Effects of Surfactants on the Toxicitiy of Glyphosate, with Specific Reference to RODEO

Submitted to:

Leslie Rubin, COTR
Animal and Plant Health Inspection Service (APHIS)
Biotechnology, Biologics and Environmental Protection
Environmental Analysis and Documentation
United States Department of Agriculture
Suite 5A44, Unit 149
4700 River Road
Riverdale, MD 20737

Task No. **5**
USDA Contract No. **53-3187-5-12**
USDA Order No. **43-3187-7-0028**

Prepared by:

Gary L. Diamond
Syracuse Research Corporation
Merrill Lane
Syracuse, NY 13210

and

Patrick R. Durkin
Syracuse Environmental Research Associates, Inc.
5100 Highbridge St., 42C
Fayetteville, New York 13066-0950

Telephone: (315) 637-9560
Fax: (315) 637-0445
CompuServe: 71151,64
Internet: SERA@interserv.com

February 6, 1997

TABLE OF CONTENTS

EXECUTIVE SUMMARY

Rodeo is an aqueous solution of the isopropyl amine salt of glyphosate. The manufacturer recommends use of a nonionic surfactant with all applications of Rodeo to improve efficacy. Surfactant formulations that are used with Rodeo include:

Agri-Dex (Setre Chemical Co.)
LI 700 (Loveland Industries, Inc.)
R-11 (Wilbur-Ellis Co.)
Latron AG-98 (Rohm and Haas Co.)
Latron AG-98 AG (Rohm and Haas Co.)

This report describes the components of the above surfactant formulations and notes those components that are listed in the U.S. EPA List of Inert Ingredients in Pesticides. All of the formulations are chemical mixtures and must be considered as mixtures in toxicity assessments. In this context, an assessment of the specific surfactants in any of the formulations or generalizations about the toxicology of surfactants as a group may not apply to the formulations. This consideration places extreme importance on data regarding the toxicity of the formulations themselves. The lack of such data will render any predictions about the effects of the formulations on glyphosate highly uncertain.

Based on the results of searches of the published literature and the Toxic Substances Control Act Test Submission (TSCATS) database, little data are available regarding the toxicity of the surfactant formulations. The available data on the formulations consist of acute lethality studies of LI 700 in rats and rabbits; dermal and eye irritation studies of LI 700 in rabbits; aquatic toxicity studies of LI 700, R-11 and Agri-Dex in fish and invertebrates; and a study of the effects of Triton AG-98 (Latron AG-98) on the fungus *Mucor mucedo* (*Mucor* rot). These studies indicate that the aquatic toxicity potency of R-11 may be considerably greater (10 to 50 times greater) than that of LI 700 and Agri-Dex.

The lack of toxicology studies of the surfactant formulations makes it difficult to predict what effects, if any, the formulations may have on the toxicity of Rodeo. The toxicologies of some of the surfactants in the various formulations were studied (summaries are provided in this report); however, the studies alone do not provide a basis for predicting either the toxicity of the formulations or their effects on the toxicity of Rodeo.

Information regarding the surfactants in the glyphosate formulations Roundup and Roundup Pro is summarized in this report; the summary was extracted from the draft report, *Selected Commercial Formulations of Glyphosate - Accord, Rodeo, Roundup and Roundup Pro. Risk Assessment Final Report*, prepared for USDA by Syracuse Environmental Research Associates (SERA, 1996). There is some uncertainty in the interpretation of the toxicity data on Roundup with respect to the potential significance of the surfactant; however, since the available toxicity data on Roundup are adequate for identifying toxic thresholds, the

uncertainty regarding the surfactant is a relatively minor one. There is much less toxicological information available on Roundup Pro and the surfactant used in Roundup Pro. Based on a comparison of the available toxicity data on Roundup Pro with corresponding data on Roundup, it appears that these two formulations will have similar toxicological properties, although Roundup Pro may be somewhat less irritating to the skin and eyes.

A major qualitative difference between the effect of glyphosate and glyphosate formulations on aquatic and terrestrial organisms concerns the surfactant used in Roundup. The surfactant is much more toxic than glyphosate to aquatic organisms. Unlike glyphosate, the surfactant is more toxic in alkaline water than in acidic water. Thus, the relative potency of the surfactant with respect to glyphosate is pH dependent. There is relatively little information regarding the toxicity of Roundup Pro to aquatic species. Nonetheless, the acute lethal potencies of Roundup and Roundup Pro are similar.

1. INTRODUCTION

Rodeo is an aqueous solution of the isopropyl amine salt of glyphosate. The manufacturer recommends use of a nonionic surfactant with all applications of Rodeo to improve efficacy. Surfactant formulations that are used with Rodeo include:

Agri-Dex (Setre Chemical Co.)
LI-700 (Loveland Industries, Inc.)
R-11 (Wilbur-Ellis Co.)
Latron AG-98 (Rohm and Haas Co.)
Latron AG-98 AG (Rohm and Haas Co.)

All of the above formulations are mixtures containing various surfactants and other ingredients (e.g., solvents, antifoam compounds).

The term *surfactant* refers to chemicals that have pronounced surface activity in aqueous solutions. Surface activity derives from the orientation of hydrophillic and hydrophobic groups within the surfactant molecule which yield oriented films at the aqueous boundaries that can decrease surface tension. In the formulations listed above, the hydrophillic portion of the surfactants consists of an oxyethyl polymer or, in one of the surfactants in LI-700, a choline phosphate ester of glycerol. Surfactant hydrophobicity is derived from saturated hydrocarbon chains of various length and linkages to the hydrophillic group. With the exception of phosphatidylcholine choline, all of the surfactants are nonionic.

The following general issues must be considered in an assessment of the effects of surfactant formulations on the toxicity of glyphosate:

1. The formulations are chemical mixtures and must be considered as mixtures in toxicity assessments. In this context, an assessment of the specific surfactants in any of the formulations or generalizations about the toxicology of surfactants as a group may not apply to the formulations. This consideration places extreme importance on data regarding the toxicity of the formulations themselves. The lack of such data will render any predictions about the effects of the formulations on glyphosate highly uncertain.

2. The same structural characteristics that produce surface activity in aqueous solutions allow surfactants to interact with biological membranes which are, in themselves, oriented films of surfactants, mainly, lipid phosphatides (Edidin and Sessions 1983). Thus, surfactants can be expected to interact with and perturb the structure, physical properties, and function of membranes. The degree to which these interactions occur depends on the physical and chemical properties of the surfactant (Caux et al. 1993, Wax et al. 1994). The wide range of possible effects of such interactions makes it extremely difficult to predict, with any certainty, the toxicity of surfactants or their

effects on the disposition or toxicity of other chemicals based on structural, mechanistic, or theoretical considerations alone. This point underscores the importance of toxicity assays (e.g., acute, chronic, different routes) in predicting the toxicity of the surfactants on humans or other species or the effects of the surfactants on the toxicity of glyphosate.

3. Possible mechanisms by which the surfactants might exert biological effects or affect the toxicity of glyphosate include: decreasing surface tension (Ernst and Arditti 1980, Imai et al. 1994); perturbing membrane permeability or transport function of membranes or other diffusion barriers (e.g., leaf cuticle), including permeability to glyphosate (Coret and Chamel 1993, 1994, Wax et al. 1994); and interacting directly with glyphosate to alter the disposition of glyphosate. Evidence for specific mechanisms of interactions between glyphosate and surfactants is lacking.

4. The structural characteristics of extreme hydrophillicity and hydrophobicity of surfactants may result in very different interactions with hydrophobic and hydrophillic herbicides. Thus, the relatively water-soluble isopropyl amine salt of glyphosate may interact differently with surfactants than the less water-soluble parent compound or other more insoluble herbicides (Coret and Chamel, 1993, 1994). The dependence of surfactant-herbicide interactions on herbicide structure will introduce uncertainty into extrapolations based on data for other herbicides.

5. At concentrations exceeding a critical value, surfactants in aqueous solutions form micelles in which the hydrophobic groups orient toward the inside of the micelle and the hydrophillic groups are oriented toward the outside aqueous environment. The concentration at which a surfactant forms micelles varies with the structure of the surfactant and properties of the aqueous solution and is known as the critical micelle concentration (CMC). It is very likely that the toxicology and any interactions that occur between surfactants and glyphosate will be different at surfactant concentrations above and below the CMC because of the extreme differences in the physical properties of the micelle and the "free" surfactant molecule. This means that predictions that are based on toxicity assays conducted at concentrations below the CMC may not apply to conditions in which the surfactant concentration exceeds the CMC, and *vice versa*. The CMC for the surfactants cannot be estimated from the information available on the various formulations. Furthermore, the consequences of exceeding the CMC on the toxicity of the surfactants and on the toxicity of glyphosate is not predictable given our lack of understanding about mechanisms of toxicity and interactions with glyphosate.

6. The biological effects of surfactants and their interactions with glyphosate are expected to be highly sensitive to the structural characteristics that determine the absolute and relative hydrophobicity and hydrophillicity of the surfactant (Coret and Chamel 1994, Ernst and Arditti 1980, Imai et al. 1994, Knoche et al. 1992). This expectation introduces uncertainties in the predictions of the biological effects of surfactant

mixtures of varying and/or unknown structure, based on data for any given surfactant. A related issue is that product labels, manufacturer data sheets, and material safety data sheets (MSDS) do not identify the exact chemical structures of the surfactants in the various formulations.

