	30	30
Females with confirmed copulation/total paired	96.7%	100.0%	83.3%	100.0%	96.7%	86.7%
Pregnant/total paired	80.0%	93.3%	53.3%	93.3%	86.7%	83.3%
Pregnant/confirmed copulation	82.8%	93.3%	64.0%	93.3%	89.7%	96.2%
Males with confirmed copulation/total paired	86.7%	93.3%	70.0%	90.0%	83.3%	80.0%
Males impregnating females/total paired	70%	90.0%	46.7%	83.3%	80.0%	76.7%
Males impregnating females/confirmed copulation	80.8%	96.4%	66.7%	92.6%	96.0%	95.8%
Precoital length for pregnant animals (days)	3.6	2.8	3.7	3.7	3.2	2.5
Gestational length (days)	22.3	22.4	22.4	22.3	22.6	22.5
Litter size						
female	6.7	6.6	6.0	5.7	5.5	5.6
male	6.6	5.4	5.9	5.8	5.3	5.2
combined	13.3	12.0	11.9	11.5	10.8	10.7
Terminal Body weight (gm)						
males	549.6	625.0		503.5**	543.4**	
females	296.3	316.2		265.9**	284.8**	
Organ weights (gm)						
ovary (ies)	0.1343	0.1579		0.1269	0.1587	
testis (es)	5.9959	6.6090		5.7905	6.3857	
Histopathology of tissue/organs:						
Epididymis (ides)						
vacuolation, duct epithelium	1 (30)[b]					
inflammation, mononuclear, interstitial		1(30)		5 (30)		
chronic inflammation, fibrosis					1 (29)	

Table 4 Summary of Reproductive and Microscopic Findings in a 2-Generation Rat Reproduction Study with Glyphosate [a]

Dose Levels (ppm)	0			30,000		
Generation	FO	F1A	F1A-Remate	FO	F1A	F1A-Remate
periepididymal adipose tissue, inflammation, granulomatous					1 (29)	
hypospermia, unilateral					1 (29)	
Testis						
hypoplasia/atrophy seminiferous tubule, bilateral	2 (30)	1 (30)		1 (30)		
Degeneration seminiferous tubules, unilateral		1 (30)			1 (29)	
hemorrhage		1 (30)				
granuloma, spermatic					1 (29)	
Ovary (ies)						
cyst (s)		3 (30)		1 (30)	3 (30)	
inactive		1 (30)				
Uterus						
remnant, implantation site	10 (29)	11 (29)		7 (29)	13 (29))	
mesometrium, calcified implantation remnant	1(29)					
dilation of uterine lumen (hydrometra)	5 (29)	5 (29)		9(29)	7 (29)	
pigment deposition		3 (29)			7(29)	
mononuclear infiltrate endometrium		1 (29)			1 (29)	
vascular necrosis mesometrium		1 (29)				
Vagina						
mononuclear cell infiltrate					1 (29)	
Prostrate						
chronic inflammation	14 (30)	4 (29)		12 (30)		
mononuclear cell inflitrate		1 (29)			1 (29)	
edema		2 (29)				
Seminal vesicle						

Table 4 Summary of Reproductive and Microscopic Findings in a 2-Generation Rat Reproduction Study with Glyphosate [a]

Dose Levels (ppm)	0			30,000		
Generation	**FO**	**F1A**	**F1A-Remate**	**FO**	**F1A**	**F1A-Remate**
mononuclear cell infiltrate		1 (29)			1 (29)	
Pituitary						
cyst (s)		2 m (30) 2 f (30)			2 m (28) 3 f (23)	
adenoma - pars distalis		1 f (30)				
Mammary gland						
galactocele		1 f (28)				
mononuclear cell, infiltrate		1 m (25)			1 f (30)	

[a] Data from Reyna, 1990

[b] 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)

