The Toxicity Profiles of Five Surfactants Used in Roundup Branded Agricultural Herbicides

Donna R. Farmer,[1] Charles E. Healy, [redacted] and Terry A. Kaempfe
Monsanto Company, St. Louis Missouri USA 63167

[1]To whom correspondence should be addressed at Monsanto Company, 800 N. Lindbergh Blvd., St. Louis, Missouri, USA 63167. Fax: 314-694-4028. E-mail: [redacted]@monsanto.com

ABSTRACT

Herbicide formulations are comprised principally of active ingredient(s), water, and a surfactant. In Roundup® branded herbicides, glyphosate is the active ingredient. An extensive toxicology database exists for glyphosate. Additional toxicology data has been developed for five of the surfactants used in a number of Roundup branded products. Four of these surfactants contain ethoxylated alkyl amine compounds, similar to those used in various consumer and industrial products; the fifth is a linear alkyl sulfate. Study results demonstrated that the surfactants were no more than slightly toxic after acute oral ingestion but did produce significant eye and skin irritation. Effects that appear related to gastrointestinal irritation and decreased palatability of the diet/surfactant mixture only were noted in subchronic rodent and dog studies; there was no indication of specific target organ toxicity. The only significant finding in developmental toxicity studies was fetal growth retardation at maternally toxic doses. No genotoxicity was observed. Since surfactants are designed to be surface-active materials, they can interact with and damage the structural integrity of cellular membranes. Thus, the effects observed in these studies (i.e., eye, skin and gastrointestinal irritation) are attributed to non-specific physicochemical properties rather than a specific toxicological mode of action. These same effects would be expected from many other surfactants used in common household products and are not specific to pesticides. The results of these studies support the conclusion that the use of these surfactants in Roundup branded herbicides is unlikely to produce significant adverse effects to humans or animals under normal conditions of exposure.

Key Words: Herbicide, Surfactant, Toxicology

INTRODUCTION

Commercially available pesticide products are comprised of two major types of ingredients - those that are "active" and those that are "inert." Active ingredients are purposely meant to have adverse effects on plants (herbicides), insects (insecticides), or fungi (fungicides). Inert ingredients also referred to as "other ingredients", on the other hand, have purposes separate from those of active ingredients (United.States Environmental Protection Agency (U.S. EPA), 2007). They may function as solvents, fillers, carriers, safeners, or surfactants. Even water, which can be found in many commercial formulations, is considered an inert ingredient. While these formulated materials manifest various physical/chemical properties, they are "inert" from the standpoint of not purposefully having any direct pesticidal properties. Only those inerts specifically listed on the U.S.EPA's approved list may be used in pesticide formulations. The primary components of Monsanto's Roundup® branded herbicide formulations are the active ingredient (glyphosate), water, and a surfactant.

Under the pesticide regulations in the United States, unless an inert ingredient is considered highly toxic detailed information, pesticide manufacturers do not have to disclose the chemical constituents, either by name or percentage of composition, that comprise the inert components of a pesticide formulation. All that is currently required on the label, at least in the United States, is reporting of the total percentage of inert ingredients in a pesticide formulation (U.S. EPA, 2007).

In addition to the extensive database on glyphosate (U.S. EPA, 1993; European Commission, 2002; and WHO/FAO, 2004), Monsanto Company, as part of an active stewardship program, has developed a core toxicology data set on surfactants that are used in a number of Roundup-branded herbicide products. These proprietary surfactants are primarily ethoxylated alkyl amines designated herein as Alkylamine (AA), Phosphate Ester (PE), Alkylamine Derivative 1 (AAD-1), Alkylamine Derivative 2 (AAD-2) and Sulfated Alcohol (SA) have been developed through extensive research and testing to enhance the performance of the final product for the consumer. This paper describes the mammalian toxicity database for these five key surfactants used in a number of Roundup branded herbicide formulations. Four of these surfactants contain ethoxylated alky amine compounds, which are similar to those used in various consumer and industrial products, and the fifth is a linear alkyl sulfate.

TOXICOLOGY STUDIES

Acute Toxicity and Irritation Studies

Materials and Methods

Standard acute oral toxicity studies were conducted in Sprague Dawley rats with AA (Birch, 1977; Evans, 1985), AAD-1 (Mallory, 1995), AAD-2 (Harrod, 1998a), and SA (Branch, 1980a). In each study, the test substance was administered as a single dose via oral gavage to five animals per sex per dose group. Distilled water was used as the vehicle to deliver AAD-1 at a constant volume of 5 ml/kg while the other substances were administered undiluted. Animals were weighed weekly and observed at least once daily for 14 days. All animals received a gross

necropsy. The LD_{50} and 95% confidence intervals were calculated using either the method of de Beer (1945) or Litchfield and Wilcoxian (1949).