The above considerations reinforce the importance of bioassay data on the surfactant formulations to support assessments of toxicity or interactions with glyphosate. For this reason, the emphasis of this report is on characterizing the types of toxicological data that are available for making such assessments.

Chapter 2 of this report, which follows the Introduction, describes the components of the various surfactant formulations and notes those components that are listed in the U.S. EPA List of Inert Ingredients in Pesticides. Chapter 3 summarizes data that are available on the toxicity of the formulations and on the surfactants in the formulations. The objective of the summary is to present an overview of the types of data available on the formulations and surfactants and is not intended to be an in depth evaluation or analysis of the data. Chapter 4 summarizes information on the surfactants that are in the glyphosate formulations Roundup and Roundup Pro. This summary was extracted from the draft report, *Selected Commercial Formulations of Glyphosate - Accord, Rodeo, Roundup and Roundup Pro. Risk Assessment Final Report*, prepared for USDA by Syracuse Environmental Research Associates (SERA, 1996). References are provided in Chapter 5.

2. CONSTITUENTS OF SURFACTANT FORMULATIONS USED WITH RODE0

Constituents of the surfactant formulations Agri-Dex, LI-700, R-11, Latron AG-98 and Latron AG-98 AG are presented in Table 2-1. Those constituents that appear on the U.S. EPA Lists of Inert Ingredients in Pesticide Products (U.S. EPA, 1987, 1994) are noted. U.S. EPA (1987) established four categories of inert ingredients of pesticide products. List 1 includes inerts of toxicological concern; List 2 includes potentially toxic inerts with high priority for testing; List 3 includes inerts of known toxicity; and List 4 includes inerts of minimal concern. In 1994, U.S. EPA (1994) further subdivided List 4 into List 4A, which contains all chemicals on the 1987 List 4, and List 4B, which includes inerts for which EPA has concluded that there is *"sufficient information to conclude that current use patterns in pesticide products will not adversely affect public health and the environment*." The empirical bases for the above conclusions were not available for this assessment and presumably include toxicology and ecotoxicology assays (Levine, 1996).

2.1. AGRI-DEX

According to the Material Safety Data Sheet (MSDS), Agri-Dex is a blend of polyoxyethylated polyol fatty acid ester, polyol fatty acid ester, and paraffin base petroleum oil (Setre Chemical Co.). The surfactant activity of the formulation is provided by the polyoxyethylated polyol fatty acid ester and polyol fatty acid ester. The name polyoxyethylated polyol fatty acid ester refers to a group of chemicals that consist of unspecified fatty acid esters of unspecified polyoxyethylated alcohols. Similarly, the name polyol fatty acid ester refers to unspecified fatty acid esters of unspecified alcohols. A more specific identification of these surfactants was not available.

The paraffin base petroleum oil assigned the CAS numbers in Table 1 is described in the Registry File of Chemical Abstracts (STN, International 1997) as a solvent refined paraffinic distillate containing a mixture of hydrocarbons having carbon numbers predominantly in the range C20-C50 (heavy paraffinic, 64741-88-4) or C15-C30 (light paraffinic, 64741-89-5). The paraffinic oil mixtures are not on the U.S. EPA lists of Inert Ingredients of Pesticides, although other paraffinic oils are on the list. For example, light (C15-C30) and heavy (C20-C50) paraffinic oils produced by vacuum distillation of the residue from atmospheric distillation of crude oil (CAS Nos. 64741-50-0 and 64741-51-1) are on List 2. The reason why certain paraffinic oils are on the U.S. EPA inerts list and others are not is not apparent.

2.2. LI-700

According to the manufacturer data sheet, LI 700 is a mixture of phosphatidylcholine, propionic acid, and alkylpolyoxyethylene ether (Loveland Industries, Inc.). These ingredients comprise 80% of the formulation; the remaining 20% is identified as *"constituents ineffective as adjuvant."* The MSDS for LI 700 lists only phosphatidylcholine and propionic acid as constituents and lists only propionic acid as a hazardous component of the mixture. The surfactant activity of the formulation is provided by phosphatidylcholine and alkylpolyoxyethylene ether.

Table 2-1. EPA inerts list classification for constituents of selected surfactants[a]

Surfactant Product	Chemical Constituents[b]	CAS No.	EPA Inerts Classification
Agri-Dex	Polyol fatty acid esters[c]	NA	--
	Polyoxyethyl polyol fatty acid esters[c]	NA	--
	Paraffin base petroleum oil	64741-88-4 64741-89-5	NL NL
LI 700	Phosphatidylcholine	8002-43-5[d]	4A
	Propionic acid	74-09-4	4B
	Alkylpolyoxyethylene ether[c]	NA	--
R-11	Octylphenoxypolyethoxyethanol	9036-19-5[e]	4B
	n-Butanol	71-36-3	4B
	Compounded silicone[c]	NA	--
Latron AG-98	Nonylphenoxypolyethoxyethanol	68412-54-4	3
	n-Butanol	71-63-3	4B
	Silicone antifoam compound[c]	NA	--
Latron AG-98 AG	Octylphenoxypolyethoxyethanol	9036-19-5	4B
	Isopropanol	67-63-0	4B
	Polydimethylsiloxane	63148-62-9	4B

[a]U.S.EPA lists of Inert Ingredients in Pesticides (U.S. EPA, 1994): List 1 = Toxicological concern; List 2 = Potentially toxic with high priority for testing; List 3 = Unknown toxicity; List 4 = minimal concern (further subdivided into List 4A = classified in U.S. EPA, 1987 as List 4 and List 4B = *sufficient information to conclude that current use patterns in pesticide products will not adversely affect public health and the environment*.

[b]Sources: Technical data sheets, material safety data sheets for surfactant products.

[c]Not sufficiently described to identify the specific consituent(s).

[d]lecithins

[e] polyoxyethylene (1,1,3,3-tetramethylbutyl)phenyl ether

NA = Not available; NL = not listed.

The name phosphatidylcholine refers to a group of chemicals that consist of unspecified fatty acid diglycerides linked to the choline ester of phosphoric acid. This group includes the

naturally occurring lecithins which are prominent phospholipids in biological cell membranes (Edidin and Sessions, 1983). The U.S. EPA lists lecithins (CAS No. 8002-43-5) and soya lecithins (CAS No. 8030-76-0) on List 4A of Inert Ingredients of Pesticides. Soya lecithins are used as emulsifiers in a variety of food products. The exact identity of the phosphatidylcholine in LI 700 could not be determined from the manufacturer data sheet or the MSDS.

The name alkylpolyoxyethylene ether refers to a chemical group of unspecified alkyl ethers of polyoxyethylene. A more specific identification of these surfactants was not available.

Propionic acid, a third constituent of LI 700, is on List 4B of the U.S. EPA List of Inert Ingredients of Pesticides.

2.3. R-11

According to the manufacturer data sheets, R-11 is a mixture of octylphenoxypolyethoxyethanol, n-butanol and compounded silicone (Wilbur-Ellis Co.). These ingredients comprise 90% of the formulation; the remaining 10% is identified as *"constituents ineffective as spray adjuvant."* The MSDS for R-11 lists the hazardous ingredients as 20% butyl alcohol and 80% nonionic surfactants. The surfactant activity of the formulation is provided by octylphenoxypolyethoxyethanol.

The name octylphenoxypolyethoxyethanol refers to a group of chemicals that consist of an 8-carbon alkylphenol linked to an ethoxyethanol polymer of unspecified length. A search of the Registry File of Chemical Abstracts (STN, International 1997) for the name octylphenoxypolyethoxyethanol found polyoxyethylene (1,1,3,3-tetramethylbutyl)phenyl ether, CAS No. 9036-19-5. This chemical is on List 4B of the U.S. EPA List of Inert Ingredients of Pesticides. It is possible that R-11 contains octylphenoxypolyethoxyethanols other than polyoxyethylene (1,1,3,3-tetramethylbutyl)phenyl ether.

n-Butanol is on List 4B of the U.S. EPA List of Inert Ingredients of Pesticides.

A more specific identification of the compounded silicone constituent of R-11 is not available. It may be the same (or similar) antifoam compound as that in Latron AG-98 AG, polydimethylsiloxane, which is on List 4B of the U.S. EPA List of Inert Ingredients of Pesticides.

2.4. LATRON AG-98

According to the manufacturer data sheets, Latron AG-98 is a mixture of nonylphenoxypolyethoxyethanol, n-butanol and a silicon antifoam compound (Rohm and Haas Co.). Nonylphenoxypolyethoxyethanol and n-butanol make up 90% of the formulation with 10% being *"constituents ineffective as spray adjuvants,"* which presumably includes the silicon antifoam compound. The surfactant activity is provided by nonylphenoxypolyethoxyethanol.