Nature/Source Of Exposure	Glyphosate				AMPA				POEA			
	Female adult applicator		1-6 year female child		Female adult applicator		1-6 year female child		Female adult applicator		1-6 year female child	
	acute	chronic	acute	chronic	acute	chronic	acute	chronic	acute	chronic	acute	chronic
Routine												
- Dietary	23.8	23.8	51.9	51.9	4.8	4.8	10.4	10.4	11.9	11.9	26	26
- Application	56.2	8.5	--	--	--	--	--	--	128	20.6	--	--
Occasional												
- Drinking Water	3.6x10^{-2}	2.1x10^{-3}	0.11	6.7x10^{-3}	2.1x10^{-3}	2.1x10^{-3}	6.7x10^{-3}	6.7x10^{-3}	1.8x10^{-2}	1.1x10^{-3}	5.5x10^{-2}	3.3x10^{-3}
- Reentry	--	--	26	--	--	--	--	--	--	--	65.0	--
- Bystander	--	--	4.4	--	--	--	--	--	--	--	9.8	--
Infrequent/ rare												
- Swimming	1.28	--	6.5	--	--	--	--	--	0.64	--	3.2	--
- Wild Foods	45	--	45	--	--	--	--	--	23	--	23	
Aggregate*	125	32.3	97	52	4.8	4.8	10.4	10.4	162.9	32.5	91.1	26

* 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 6 Glyphosate: NOAELs for Toxicological Endpoints

Type of Study and Species Tested	NOAEL (mg/kg/day)	Comments	Study Reference
Subchronic toxicity			
Mouse, 90-day	2310	based on decreased b.w.[a] gain	Tierney, 1979
Mouse, 90-day	630	based on salivary gland lesions	NTP, 1992
Rat, 90-day	≥1445	no adverse effects at HDT[b]	Stout, 1987
Rat, 90-day	209	salivary gland changes at the lowest dose tested not considered toxicologically significant	NTP, 1992
Dog, 12-month	≥500	no adverse effects at HDT	Reyna and Ruecker, 1985
Chronic toxicity			
Mouse, 24-month	885	based on liver effects	Knezevich, 1983
Rat, 26-month	≥33	no adverse effects at HDT	Lankas, 1981
Rat, 24-month	409	based on decreased b.w. gain and ocular lesion	Stout, 1990a
Developmental toxicity			
Rat	1000	based on maternal and fetal effects	Tasker, 1980a
Rabbit	175	based on maternal toxicity	Tasker, 1980b
Reproductive toxicity			
Rat	≥30	no adverse effects at HDT	Schroeder, 1981
Rat	694	based on systemic toxicity; no reproductive effect	Reyna, 1990

[a] b.w. = body weight
[b] HDT - highest dose tested

Table 7 AMPA NOAELs for Toxicological Endpoints

Type of Study and Species Tested	NOAEL (mg/kg/day)	Comments	Study Reference
Subchronic toxicity			
Rat, 90-day	400	based on urinary tract infection	Estes, 1979
Dog, 90-day	263	no adverse effects at HDT	Tomkins, 1991
Chronic toxicity	>2.8	AMPA present at	Stout, 1990a
Rat, 24 month		0.68% in glyphosate study; no effects at mid-dose	
Developmental toxicity			
Rat	400	based on maternal and fetal b.w.[a] effects	Holson, 1991
Reproductive toxicity			
Rat	>4.2	AMPA present at 0.61% in glyphosate study; no effects at mid-dose	Reyna, 1990

[a] b.w. = body weight

Table 8 POEA NOAELs for Toxicological Endpoints

Type of Study and Species Tested	NOAEL (mg/kg/day)	Comments	Study Reference
Subchronic Toxicity			
Rat, 1-month	57	based on decreased b.w.[a] gains	Ogrowsky, 1989
Rat, 3-month	36	based on decreased b.w. and intestinal irritation	Stout, 1990b
Dog, 14-week	<30	based on reduced b.w. and gastrointestinal irritation	Filmore, 1973
Developmental Toxicity			
Rat	15	based on slight decrease in food consumption and mild clinical signs	Holson, 1990

[a] b.w. = body weight

Table 9 Summary of no-observed-adverse-effect levels (NOAEL), worst-case exposure estimates and Margins of Exposure (MOE) for glyphosate, AMPA, and POEA

Chemical	NOAEL (mg/kg/day)	Basis of NOAEL	Worst-case chronic exposure (mg/kg/day)		Margin of exposure[a]	
			Adults	Children	Adults	Children
Glyphosate	175	Maternal toxicity in developmental toxicity study	0.0323	0.052	5,420	3,370
	209	90-day rat study			--	4,020
AMPA	400	90-day rat and developmental toxicity studies	0.0048	0.0104	83,300	38,500
	>2.8	Based on AMPA content in glyphosate used for chronic rat study			>583	>269
POEA	15	Maternal toxicity in developmental toxicity study	0.0325	0.026	461	577
	36	90-day rat study			--	1380

[a] All MOE values rounded to 3 significant figures.