Dermal toxicity studies were conducted in albino rabbits with AA (2/sex/dose group) (Birch, 1977), AAD-1 (5/sex/dose group) (Mallory, 1996), and SA (4/sex/dose group) (Branch, 1980b). The undiluted material was applied to intact skin under an occlusive dressing for 24 hours. The rabbits were weighed weekly and observed at least once daily for 14 days. A gross necropsy was conducted on all animals. The LD_{50} and 95% confidence intervals for AA were calculated using the method of de Beer (1945) and for males dosed with SA by the method of Finney (1971).

The eye irritation potential of AA (Birch, 1977), AAD-1 (Mallory, 1994), and SA (Branch, 1980c) was tested in the eyes of albino rabbits. One eye of each test animal was instilled with 0.1 ml of test substance. The opposite eye was left untreated and served as a negative control. The eyes were scored according to the method of Draize *et al.* (1944) at 1, 24, 48, and 72 hours and at 7, 14, and 21 days after treatment or until the scores indicated that the material was corrosive or that the eyes had returned to normal.

Dermal irritation studies were performed on AA (Birch, 1977), AAD-1 (Mallory, 1999), AAD-2 (Harrod, 1998b), and SA (Branch, 1980d) by applying the test substances under semi-occlusive dressing for 4 hours to intact skin on the shaved backs of albino rabbits. The irritation was graded according the method of Draize *et al.* (1944) at 1, 24, 48, and 72 hours after patch removal. Additional irritation scores were determined 7 days (AA), 7 and 14 days (AAD-2), and 4 through 14 days (AAD-1, SA) after removal of the test substance. A Primary Irritation Index (PII) was determined by dividing the sum of the erythema and edema scores from 1, 24, 48, and 72 hours by 24 (the number of test sites times the number of scoring intervals).

AA (Blasczak, 1987) and AAD-1 (Hiles, 1994) were tested for their potential to be contact sensitizers using the Buehler (1965) method. During the induction phase, Hartley-derived guinea pigs were exposed to 0.3 ml of test material once weekly for 6 hours for three consecutive weeks. AA was tested undiluted while AAD-1 was applied as a 6.0% (v/v) solution in distilled water. After a two-week rest period, the animals were challenged with test material. During the challenge phase, AA was dosed undiluted and AAD-1 was dosed as a 4.0% (v/v) solution in distilled water. The animals were rechallenged with AA one week later using a 50% (v/v) solution in acetone. Naïve irritation control animals were dosed with solutions identical to those used with the test animals at challenge and rechallenge. Animals were scored 24 and 48 hours after treatment using the Draize scale (Draize *et al.*, 1944). In the AAD-1 study, 10 animals per sex were used for the test group and 5 animals per sex for the irritation control group. Five animals per sex were used for the test, irritation control, and rechallenge irritation control groups in the sensitization study with AA.

Results

The results of the acute toxicity and irritation testing are summarized in Table 1. Clinical observations made during the 14-day post exposure observation period of the acute oral toxicity tests were consistently similar for all of the tested materials and included decreased activity, diarrhea, staining around the facial area, breathing abnormalities, abnormal gait/posture, and poor grooming/appearance. Survivors generally gained weight throughout the study. Notable abnormal necropsy findings that occurred in the animals that died prior to study termination included reddened or discolored lungs, livers, and kidneys and inflammation or distension of the

gastrointestinal tract. There were no notable findings in the surviving animals at study termination.

Notable clinical observations made during the acute dermal toxicity studies included lethargy, abnormal gait/posture, poor grooming/appearance, and decreased muscle tone. Body weight effects varied between studies. Gross necropsy findings in the animals that died prior to study termination included reddened or discolored lungs, liver, and kidney and inflammation of the gastrointestinal tract. There were no abnormal findings in the surviving animals at study termination.

The AA eye irritation studies both resulted in findings of "corrosive" while "moderate irritation" was observed with AAD-1. There were no corneal or iris effects with any of the materials at 1 hour. However, corneal opacity, iritis, and conjunctivitis were observed with AA 24 hours after instillation. All of these findings increased in severity until it was determined that irreversible damage had occurred in all of the animals within 72 hours after instillation. AAD-1 caused few corneal effects or iritis at any observation point. Conjunctival effects, including slight to marked erythema and substantial erythema, were observed 1 hour after instillation of AAD-1. These findings persisted until at least 7 days post dosing. All irritation cleared 7 to 21 days after instillation.