The name nonylphenoxypolyethoxyethanol refers to a group of chemicals that consist of a 9-carbon alkylphenol linked to an ethoxyethanol polymer of unspecified length. Nonylphenoxypolyethoxyethanol is on List 3 of the U.S. EPA List of Inert Ingredients of Pesticides. n-Butanol is on List 4B of the U.S. EPA List of Inert Ingredients of Pesticides. The antifoam compound could not be identified from the manufacturers' literature or MSDS. It may be polydimethylsiloxane which is a component of a similar formulation, Latron AG-98 AG (Rohm and Haas, Co.) and which is on List 4B.

2.5. LATRON AG-98 AG

According to the manufacturer data sheets, Latron AG-98 AG is a mixture of octylphenoxypolyethoxyethanol, isopropanol and polydimethylsiloxane (Rohm and Haas Co.). Surfactant activity of the mixture is provided by octylphenoxypolyethoxyethanol. As previously noted for R-11, the octylphenoxypolyethoxyethanol, polyoxyethylene (1,1,3,3-tetramethylbutyl)phenyl ether (CAS No. 9036-19-5), is on List 4B of the U.S. EPA List of Inert Ingredients of Pesticides. However, it is not clear from available information if the surfactant in Latron AG-98 is the same octylphenoxypolyethoxyethanol mixture as in R-11. Surfactants in the two formulations could have different structural features that might affect their toxicity and/or their effect on glyphosate toxicity. For example, there could be differences in the degree of branching of the hydrophobic alklyphenylether portion of the surfactant(s) or length of the hydrophyllic polyoxyethylene chain.

Isopropanol and polydimethylsiloxane(s) are on List 4B.

3. INFORMATION ON EFFECTS OF SURFACTANT FORMULATIONS ON THE TOXICITY OF RODEO

3.1. HUMAN HEALTH

A search of the scientific literature (AGRICOLA, TSCATS, EMIC, EMICBACK and TOXLINE) was conducted to find studies on the toxicology of the surfactant formulations described in Chapter 2. No studies were found.

The only available toxicity studies of any of the formulations are acute lethality studies and a dermal irritation study of LI 700; these are described in attachments to a letter from Rudy Lapurga of the California EPA to John Borrecco of USDA (Lapurga 1996) (described below). As noted in Chapter 1 (Introduction), the lack of toxicology studies regarding the formulations themselves makes it difficult to predict what effects, if any, the formulations may have on the toxicity of Rodeo. The toxicologies of some of the surfactants in the various formulations have been studied; however, these studies alone do not provide a basis for predicting either the toxicity of the formulations or their effects on the toxicity of Rodeo.

Searches were conducted to find toxicological studies of the surfactants in the various surfactant formulations to determine if a basis might exist for attempting a component-based risk assessment of the formulations. No attempt was made to review the individual studies for quality or applicability to quantitative risk assessment. Nevertheless, the results of the search give some idea of the type of data that are available on the toxicology of the surfactants. Based on this cursory survey, combined with uncertainty about the exact chemical structures of the surfactants in the formulations, there does not appear to be adequate data to develop quantitative risk assessments of the surfactants. It should be kept in mind that this survey of the literature did not include direct queries to the manufacturers; it is possible that more adequate data exist that are not publicly available. The results of the search are summarized below (searches of the surfactants in Agri-Dex were not attempted because the identity of the surfactant compounds in the formulation could not be determined).

3.1.1. LI 700. Acute lethality studies and a dermal irritation study of LI 700 are described in attachments to a letter from Rudy Lapurga of the California EPA to John Borrecco of USDA (Lapurga 1996). The single-dose oral LD_{50} (observation period not specified) was reported as > 5.0 and 5.9 g/kg in male and female rats, respectively. Animals that died had congested kidneys and gastrointestinal irritation. The dermal LD_{50} for a 24-hour exposure was reported as > 5.0 g/kg in rabbits. Animals that died had discoloration of the lungs and pleural cavity, discoloration of the liver, congestion of the kidneys, and bloody urine. Eye and dermal irritation assays in the rabbit showed that LI 700 produced eye irritation when introduced into the eyes and dermal irritation when applied to the skin.

3.1.2. Phosphatidylcholine. Phosphatidylcholine is a surfactant component of LI 700. The human nutritional and clinical experience with the phosphatidylcholine, lecithin, is quite extensive. In 1974, the World Health Organization (WHO, 1974) reported that the average

diet provides 1-5 g lecithin/day and concluded that the acceptable daily intake was *"not limited"* based on nutritional experience with humans. (Note, there appears to be some confusion in the literature regarding the terminology applied to phosphatidylcholine and lecithin and this needs to be considered in any cursory review of literature. In some cases, the terms phosphatidylcholine and lecithin are used interchangeably to refer to a specific class of phospholipids associated with cell membranes. The term lecithin also is applied to commercial formulations of lecithins that contain phosphatidylcholine and other lipid phosphatides.) The Life Sciences Research Office of the Federation of the American Societies for Experimental Biology, at the request of the Food and Drug Administration, conducted a review of the status of lecithin, phosphatidylcholine and choline therapy as dietary supplements (Wood et al., 1981). In a summary report of this review, Wood and Allision (1982) reported that the clinical case literature includes cases in which oral dosages of 20-40 g/day phosphatidylcholine (in commercial lecithin formulations) was *"tolerated"* without adverse *"side effects."*

Liposomes (micelles) composed of phosphatidylcholine were explored as inhalant vehicles for delivering drugs into the respiratory tract; acute inhalation toxicology studies relevant to this use are available in the literature (e.g., Thomas et al., 1991).

3.1.3. Alkylpolyoxyethylene ether. Berberian et al. (1965) studied the acute toxicity of a polyoxyethyene dodecyl ether, Laureth 9, a spermicidal agent intended for use in contraceptive foams. The acute 24-hour LD_{50} in mice was 3300 mg/kg and the 7-day LD_{50} was 3050 mg/kg. The LD_{50} for a 5-day oral exposure was 1190 mg/kg/day. Daily dosages of 390 mg/kg/day for 22 days resulted in depressed growth rates in mice and a dosage of 780 mg/kg/day resulted in 20% mortality. Grubb et al. (1960) reported 7-day LD_{50} values for a polyoxyethyene dodecyl ether of 1170 mg/kg in mice and 4150 mg/kg in rats.

Ernst and Arditti (1980) examined the structure-activity relationships for the cytotoxicity of nonionic alkylpolyethoxyethanol surfactants in isolated human skin fibroblast cell cultures (HeLa cells). Cytotoxic potency decreased with increasing polyoxyethyl chain length (increasing hydrophobicity) when the alkyl chain length remained constant. When the polyoxyethyl chain length was held constant, cytotoxicity increased with increasing alkyl chain length (increased hydrophobicity). This study and other structure-activity studies (Coret and Chamel 1993, 1994, Knoche et al. 1992) suggest that biological activity of nonionic surfactants will vary with size of the hydrophobic and hydrophillic domains of the surfactants.

3.1.4. Octylphenoxypolyethoxyethanol. Octylphenoxypolyethoxyethanol is a surfactant in AN R-11 and Latron AG-98 AG. Octylphenoxypolyethoxyethanol also is the sole component of the surfactant formulations, Triton X-100, Triton X-102, and Triton X-15 (Union Carbide Chemical and Plastics Company, Inc.). Toxicology studies of various Triton X formulations have been reported in submissions to the U.S. EPA under Section 8E of the Toxic Substances Control Act and were located by searching the TSCATS database (Union Carbide, 1992a,b,c,d). These studies suggest that octylphenoxypolyethoxyethanol is a skin irritant and eye irritant in rabbits. The LD_{50} for a single oral dose in the rats was approximately 1-5 g/kg.

The LD_{50} for a single percutaneous dose in rabbits was approximately 12 g/kg. Administration of percutaneous octylphenoxypolyethoxyethanol (Triton X-100) to pregnant rats on days 6-15 of gestation (0.4 - 4 g/day) resulted in skeletal abnormalities in pups (extra ribs and enhanced ossification). The same types of skeletal effects were observed in pups when rats were exposed to Triton X-100 in the diet (0.06% or 0.3%) on days 6-17 of gestation.

Thompson and Gibson (1984) reported a single-dose oral 14-day LD_{50} for Triton X-15 (octylphenoxypolyethoxyethanol) of 11.6 and 4.2 g/kg in male and female Sprague-Dawley rats, respectively (the authors observed higher LD_{50} values in male rats for six other chemicals tested but provided no explanation). Rats were administered a single oral dose of Triton X-15 at the estimated LD_{01}, $LD_{10,}$ and LD_{30} level and killed 20 hours after dosing. Bone marrow cells were collected for assessment of chromosomal abnormalities and suppression of mitosis (mitotic index). No chromosomal abnormalities were observed in rats that received Triton X-15. Male rats that received an LD_{10} or LD_{30} (10.2 or 11.0 g/kg) had significantly depressed mitotic indices ($p <= 0.05$, approximately 50% reduction from controls). Female rats that received a single LD_{10} or LD_{30} dose (3.0 or 3.7 g/kg) had no significant depression of mitotic index.

Long et al. (1982) reported an *in vitro* study of transforming activity of Triton X-100 (octylphenoxypolyethoxyethanol) in mouse BALB/3T3 and 10T1/2 fibroblasts. Triton X-100 was inactive in both systems.