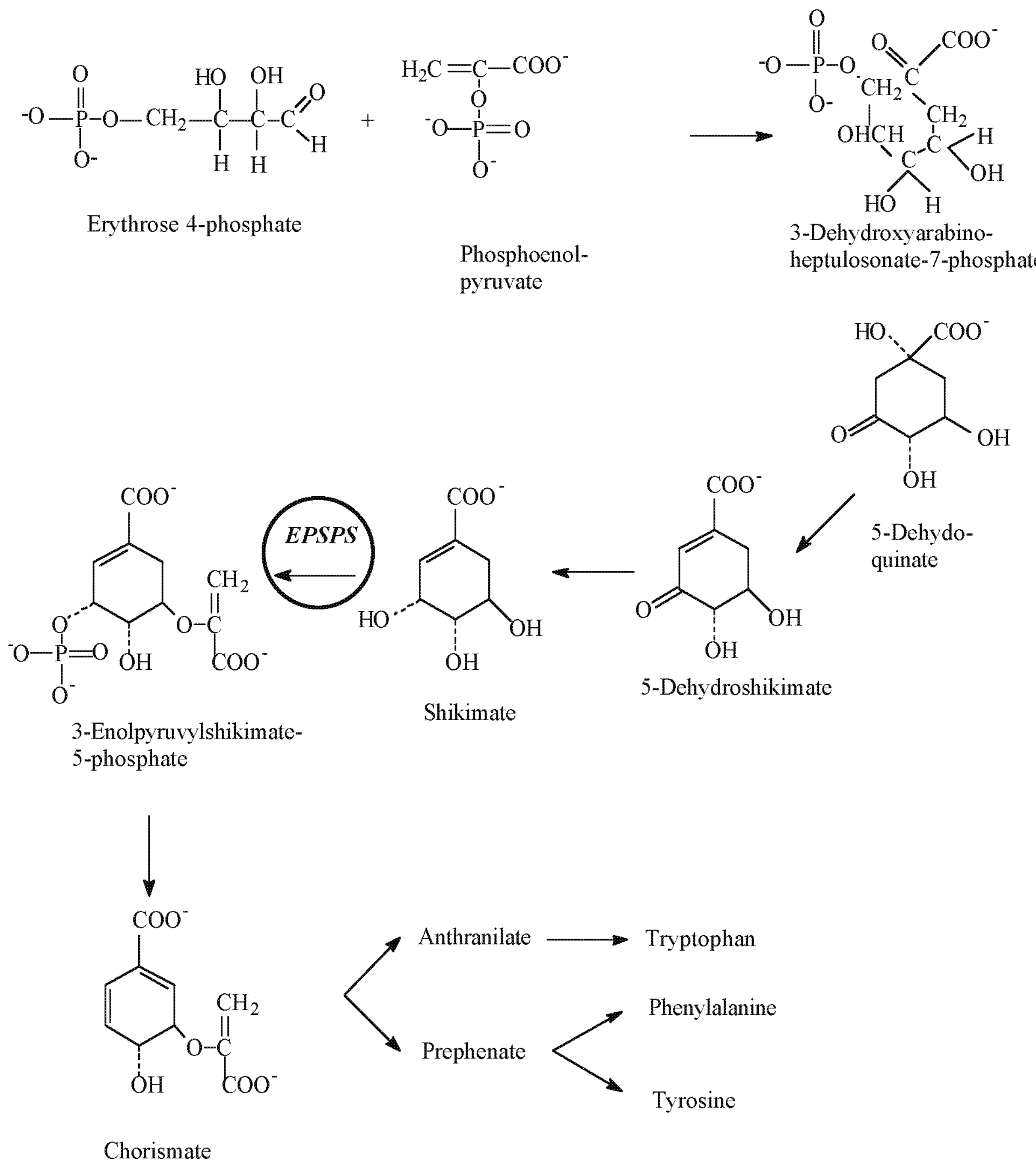

Figure 1: **Mechanism of action for glyphosate in plants. Glyphosate inhibits synthesis of essential aromatic aminoacids by competitive inhibition of the enzyme enolpyruvylshikimate phosphate synthase (EPSPS).**