When tested for skin irritation, one study with AA showed no irritation while the other study with AA and studies with AAD-1 and AAD-2 all produced findings of severe irritation, with PIIs of 5.3, 5.4, and 5.3, respectively. At the one-hour scoring interval, very slight to moderate erythema and very slight to severe edema were noted. These findings increased and reached a maximum at 48 hours (AAD-2 and AA) or 72 hours (AAD-1); the irritation caused by AA and AAD-2 decreased from this time point on. The skin irritation cleared by day 7 for AA and essentially cleared by day 14 for AAD-2. The maximum irritation caused by AAD-1 (severe erythema and severe edema in 6/6 animals) persisted until study termination in two animals and decreased to barely perceptible to slight erythema with no edema by day 14 in the other four animals. Additional findings of necrosis, fissuring, and sloughing were also noted.

At challenge in the delayed contact hypersensitivity study, one of ten guinea pigs treated with AA had positive erythema at the 24-hour scoring interval compared to two irritation control animals that had more severe erythema. At the 48-hour scoring interval, five AA-treated animals had positive erythema scores, two of which also exhibited edema, while five-irritation control animals had positive scores. In the rechallenge assay, none of the irritation control animals showed any erythema or edema, while six test animals at 24 hours and seven test animals at 48 hours exhibited some irritation. Based on these results, AA is considered to have the potential to produce dermal sensitization. In the study with AAD-1, 25% of the challenge animals exhibited a response compared to 20% of the irritation control animals. Since the response was similar, AAD-1 is not considered to have the potential to elicit a delayed contact hypersensitivity response.

Subacute and Subchronic Toxicity Studies

Material and Methods

Rats

One-week dermal study in rats

AAD-1 (Bechtel, 1996) was tested in Sprague-Dawley rats at dose levels of 0, 100, 300, and 1000 mg/kg body weight/day. AAD-1 was applied for 5 days over a one-week period to an approximately 25-35 cm^2 area on the shaved back of the rats and covered with a gauze wrap and nonirritating tape. A collar was applied to each rat to minimize the potential for ingestion of the test article. After a 6-hour exposure period, the dressing was removed and the excess test article was removed using gauze and distilled water. The health status of the animals was checked twice daily. Detailed observations for signs of toxicity were made once near the end of the exposure period. Body weights were recorded on the first day of dosing and one week later. Food consumption was determined over the course of the experiment. A gross necropsy was performed on all animals at study termination.

One month oral (feeding and gavage) studies in rats

SA (Reyna, 1982a) was administered orally by gavage to groups of 5 Sprague-Dawley rats per sex at daily doses of 0, 100, 500, 1000 and 2000 mg/kg for 30 days. Each of the four other surfactants (AA (Ogrowsky, 1989), PE (Stout, 2001), AAD-2 (Dudek, 2000), and AAD-1 (Stout, 1997)) was tested in one-month feeding studies in which Sprague-Dawley rats were administered the test article in feed for four weeks (Table 2). For all five studies, mortality, moribundity, and overt signs of toxicity were checked twice daily. Body weights and food consumption measurements and detailed observations for clinical signs of toxicity were made weekly. At the end of the four-week exposure period the animals were sacrificed. Blood was collected (AAD-2 and AAD-1) from the posterior vena cava for hematology and serum chemistry evaluations. All animals were given a gross necropsy in which the internal organs and cavities were examined. Selected organs were weighed, including at least the kidneys, liver, spleen, testes, and thyroid (exception: SA). For AAD-2, histopathological examination of approximately 40 retained tissues was conducted for the control and high-dose (3000 ppm) groups and clinical pathological evaluations were preformed (Tables 3 and 4).

Three-month oral (feeding and gavage) studies in rats

AA (Stout, 1990), PE (Stout and Thake, 2001), and AAD-1 (Stout and Thake, 1997) were tested in 3-month feeding studies in Sprague-Dawley rats (10/sex/group) (Table 5). SA (Reyna and Thake, 1983a) was administered orally by gavage to 20 rats/sex/group at daily doses of 0, 25, 100, and 400 mg/kg/day. A 3-month feeding study was not conducted with AAD-2. For all four 3-month studies, checks for mortality, moribundity, and overt signs of toxicity were conducted twice daily. Detailed observations for signs of toxicity and determination of body weights and food consumption were made weekly. An ophthalmic examination was conducted prior to the start of each study and during week 13 (exception: SA). All animals were examined at both time points in the studies with PE and AAD-1. All animals were examined prior to study initiation in the AA study while only the control and high dose animals were examined during week 13. At the termination of each study, all animals were fasted overnight, and then sacrificed and gross necropsies were performed. Blood samples were collected from the posterior vena cava and hematological, clinical blood chemistry, and clotting potential analyses were performed (Table 3). Blood samples for SA were collected at pretest and weeks 7 and 13 after an overnight fast. Urine samples for SA were collected at weeks 7 and 13. The urine was assayed to determine pH and the presence of protein, blood, glucose, ketone, bilirubin, and urobilinogen. Selected organs were removed and weighed and tissues were collected and retained (Table 4).