3.1.5. Nonylphenoxypolyethoxyethanol. Nonylphenoxypolyethoxyethanol is a surfactant component of Latron AG-98. It also is the sole component of the surfactants Triton N-60, IGEPAL C0-630 (also known as NP-9). Toxicology studies of these formulations have been reported in submissions to the U.S. EPA under Section 8E of the Toxic Substances Control Act and were located by searching the TSCATS database (Rhone-Poulenc, Inc. 1991, Rohm and Haas Co. 1992). These studies suggest that nonylphenoxypolyethoxyethanol is a skin and eye irritant in the rabbit.

A search of the published toxicological literature found an *in vitro* study of transforming activity in mouse BALB/3T3 and 10T1/2 fibroblasts (Long et al. 1982). The nonylphenoxypolyethoxyethanol mixture (NP-9, also known as IGEPAL-630) induced transformations in both cell cultures.

Nonylphenoxypolyethoxyethanol has been used as a spermicide in vaginal contraceptive foams. Toxicological studies of the effects of vaginal applications of nonylphenoxypolyethoxyethanol on vaginal physiology have been reported (e.g., Levin 1987). Anticipated human exposure to surfactants from pesticide applications is not analogous to the type of exposure that would occur from use of the surfactant as a contraceptive foam (i.e., intravaginal).

Long et al. (1982) reported an *in vitro* study of transforming activity of NP-9 (also known as IGEPAL-630) in mouse BALB/3T3 and 10T1/2 fibroblasts. NP-9 induced transformations in both mouse cell transformation systems.

3.2. ECOLOGICAL EFFECTS

Aquatic toxicity studies of LI 700 were described in attachments to a letter from Rudy Lapurga of the California EPA to John Borrecco of USDA (Lapurga 1996) (Table 3-1). The acute 96-hour LC_{50} (nominal concentration, static exposure conditions) in juvenile Bluegill sunfish (*Lepomis macrochirus*) was 210 mg/L, and 130 mg/L in juvenile Rainbow trout (*Oncorhynchus mykiss*). The acute 48-hour LC_{50} in *Daphnia magna* was190 mg/L.

The same letter (Lapurga 1996) describes studies of the aquatic toxicity of R-11. The acute 96-hour LC_{50} (nominal concentration, static exposure conditions) in juvenile Bluegill sunfish (*Lepomis macrochirus*) was 4.2 mg/L, and in juvenile Rainbow trout (*Oncorhynchus mykiss*), 3.8 mg/L. The acute 48-hour LC_{50} in *Daphnia magna* was 19 mg/L.

Acute toxicity data on LI 700, R-11 and Agri-Dex are summarized in a report entitled *Use of the Registered Aquatic Herbicide Fluridone (SONAR) and the Use of the Registered Aquatic Herbicide Glyphosate (Rodeo and Accord) in the State of New York*, prepared by McLaren/Hart for DowElanco and Monsanto (McLaren/Hart 1995) (Table 3.2). Note that the values for LI 700 and R-11 are nearly identical to those reported in Lapurga (1996), except that the 48-hour LC_{50} for LI 700 in *Daphnia magna* is reported as 170 mg/L in McLaren/Hart (1995) and 190 mg/L in Lapurga (1996). The similarities between the data in the two reports are so striking that the possibility that they represent the exact same studies should be considered.

The above studies indicate that the toxicity potency of R-11, as assessed by lethality in these species, is considerably greater than LI 700 or Agri-Dex (10 to 50 times greater). Considerable uncertainty would be attached to any extrapolations of these results to other species or exposure durations.

A search of the scientific literature (AGRICOLA, TSCATS, EMIC, EMICBACK and TOXLINE) was conducted to find studies on the ecotoxicology of the surfactant formulations described in Chapter 2. The search found one study on the effect of Triton AG-98 (Latron AG-98) on *in vitro* spore germination and germ tube growth of the fungus, *Mucor mucedo*, the causative agent of a fungal rot in stored fruit (Reyes 1992). As noted for the survey of data relevant to the assessment of human health effects, the lack of toxicology studies on the formulations themselves makes it difficult to predict what effects, if any, the formulations may have on the ecotoxicity of Rodeo. A search was conducted for studies concerning the toxicity of individual surfactants in the formulations to determine whether there might be a basis for a component-based assessment of the formulations. A study of toxicity in marine shrimp and a study of toxicity in aphids were found as well as several studies on the phytotoxicity of various surfactants. No studies were found regarding toxicity to aquatic or terrestrial vertebrates

(other than those on rats and rabbits described in Section 3.1 on human health). Therefore, data appear to be inadequate for a quantitative assessment of ecological effects of the surfactants. Here again, this survey did not query the manufacturers for data that might not be publicly available. The available studies are summarized below.

A study of the acute toxicity of IGEPAL CO-210 (GAF Corporation) to the opossum shrimp (*Mysidosis Bahia*) was submitted to the U.S. EPA under Section 8D of the Toxic Substances Control Act (Quantum Chemical Corp. 1992). Nonylphenoxypolyethoxyethanol is the sole ingredient of IGEPAL CO-210. An acute (48-hour) LC_{50} of 0.26 mg/L was reported.

Table 3-1. Lethality of surfactant formulations LI 700 and R-11 to aquatic species[a]

Genus species	LC_{50} (mg/L)[b]	
	LI 700	**R-11**
Lepomis macrochirus (Bluegill sunfish)	210	4.2
Oncorhynchus mykiss (Rainbow trout)	130	3.8
Daphnia magna	190	19

[a]Source: Lapurga 1996

[b]Nominal concentration under static conditions. 96-hour exposure of *Lepomis macrochirus* and *Oncorhynchus mykiss*; 48-hour exposure of *Daphnia magna*.

Table 3-2. Lethality of surfactant formulations LI 700, R-11 and Agri-Dex to aquatic species[a]

Genus species	LC_{50} (mg/L)[b]		
	LI 700	**R-11**	**Agri-Dex**
Lepomis macrochirus (Bluegill sunfish)	210	4.2	> 1000
Oncorhynchus mykiss (Rainbow trout)	130	3.8	> 1000
Daphnia magna	170	19	> 1000

[a]Source: McLaren/Hart 1995

[b]96-hour exposure of *Lepomis macrochirus* and *Oncorhynchus mykiss*; 48-hour exposure of *Daphnia magna*.

Imai et al. (1994) compared the lethality of various surfactants in laboratory cultures of green peach aphid, *Myzus persicae*. Lethality varied considerably among various preparations of nonly- and octylphenoxypolyethoxyethanols, and alkylpolyoxyethyl ethers. Lethality was

highly correlated with surface activity of the preparations; that is, lethality increased with decreasing surface tension of the surfactant preparations.

Caux et al. (1993) examined membrane structure and composition of *Lemna minro L.* plants (a macrophyte commonly used in aquatic toxicity studies) that were exposed to one of several Triton X formulations, including Triton X-15 and X-100 in which the sole component is octylphenoxypolyethoxyethanol. Exposures to Triton X-15 and Triton X-100 produced changes in the phospholipid composition of membranes and increased fluidity of chloroplast membranes. Exposures to radiolabeled Triton X-100 resulted in incorporation of surfactant lipid into chloroplast membranes. This study indicates that surfactant lipids are incorporated into biological membranes and can affect membrane composition and function.

Knoche et al. (1992) reported that the phytotoxicity of octylphenoxypolyethoxyethanols and nonylphenoxypolyethoxyethanols varied with the oxyethyl chain length. Phytotoxicity was highest in surfactants that had 9 to 10 oxyethyl groups, with phytotoxicity decreasing with shorter or longer oxyethyl chain lengths. There are numerous studies regarding structure-activity relationships for the effects of polyethoxyethyl surfactants on the penetration of herbicides into leaf cuticles (e.g., Corbet and Chamel 1992, 1994). The studies show that the effect of the surfactant on herbicide penetration will vary with the oxyethyl chain length and that the physical and chemical properties of the pesticide may affect the structure-activity relationship (e.g., differences in water solubility). These studies have important implications for the assessment of the effects of surfactant formulations on the toxicity of Rodeo because they suggest that certain effects of the octylphenoxypolyethoxyethanols and nonylphenoxypolyethoxyethanols may vary with the length of the oxyethylene moiety, which may be different in the various formulations. This point underscores the importance of data on the toxicity of the formulations and of information on the exact chemical structure of the surfactant components of the formulations.

Knoche and Bukovac (1992) examined the effects of a variety of surfactant formulations, including Triton AG-98 (Latron AG-98) on penetration of gibberellic acid into sour cherry leaves (*Prunus cerasus L. cv Montmorency*). Triton AG-98 enhanced gibberellic acid penetration.

Lewis and Hamm (1987) compared the potencies of various surfactants to depress photosynthesis and growth of freshwater algae. For *in situ* depression of photosynthesis, the 3-hour EC_{50} values were 28.7 mg/L for octylphenoxypolyethoxyethanol and 2.1 mg/L for alkylethoxyethanol. The mean 96-hour EC_{50} values for growth depression in laboratory cultures of green algae (*Selenastrum capricornutum*), blue-green algae (*Microcystis aeruginosa*) and diatoms (*Naviculla pelliculosa*) were 0.21 mg/L for octylphenoxypolyethoxyethanol and 0.09 mg/L for alkylethoxyethanol.