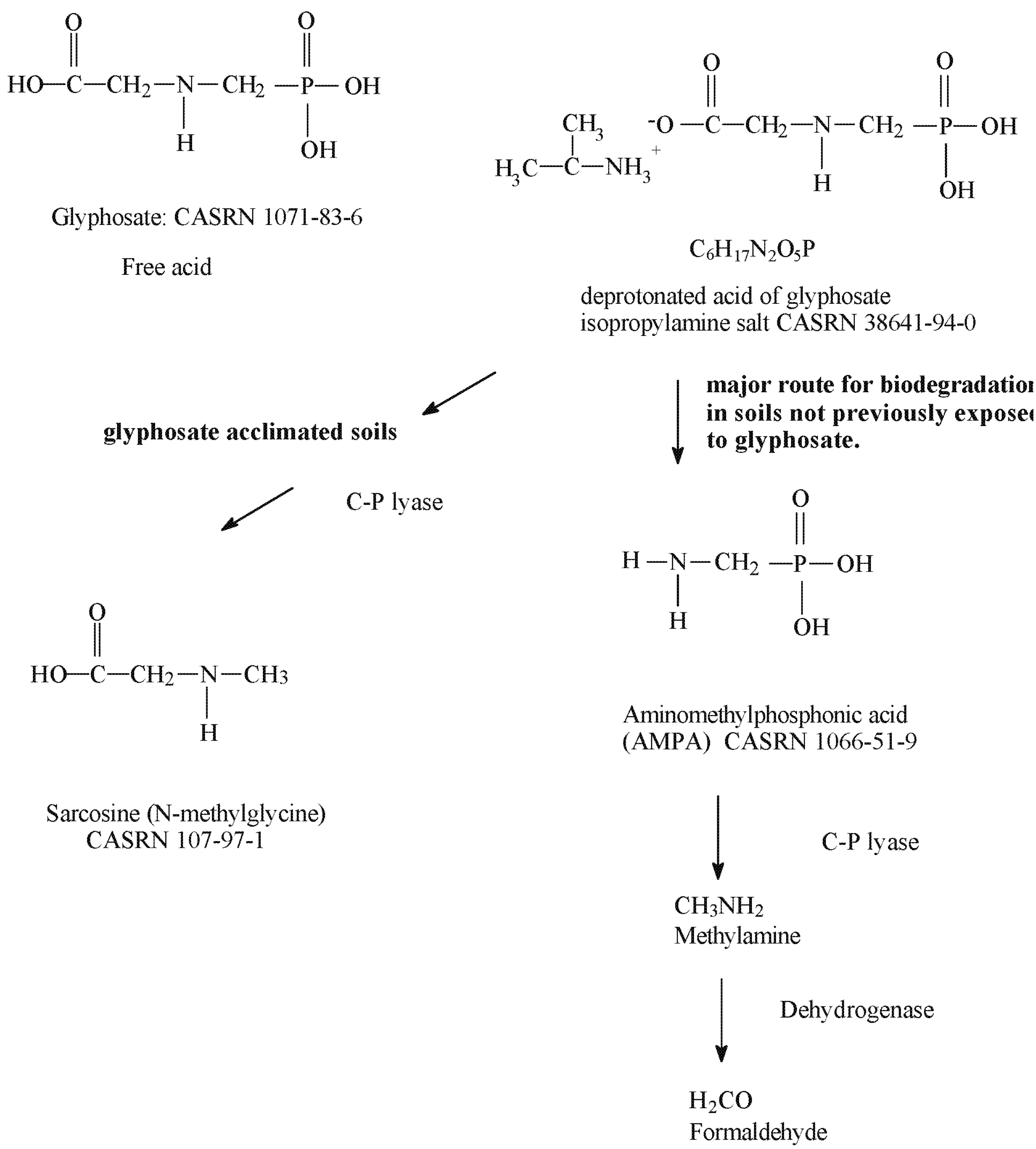

Figure 2: A simplified pathway for degradation of glyphosate in the terrestrial environment. Adapted from R. Wiersema, M. Burns, and D. Hershberger. University of Minnesota, 1997.

Guidance for preparing a Weight-of-Evidence analysis for mutagenicity data for a chemical.

Elements of Analysis

<u>**LOW WEIGHTING**</u> <u>**HIGH WEIGHTING**</u>

Assay System Validation

Weak → **Strong**

Reproducibliity /Consistency of Data

Variable → **Consistent**

Endpoint measured

Indirect/DNA damage → **Heritable Mutation**

Species/metabolism

***In vitro*/eucaryote** → ***In vivo* mammal**

Magnitude of Effect/Dose Level

Weak/Toxic dose → **Strong/Nontoxic dose**

Figure 3: Weight-of-evidence data hierarchy organization for evaluation and preparation of a statement of the potential for mutagenic activity of a compound.