4. SURFACTANTS IN THE GLYPHOSATE FORMULATIONS ROUNDUP AND ROUNDUP PRO

Roundup is an aqueous solution of the isopropylamine salt of glyphosate with a polyethoxylated tallow amine surfactant. This material is referred to in the literature as MON 0139, with the MON presumably referring to Monsanto, or polyoxyethyleneamine (POEA) (Smith and Oehme 1992). The surfactant in Roundup is present at 15% (Hoogheem 1987, Sawada et al. 1988) or 150 g/L assuming that the 15% value refers to the level in terms of weight per unit volume. Presumably, the Roundup surfactant is a derivative of tallow, a complex mixture of fat from the fatty tissue of cattle or sheep. Tallow contains a variety of fatty acids including oleic (37–43%), palmitic (24–32%), stearic (20–25%), myristic (3–6%), and linoleic (2–3%) acids as well as small amounts of cholesterol, arachidonic, elaidic, and vaccenic acids (Budavari 1989). An ethoxylated tallow amine (CAS No. 61791-26-2), is on the U.S. EPA List 3 of Inert Ingredients of Pesticides (U.S. EPA 1987, 1994). The surfactant used in Roundup contains 1,4-dioxane as an impurity. The upper limit of this compound in Roundup is about 0.03% (Monsanto 1990). In a previous review, the U.S. Forest Service determined that the amount of exposure to 1,4-dioxane is toxicologically insignificant (Borrecco and Neisess 1991).

Roundup Pro is a recently introduced formulation of glyphosate that contains a phosphate ester neutralized polyethoxylated tallow amine surfactant at a level of 14.5% (Monsanto 1995a,b, 1996) or 145 g/L. Other than the specification that the tallow amine surfactant in Roundup Pro is a phosphate ester of POEA, there is no published information regarding the chemical differences between the surfactant in Roundup and Roundup Pro.

4.1. HUMAN HEALTH

The potential role of the surfactant in the toxicity of Roundup was first emphasized by Sawada et al. (1988) in their analysis of poisoning cases in humans. They indicate that the acute LD_{50} of POEA is *"less than one-third that of roundup and its active ingredient"* and reference this statement to a chapter by Atkinson (1985) in *The Herbicide Glyphosate* (Grossbard and Atkinson 1985). The Sawada reference has been quoted in turn by Martinez and Brown (1991) as indicating that *" . . . POEA by itself has a LD_{50} of 1-2 g/kg*." The original experimental reference documenting these statements was not identified in the process of preparing this risk assessment.

Atkinson (1985) does cite an LD_{50} of 4.3 g/kg for glyphosate [a rounding of the rat oral LD_{50} of 4320 mg/kg reported in U.S. EPA (1986) and earlier U.S. EPA reports] and indicates that this is about the same as the acute oral LD_{50} in for isopropylamine salt in rats, 4.9 g/kg. Atkinson (1985), however, does not give an acute oral LD_{50} for POEA or any other surfactant.

An acute oral LD_{50} for POEA of 1200 mg/kg was reported in the previous EIS (USDA 1989b, p. 3-49, Coastal Plain/Piedmont Appendices). This value has been verified by Monsanto Co.

and is consistent with the acute toxic potency of other surfactants: in general, acute LD_{50} values for surfactants range from several hundred to several thousand mg/kg (Kosswig 1994, Grayson and Eckroth 1983).

The acute oral toxicity of Roundup (glyphosate and surfactant, LD_{50} in rats of 5400 mg/kg) is almost the same as that of glyphosate (LD_{50} in rats of 5600 mg/kg) (Monsanto Co. 1982a,b, 1983a). Based on these LD_{50} values, the LD_{50} of the surfactant can be estimated under the assumption of dose addition (Finney 1971). This assumption requires that the components in the mixture have the same mode of action. This assumption is not certain, but it is consistent with the observation by Talbot et al. (1991) that both glyphosate and POEA may exert some of their acute toxicity via irritation of biological membranes. The assumption of dose addition is also not interactive—that is, it assumes that the components in the mixture do not influence the toxicity of one another. This assumption is conservative, compared with other non-interactive models of joint action (Mumtaz et al. 1994).

For some uniform measure of toxicity (ζ) (e.g., LD_{50}), the toxicity of any mixture (ζ_M) is predicted, under the assumption of dose addition, by:

$$\zeta_M = \frac{\zeta_1}{(\pi_1 + \rho\pi_2)} \qquad \textbf{(4-1)}$$

where ζ_1 is the effective exposure (e.g., LD_{50} or LD_{95} values) for one compound, π_1 and π_2 are the proportions of each compound in the mixture, and ρ is the potency defined as $\zeta_1 \div \zeta_2$. Furthermore, given the toxicity of a defined mixture (ζ_M) and one of the components (ζ_1), the potency of the second component can be calculated as:

$$\rho = \frac{(\zeta_1 / \zeta_M) - \pi_1}{\pi_2} \qquad \textbf{(4-2)}$$

Here, the term *defined mixture* indicates that π_1 and π_2 are known. From this relationship, the effective exposure (i.e., toxic potency) of the second component (ζ_2) can be estimated as:

$$\zeta_2 = \frac{\zeta_1}{\rho} \qquad \textbf{(4-3)}$$

Using the nominal LD_{50} for Roundup of 5400 mg/kg, a π_1 of 0.356 for glyphosate (356 g/L), and π_2 of 0.15 for POEA (150 g/L), the estimated LD_{50} for POEA would be almost exactly 1200 mg/kg, consistent with dose additivity. This approach, however, would be a misapplication of the above equations.

To estimate the toxicity of POEA from the Roundup (glyphosate + POEA) LD_{50}, this LD_{50} must be converted from units of glyphosate to total mixture mass (glyphosate + POEA). In other words, an LD_{50} of 5400 mg glyphosate/kg bw is equivalent to a combined mass

(glyphosate and POEA) of about 7560 mg [1.4·5,400 mg], since the ratio of POEA to glyphosate is approximately 0.4 [150 g/L ÷ 356 g/L]. Similarly, the correct π_1 for glyphosate is about 0.7 [356 ÷ (150 + 356)] and the correct π_2 for POEA is about 0.3 [150 ÷ (150 + 356)]. Using this approach, the potency of POEA relative to glyphosate is about 0.14 and the estimated oral LD_{50} in rats is for POEA is about 40,000 mg/kg [5,600 mg/kg ÷ 0.14]. This estimate is consistent with the published results of Martinez, summarized in the following paragraph, in which no mortality was noted in rats after oral doses of up to 14,286 mg/kg POEA.

Martinez and coworkers (Martinez and Brown 1991; Martinez et al. 1990) conducted a series of experiments specifically designed to assess the role of the surfactant in the acute toxicity of Roundup. In these studies, compounds were administered to groups of five rats either by gavage (direct instillation into the stomach) or direct installation into the trachea. Oral exposure to Roundup at doses of 1, 3, and 5 mL/animal caused 0%, 40%, and 100% mortality, respectively, over a 24-hour observation period. Taking an average body weight of 350 g/rat reported by Martinez and Brown (1991), the mid-dose level corresponds to approximately 3050 mg/kg [3 mL · 356 mg a.e./mL ÷ 0.350 kg], only somewhat less than and consistent with the reported LD_{50} for Roundup of 5400 mg/kg (Monsanto Co. 1982a,b). POEA, administered by gavage, caused no deaths at doses of 1, 3, and 5 mL/animal. Since ethoxylated surfactants generally have a density of about 1 g/mL (Kosswig 1994, p. 789), the doses of POEA correspond to approximately 2857, 8571, and 14,286 mg/kg. The low acute oral toxicity of POEA is consistent with the similarity between the acute oral toxicity of glyphosate and Roundup, discussed above.

In the earlier study by Martinez et al. (1990), an oral dose with Roundup RTU or Roundup concentrate caused delayed (6 hours) pulmonary edema, consistent with clinical observations in humans, as summarized above. The authors concluded that *" . . . delayed pulmonary edema combined with blood stained weeping from the nose, diarrhea, distended GI tract, and ascites is in excellent agreement with . . . The clinical picture of . . . hypovolemic shock,"* as described by Sawada et al. (1988). In the individuals involved in the Taiwan studies of glyphosate poisoning, however, hematocrit, blood urea nitrogen, and central venous pressure determinations were not consistent with hypovolemia.

Intratracheal instillations in rats resulted in much more toxic effects at much lower dose levels. Roundup at doses of 0.1, 0.2, and 0.4 mg/animal caused 80% mortality at the low dose and 100% mortality at the two higher doses as well as an increase in lung weights. POEA, at the same dose levels, caused 20%, 70%, and 100% mortality as well as increases in lung weights, although the increases were less than those observed with Roundup (Martinez and Brown 1991, Table 1, p. 44). Pathological examinations indicated that both Roundup, and to a lesser extent POEA, cause hemorrhaging and congestion of the lungs after intratracheal instillations. Martinez and Brown (1991) conclude that POEA potentiates the pulmonary toxicity of glyphosate. Since, however, these investigators did not test glyphosate alone, the basis for their conclusion is not clear.

Based on drinking water studies of both glyphosate and Roundup (i.e., glyphosate with POEA), the surfactant does not affect the rapid elimination rate of glyphosate (NTP 1992).

Pertinent data regarding the subchronic or chronic toxicity of POEA were not located in the published literature. In a letter to the Forest Service concerning a review of the previous EIS, Monsanto provides single page summaries of two studies conducted by Bionetics on what is characterized as the *Roundup surfactant* (Long 1987, letter to Larry Gross dated March 12, 1987). In the first study, dietary concentrations of 0, 1250, 2500, or 5000 ppm (0, 1.6, 3.8, or 6.5 mg/kg/day) were fed to Sprague-Dawley rats for 13 weeks. The study is identified as LBI Project No. 2290. The results of the study are summarized as follows:

> *No toxic signs were observed except for slow acclimation to the highest dosage in the first three weeks. Clinical laboratory tests failed to reveal important differences between controls and test animals. Microscopic examination of organs collected at necropsy revealed only histiocytic infiltrations of the lamina propria of the small intestines and sinusoids of mesenteric lymph nodes at all dosage levels.*

In the second study, the surfactant was fed to dogs via gelatin capsules at doses of 0, 10, 20, and 30 mg/kg, 3 times/day, over the final 10 weeks of a 14-week study. Dosing during the first 4 weeks, if any, is not specified, and the LBI project number is not specified. The summary states:

> *The material showed no effect with regard to survival, general appearance, behavior, neurologic, electrocardiographic or histopathological parameters. Changes observed during the study included decreased food consumption, depressed growth rate, reduced serum calcium and total protein, increased relative and absolute renal and cardiac weights, and increased relative adrenal weights. None of the observed changes would preclude the use of Roundup surfactant as an adjuvant in a herbicide formulation.*

No further information is provided. Superficially, these studies increase rather than lessen concern about the presence of the surfactant.

Very little information is available regarding the surfactant in Roundup Pro. The surfactant in Roundup Pro is described as a *phosphate ester neutralized polyethoxylated tallow amine* (Monsanto 1996). Like the polyethoxylated tallow amine in Roundup, this surfactant is produced, presumably, by the ethoxylation of tallow amine with ethylene glycol. Since tallow is a complex and variable mixture of fatty acids and other minor components and since the nature and extent of ethoxylation can vary according to the conditions during synthesis, the significance of the difference between the surfactants used in Roundup and Roundup Pro is not apparent from the available chemical descriptions of these two surfactants. There is relatively little data regarding the toxicity of Roundup Pro.

4.2. ECOLOGICAL EFFECTS

A major qualitative difference between the effect of glyphosate and glyphosate formulations on aquatic and terrestrial organisms concerns a polyethoxylated tallow amine surfactant (POEA) used in Roundup. For aquatic organisms, the surfactant is much more toxic than glyphosate. Unlike glyphosate, POEA is more toxic in alkaline water than in acid water. Thus, the relative potency of POEA with respect to glyphosate is pH dependent. There is relatively little information regarding Roundup Pro, a formulation of glyphosate that contains a phosphate ester neutralized polyethoxylated tallow amine surfactant. Nonetheless, the available data suggest that this formulation is quite similar to Roundup.

There is an extensive amount of literature on glyphosate specifically (Boerboom and Wyse 1988, Clay and Lawrie 1988, Cranmer and Linscott 1991, Sherrick et al. 1986, Turner 1985) and many other compounds (Green et al. 1992, Prasad 1989) indicating that the addition of surfactants can greatly enhance the phytotoxicity of herbicides.

Two aquatic toxicity studies (Folmar et al. 1979, Wan et al. 1989) have been conducted on glyphosate, POEA, and Roundup which permit a quantitative assessment of the relative toxicities of glyphosate and POEA as well as the effects of combined exposures to these agents. Both of these studies indicate that POEA is substantially more toxic than glyphosate.

Folmar et al. (1979) conducted bioassays on four species of fish and one invertebrate (midge larvae). The LC_{50} values for glyphosate, POEA, and Roundup are presented in Table 4-1. Also shown in Table 4-1 is the relative potency (ρ) of POEA with respect to glyphosate, estimated as the LC_{50} of glyphosate divided by the corresponding LC_{50} for POEA. For example, for rainbow trout at pH 6.5, the LC_{50} for POEA is 7.4 mg/L and the corresponding LC_{50} for glyphosate is 140 mg/L. Thus, the relative potency of POEA with respect to glyphosate is about 19 [$140 \div 7.4 = 18.92$].

Table 4-1. Estimates of relative potency and toxicological interaction of glyphosate and POEA[a]

Species/Assay/Study	Observed LC_{50} values					
	Glyphosate	POEA	Roundup[b]	**D**	Predicted LC_{50}	Pred.÷ Obs.
			pH 6.5			
Rainbow trout	140	7.4	10.8	19	22	2.0
Bluegills	140	1.3	6.0	108	3.1	0.5
			pH 7.2, 96 hr unless specified			
Midge larvae, 48 hr.	55	13	25	4.2	28	1.1
Rainbow trout	140	2	11.8	70	6.5	0.6
Fathead minnow	97	1.0	3.2	97	3.2	1.0
Channel catfish	130	13	18	10	35	1.9
Bluegills	140	3.0	7.1	47	9.5	1.3
			pH 9.5			
Rainbow trout	240	0.65	2.0	369	2.1	1.1
Bluegills	220	1.0	2.6	220	3.3	1.3

[a]Data from Folmar et al. (1979).

[b]Value reported by Folmar as mg a.i multiplied 1.42 to account for added mass of surfactant.

D = LC_{50} of glyphosate ÷ LC_{50} of POEA.

In mixtures, the concept of relative potency provides an explicit tool for identifying the most significant toxic agent(s) in a mixture as well as for assessing potential interactions among agents in a mixture (Durkin 1981, Mumtaz et al. 1994). For example, for a mixture of two agents with the same potency present in a mixture in proportions of π_1 and π_2, the fractional contribution of each agent to the toxicity of the mixture is simply the proportion (π_1 or π_2) of the agent in the mixture. When the potencies differ, both agents contribute equally to the toxicity of the mixture when π_1 is equal to $\rho\pi_2$. As above, ρ is defined here as the LC_{50} of component 1 divided by the LC_{50} of component 2.

In Roundup, glyphosate is present at 356 g/L and POEA is present at 150 g/L. The proportion of glyphosate in Roundup (π_G), ignoring the only other constituent, which is water, is about 0.7 [$356 \div (356 + 150)$]. Similarly, the proportion of POEA (π_S for proportion of surfactant) in the mixture is about 0.3 [$150 \div (356 + 150)$]. Both constituents would contribute equally to the mixture if the relative potency of POEA was about 2.3 [$0.7 \div 0.3$]. The relative potency of POEA with respect to glyphosate is much greater than 2.3, at least for fish species (Table 4-1). Thus, POEA is the more significant toxic agent in the mixture.

The magnitude of the difference can be expressed in various ways, the simplest of which is the ratio of the concentrations or equivalently the ratios of the proportions adjusted for the difference in potency:

$$\frac{\rho \cdot \pi_2}{\pi_1} \qquad \textbf{(4-4)}$$

For example, if the relative potency is 70, as it is in Table 4-1 for rainbow trout at pH 7.2, POEA may be said to contribute 30 times [70 · 0.3 ÷ 0.7] more than glyphosate to the toxicity of the mixture.

This method of describing relative toxic contribution is based on the assumption that the components in the mixture do not affect one another (i.e., there are no toxicological interactions). For terrestrial plants, such interactions have been clearly documented. One method for assessing whether or not similar interactions are plausible in aquatic species is to compare the observed LC_{50} values for Roundup with the LC_{50} values that would be predicted by one model of non-interactive joint action, simple similar action (Finney 1971, Durkin 1981). Using this assumption, the expected LC_{50} can be calculated as:

$$LC_{50_{Roundup}} = \frac{LC_{50_{Glyphosate}}}{(\pi_G + \rho\pi_S)} \qquad \textbf{(4-4)}$$

where π and ρ are as defined above.

The predicted LC_{50} values for Roundup based on this assumption are presented in the second to the last column of Table 4-1, and the ratio of the predicted to observed LC_{50} values are given in the last column. Ratios > 1 suggest some form of greater than additive toxicity, and, conversely, ratios < 1 indicate less than additive toxicity. Note also that the observed LC_{50} values for Roundup are presented as the total concentration of glyphosate and POEA. In other words, the LC_{50} values for Roundup reported in Folmar et al. (1979) are multiplied by 1.42 ((352 + 150) ÷ 352) and give the LC_{50} values in units of weight of both glyphosate and POEA. These units are required for the above equation 4-2.

As indicated in Table 4-1, there is a tendency for the toxicity of glyphosate to decrease (i.e., the LC_{50} values increase—as the pH increases), although the changes are not substantial. The effect of pH on POEA is also not substantial but the effect seems to be the opposite of the effect that pH has on glyphosate. In all of the bioassays, the surfactant is more toxic than glyphosate. Because of the effect of pH on toxicity, the relative potency of POEA increases as pH increases. At all pH levels, the ratio of predicted to observed LC_{50} values for Roundup does not deviate remarkably or systematically from unity, suggesting that no substantial interactions take place between these two compounds.

Table 4-2. Estimates of relative potency and toxicological interaction of glyphosate and POEA in five species of salmonids[a]

Species/Assay/ Study	Observed 96-hour LC_{50} Values					
	Glyphosate	POEA	Roundup[a]	D	Predicted LC_{50}	Pred.÷ Obs.
		Soft Water pH 6.3				
Coho	27	4.6	32	5.9	10.9	0.34
Chum	10	2.7	20	3.7	5.5	0.28
Chinook	19	2.8	33	6.8	6.9	0.21
Pink	14	4.5	33	3.1	8.5	0.26
Rainbow	10	2	33	5	4.5	0.13
		Soft Water pH 7.2				
Coho	36	3.2	27	11.3	8.8	0.33
Chum	22	4.2	19	5.2	9.7	0.51
Chinook	30	2.8	27	10.7	7.5	0.28
Pink	23	2.8	31	8.2	7.2	0.23
Rainbow	22	2.5	15	8.8	6.6	0.44
		Hard Water pH 8.2				
Coho	210	1.8	13	117	5.9	0.45
Chum	202	1.4	11	144	4.6	0.41
Chinook	220	1.7	17	129	5.6	0.32
Pink	380	1.4	14	261	4.6	0.33
Rainbow	220	1.7	14	129	5.6	0.40

[a]Data from Wan et al. (1989)

[b]As reported by Wan et al. (1989) in units of mg product/L.

A similar analysis of the results presented by Wan et al. (1989) is summarized in Table 4-2. In general, this study agrees well with the earlier study by Folmar et al. (1979). In all cases, the surfactant is substantially more toxic than glyphosate. The effect of pH is more consistent and more substantial: the toxicity of glyphosate decreases and the toxicity of the surfactant increases with increasing pH. Consequently, the relative potency of the surfactant to glyphosate also increases with increasing pH. The LC_{50} values reported in Wan et al. (1989)

for Roundup are expressed as "*mg product/L*." In calculating the expected LC_{50} values for Roundup in Table 4-2, it is assumed that these LC_{50} values include the concentrations of both glyphosate and the surfactant. As indicated in the last column of this table, the ratio of the predicted to observed LC_{50} values for Roundup are consistently < 1, indicating a less than additive interaction.

Comparable data allowing for an assessment of the joint action of glyphosate with the surfactant used in Roundup Pro are not available. The surfactant used in Roundup Pro is a *phosphate ester neutralized polyethoxylated tallow amine* (Monsanto 1996) that is probably similar to the *polyethoxylated tallow amine* used in Roundup. Consequently, it is reasonable to assume that the surfactant in Roundup Pro will enhance the toxicity of glyphosate to aquatic species. It is not clear, however, that this enhancement will be pH dependent.

5. REFERENCES

Atkinson D. 1985. The toxicological properties of glyphosate - a summary. In: The Herbicide Glyphosate. London, England: Butterworth and Co. Ltd. pp. 127-133.

Berberian DA; Gorman WG; Drobeck HP; Coulston F; Slighter Jr. RG. 1965. The toxicology and biological properties of Laureth 9 (a polyoxyethylene lauryl ether), a new spermicidal agent. Toxicol. Appl. Pharmacol. 7: 206-14.

Boerboom CM; Wyse DL. 1988. Influence of glyphosate concentratin on glypohosate absorption and translocation in Canada thistle (*Cirsium arvense*). Weed Sci. 36(3): 291-295.

Borrecco JE; Neisess J. 1991. Risk Assessment for the impurities 2-butoxyethanol and 1,4-dioxane found in Garlon 4 and RoundUp herbicide formulations. Forest Pest Management: Pacific Southwest Region, dated February 25, 1991.. R91-2(2150): 33 pp.

Budavari S. (Ed). 1989. The Merck Index: An Encyclopedia of Chemicals, Drugs,and Biologicals, 11th ed. Merck & Co., Inc., Rahway, New Jersey.

Caux P-Y; Weinberger P. 1993. Effects of pesticide adjuvants on membrane lipid compostion and fludity in *Lemna minor*. Can. J. bot. 71(10):1291-97.

Clay DV; Lawrie J. 1988. Herbicides for grass control in forestry: Pot experiments on efficacy and crop tolerance. Asp. Appl. Biol. 16: 113-122. (Taken from Database).

Coret J; Chamel A. 1993. Influence of some nonionic surfactants o water sorption by isolated fruit cuticles in relation to cuticular penetration of glyphosate. Pesticide Sci. 38:27-32.

Coret J; Chamel A. 1994. Effect of some ethoxylated alkylphenols and ethoxylated alcohols on the transfer of [^{14}C]chlorotoluron across isolated plant cuticles. Weed Res. 34(6): 445-51.

Cranmer JR; Linscott DL. 1991. Effects of droplet composition on glyphosate absorption and translocation in velvetleaf (*Abutilon theophrasti*). Weed Sci. 39(2): 251-154.

Durkin PR. 1981. An Approach to the Analysis of Toxicant Interactions in the Aquatic Environment, in Aquatic Toxicology and Hazard Assessment, ASTM 4th Symposium on Aquatic Toxicology, pp. 388-401.

Edidin M; Sessions AV. 1983. Heterogeneity in the plasma membrane lipids of eukaryotic cells. In: Biomembranes and Cell Function. Annals of the New York Academy of Sciences, Vol. 414. Kummerow, F.A., Benga, G. and Holmes, R.P. eds. New York, NY: New York Academy of Sciences. pp. 8-18.

Ernst R; Arditti J. 1980. Biological effects of surfactants. IV. Effects of non-ionics and amphoterics on HeLa cells. Toxicol. 15: 233-42.

Finney DJ. 1971. Probit Analysis. New York: Cambridge University Press. 333 p.

Folmar LC; Sanders JQ; Julin AM. 1979. Toxicity of the herbicide glyphosate and several of its formulations to fish and aquatic invertebrates. Arch. Environ. Contam. Toxicol. 8: 269-278.

Grayson M; Eckroth DV. 1983. Surfactants and Detersive Systems. In: Kirk-Othmer Encyclopedia of Chemical Technology, 3rd ed., Volume 22. New York: John Wiley and Sons. pp. 374-424.

Green TH; Minoque PJ; Brewer CH; Glover GR; Gjerstad DH. 1992. Absorption and translocation of [14C]glyphosate in four woody plant species. Can. J. For. Res. 22(6): 785-789.

Grubb TC; Dick LC; Osher M. 1960. Studies on the toxicity of polyoxyethylene dodecanol. Toxicol. Appl. Pharmacol. 2: 133-143.

Hoogheem TJ. 1987. The safety of roundup pesticide [letter]. JAMA. 26(2): 2679.

Imai T; Tsuchiya S; Morita K; Fujimori T. 1994. Surface tension-dependent surfactant toxicity on the grean peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). Appl. Entomol and Zool. 29(3):389-93.

Knoche M; Bukovac MJ. 1992. Surfactants influence foliar absorption of gibberellic acid by sour cherry leaves. J. Am. Soc. Hort. Sci. 117(1): 80-4.

Knoche M; Noga G; Lenz F. 1992. Surfactant-induced phytotoxicity: Evidence for interaction with epicuticular wax fine structure. Crop. Prot. 11(1): 51-6.

Kosswig K. 1994. Surfactants. In: Ullmann's Encyclopedia of Industrial Chemistry, Vol. A25, VCH Verlagsgesellschaft mbH, Weinheim, Germany. pp. 747-816.

Lapurga R. 1996. Letter from Rudy Lapurga of the Califronia EPA to John Borrecco of USDA, with attachments containing descritptions of toxicitiy tests of R-11 and LI-700..

Levin RJ. 1987. Bioelectric activity as a quantifiable index of acute spermicide (nonoxynol-9) actions on rat vaginal epithelial function during the oestrous cycle. Pharmacol. Toxicol. 60(3): 175-8

Levine TE. 1996. The regulation of inert ingredients in the United States. In: Pesticide Formulation and Adjuvant Technology. Foy, C.L., Pritchard, D.W. eds. Boca Raton, FL: CRC Press. pp. 1-11.

Lewis MA; Hamm BG. 1986. Environmental modification of the photosynthetic response of lake plankton to surfactants and significance to a laboratory-field comparison. Wat. Res. 20(12)1575-82.

Long SD; Warren AJ; Little JB. 1982. Effect of nonoxynol-9, a detergent with spermicidal activity, on malignant transformation *in vitro*. Carcinogenesis (Lond). 3(5): 553-58.

Martinez TT; Long WC; Hiller R. 1990. Comparison of the toxicology of the herbicide Roundup by oral and pulmonary routes of exposure. Proc. Western Pharmacol. Society. 33: 193-197.

Martinez TT; Brown K. 1991. Oral and pulmonary toxicology of the surfactant used in roundup herbicide. Proc. Western Pharmacol. Soc. 34: 43-46.

McLaren/Hart. 1995. Use of the Registered Aquatic Herbicide Fluridone (SONAR) and the Use of the Registered Aquatic Herbicide Glyphosate (Rodeo and Accord) in the State of New York, prepared by McLaren/Hart Environmental Engineering Corproation for DowElanco and Monsanto. pp. 12-8 - 12-10.

Mitre Corporation. 1989. Glyphosate: Herbicide Background Statement. Pesticide Background Statements. Volume 1: Herbicides. : G1-G72.

Monsanto Co. 1982a. Material safety data sheet, glyphosate. Monsanto Company, St. Louis, MO. (Cited in Mitre 1989).

Monsanto Co. 1982b. Material safety data sheet, Roundup. Monsanto Company, St. Louis, MO. (Cited in Mitre 1989).

Monsanto Co. 1983a. Rodeo herbicide bulletin No. 1, January. Monsanto Co., St. Louis, MO. (Cited in Mitre 1989).

Monsanto Co. 1990. Correspondence of James A. Chamber to Hohn Borrecco, USDA Forest Service concerning information on the impurity 1,4-dioxane present in Roundup herbicide. Feb. 23, 1990.

Monsanto Co. 1995a. Roundup Pro product label. Monsanto Company, Agricultural Products, St.Louis, MI.

Monsanto Co. 1995b. Roundup Pro material safety data sheet. Dated November 1995. Monsanto Company, Agricultural Products, St.Louis, MI.

Monsanto Co. 1996. Roundup Ultra ingredients summary sheet. Monsanto Company, Agricultural Products, St.Louis, MI.

Mumtaz MM; DeRosa C; Durkin PR. 1994. approaches and challenges in risk assessments of chemical mixtures. In: Toxicology of Chemical Mixtures. Yang, R. ed. Chicago, IL: Academic Press. pp. 565-597.

NTP Working Group. 1992. Toxicity studies of glyphosate. National Toxicology Program; TOX 16, 39 p, 1992.

Prasad R. 1989. Crop toerance of three west coast conifer species to lyphosate. FDRA report no. 063. pp. 186-196.

Quantum Chemical Corp. 1992. Initial submission: Acute toxicity of Igepal CO-210 on *Mysidopsis bahia* for 48-hour exposure (final report) with cover letter dated 02/11/92. U.S. EPA/OPTS Public Files. Fiche No. OTS0535390. Document No. 88-920000892. Sect 8ECP.

Reyes AA. 1992. Comparative effects of an antitranspirant, surfactants and fungicides on *Mucor* rot of tomatoes in storage. Microbios. 71(288-289): 235-41.

Rhone-Poulenc, Inc., 1991. Initial submission: Prmary dermal and eye irritation of poly(oxy-1,2-ethanediyl),alpha-(nonylphenyl)-omega-hydroxy-branched with cover letter dated 09/15/92. U.S. EPA/OPTS Public Files. Fiche No. OTS0571085. Document No. 88-920009333. Sect 8ECP.

Rohm and Haas Co. 1992. Initial submission: Primary occular irritation in rabbits (final report) with cover letter dated 11/22/91. U.S. EPA/OPTS Public Files. Fiche No. OTS0534772. Document No. 88-920000321. Sect 8ECP.

Sawada Y; Nagai Y; Ueyama M; Yamamoto I. 1988. Probable toxicity of surface-active agent in commercial herbicide using glyphosate [letter]. Lancet. 1(8580): 299.

SERA (Syracuse Environmental Research Associates). 1996. Selected Commercial Formulations of Glyphosate - Accord, Rodeo, Roundup and Roundup Pro. Risk Assessment Final Report. Prepared by Syracuse Environmental Research Associates for the Animal and Plant Health Inspection Service (APHIS). SERA TR 96-22-02-01c.

Sherrick SL; Holt HA; Hess FD. 1986. Absorption and translocation of MON 0818 adjuvant in field bindweed (*Convolvulus arvensis*). Weed Sci. 34(6): 817-823.

Smith EA; Oehme FW. 1992. The biological activity of glyphosate to plants and animals: a literature review. Vet. Hum. Toxicol. 34(6): 531-543.

Talbot AR; Shiaw MH; Huang JS; Yang SF; Goo TS; Wang SH; Chen CL; Sanford TR. 1991. Acute poisoning with a glyphosate-surfactant herbicide ('Roundup'): a of 93 cases. Hum. Exp. Toxicol. 10(1): 1-8.

Thomas DA; Myers MA; Wichert B; Schreier H; Gonzaliz-Rothi RJ. 1991. Acute effects of liposome aerosol inhalation on pulmonary fucntion in healthy volunteers. Chest 99(5):1268-70.

Thompson ED; Gibson DP. 1984. A method for determining the maximum tolerated dose for acute *in vivo* cytogenic studies. Food Chem. Toxicol. 22(8): 665-76.

Turner DJ. 1985. Effects on glyphosate performance of formulation, additives and mixing with other herbicides. In: The Herbicide Glyphosate. Grossbard, E; Atkinson, D; eds. London England: Butterworths Co., Ltd.

Union Carbide Corp. 1992a. Initial submission: Letter submitting six enclosed acture toxicity studies on Triton X-100. U.S. EPA/OPTS Public Files. Fiche No. OTS0536084. Document No. 88-920001408. Sect 8ECP.

Union Carbide Corp. 1992b. Supplement: Letter from Union Carbide Corporation submiiting inofrmation related to testng with Triton CF-10, CF-54, DF-16 and X-100 surfactants in rabbits with attachment. U.S. EPA/OPTS Public Files. Fiche No. OTS05134210. Document No. 89-920000129. Sect 8E.

Union Carbide Corp. 1992c. Support: Trition X-15 surfactant: Acute Toxicity and irritancy testing using the rat (peroral toxicity) and the rabbit cutaneous and eye tests with cover letter dated 10/23/92. U.S. EPA/OPTS Public Files. Fiche No. OTS05134211. Document No. 89-920000016. Sect 8E.

Union Carbide Corp. 1992d. Support: Acute toxicity and irritancy testing of Trition X-100 surfactant using the rate (peroal toxicity) and the rabbit(cutaneous and eye tests) with cover letter dated 02/22/93. U.S. EPA/OPTS Public Files. Fiche No. OTS05134212. Document No. 89-93000011. Sect 8E.

Union Carbide Corp. 1994. Support: Letter from Union carbode Corporation to U.S. EPA regarding preliminary resuolts from developmental toxicity study of Trition X-100 in rats with attachments date 04/27/94. U.S. EPA/OPTS Public Files. Fiche No. OTS053804-1. Document No. 89-940000192. Sect 8E.

Union Carbide Corp. 1995. Support: Developmental toxicity study of Triton X-100 surfactant by cutaneous administration to CD rats with cover letter dated 01/30/95. U.S. EPA/OPTS Public Files. Fiche No. OTS053804-1. Document No. 89-940000121. Sect 8E.

USDA (U.S. Department of Agriculture). 1989b. Draft Environmental Impact Statement: Vegetation Management in the Ozark/Ouachita Mountains, Management Bulletin R8-MB-23, dated June, 1989. 499 pp.

U.S. EPA. 1986. Guidance for the Registration of Pesticide Products Containing Glyphosate as the Active Ingredient. Case No. 0178, June 1986. PSD-HC-8723 Nos. 0153374, 0153376, 46363, 132681, 137640, 0132683, 0132683, 0132686, 130406. (Cited in U.S. EPA 1992).

U.S. EPA. 1987. EPA Issues Policy Statement on Reduction of Potential Adverse Effects of Pesticides Containing Toxic Inert Ingredients; Tables. Fed. Reg. 52(77): 13305.

U.S. EPA. 1992. Drinking Water Criteria Document for Glyphosate. U.S. EPA, Office of Assistant Administrator, Washington, DC. NTIS PB92-173392.

Wax, L.M., Leibl, R.M., Bush, D.R. 1994. Surfactant-increased glyphosphate uptake into plant membrane vesicles isolated from common lambsquateres leaves. Plant Physiol. 105(4): 1419-25.

U.S. EPA. 1994. Inert Ingedients in Pesticide Products; List of Minimal Risk Inerts. U.S. Environmentla Protection Agency. Fed. Reg. 59(187)49400-01.

Wan MT; Watts RG; Moul DJ. 1989. Effects of different dilution water types on the acute toxicity to juvenile Pacific salmonids and rainbow trout of glyphosate and its formulated products. Bull. Environ. Contam. Toxicol. 43(3): 378-385.

WHO (World Health Organization). 1974. Emulsifiers. Lecithin. In: Toxicological Evalaution of Some Food Additives Including Anticaking Agents, Antimicrobials, Antioxidants, Emulsifiers and Thickening Agents. Geneva: World Health Organization. W1 W14H No. 5. pp. 234-5.

Wood JL; Allision RG. 1981. Effects of consumption of choline and lecithin on neurological and cardiovascular systems. Life Sciences Research Office, Federation of American Societies for Experimentla Biology. PB82-133257.

Wood JL; Allision RG. 1982. Effects of compposition of choline and lecithin on neurological and cardiovascular systems. Fed. Proc. 41(14):3015-21.