Retained tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, processed, and stained with hematoxylin and eosin. All retained tissues from the control and high-dose animals were examined microscopically. Selected tissues from the other dose groups were also examined (Table 4). Additionally, in the PE study, the uterus, vagina, ovaries, and mammary tissue from all groups were histopathologically examined.

Dogs

One-month oral (capsule) studies in dogs

SA (Reyna, 1982b) was administered orally by gelatin capsule to 2 beagle dogs/sex/group at daily doses of 0, 50, 150, or 500 mg/kg for four weeks. AAD-1 (Wolford, 1997a) was also administered in gelatin capsules for four weeks to beagle dogs. Two animals/sex/group were treated with 0, 5, 15, and 30 mg/kg body weight per day. The dose of AAD-1 for a fifth group was increased weekly from 30 to 45 to 60 to 90 to120 to 200 mg/kg/day during the four weeks. The dose escalation was done to determine the maximum tolerated dose. The daily doses for AAD-1 were split and separated by approximately four hours. Checks for mortality and overt signs of toxicity were conducted twice daily. Detailed clinical observations, body weights, and food consumption (AAD-1 only) were performed weekly. Blood samples were collected from the AAD-1 animals pretest and after 4 weeks for hematology and clinical chemistry tests. All animals were necropsied at study termination. Selected organs were weighed and selected tissues were retained from AAD-1 animals only.

Three-month oral (capsule) studies in dogs

Beagle dogs were administered AA (Fillmore, 1973), AAD-1 (Wolford, 1997b), and SA (Reyna and Thake, 1983b) in gelatin capsules for three months. AA was given to 4 animals/sex/group in increasing doses during the first four weeks and then maintained at 0, 30, 60, or 90 mg/kg/day (t.i.d.) for the final ten weeks of the study. AAD-1 was administered to 5 animals/sex/group at doses of 0, 10, or 30 mg/kg body weight per day. The dose of AAD-1 for a fourth group was increased weekly from 30 to 45 to 60 to 90 mg/kg/day during the first four weeks. The dose escalation was done to determine the maximum tolerated dose. Due to significant toxicity at 90 mg/kg/day included mortality the dose was reduced to 75 mg/kg/day for weeks 5 through 8 and further reduced to 60 mg/kg for the remainder of the study. In order to achieve the daily doses for AAD-1 the doses were split and separated by approximately four hours. SA was administered to 6 animals/sex/group at daily doses of 0, 20, 80, and 300 mg/kg/day. For all three studies, checks for mortality and moribundity and observations for signs of toxicity were conducted at least once a day. Body weights were measured weekly. In the AA study, food consumption was monitored daily and water consumption was measured 4 days/week. Food consumption was measured weekly for AAD-1 and SA. Electrocardiograms were taken on each dog pretest and (AA only) at the end of the study. Ophthalmic examinations were performed prior to treatment initiation and just prior to termination (exception: AA). Blood and urine samples were collected prior to initiation of treatment and at the end of the study. Blood was collected from the jugular vein. Urine was collected following overnight (approximately 16 hours) fasting. Hematology and clinical chemistry analyses and urinalysis endpoints evaluated are presented in Tables 3 and 4, respectively. At termination, the animals were given physical exams, sacrificed and a gross necropsy performed. (AA only underwent a neurological examination.) Selected organs were removed and weighed (Table 4). Additionally,

tissues from all animals were retained in neutral buffered formalin, embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically.

Statistical Analyses

One-week dermal study in rats

Body weights and food consumption were analyzed using Dunnett's Multiple Comparison Test (two-tailed) to compare treated animals to controls.

One month and three-month oral (feeding and gavage) studies in rats

Body weights, body weight changes, and food consumption were analyzed using Dunnett's Multiple Comparison Test (two-tailed). Hematology, blood chemistry and clotting-potential data, terminal body weights, absolute organ weights, and organ/body weight ratios were evaluated by a decision-tree analysis that, depending on the results of tests for normality and homogeneity of variances (Bartlett-Box test), utilized either parametric (Dunnett's test and linear regression) or nonparametric (Kruskal-Wallis, Jonckheere's and/or Mann-Whitney tests) routines to detect differences and analyze for trend (two-tailed). Fisher's Exact Test (one-tailed) with Bonferoni Inequality Procedure was used to evaluate the incidence of microscopic lesions.

Three-month oral (capsule) studies in dogs

One-way analysis of variance (ANOVA) was used to analyze initial body weights, cumulative body weight gains; food consumption; clinical chemistry and hematology data; urine pH, volume and specific gravity; absolute organ weights; organ/body weight ratios; and organ/brain weight ratios. The variance homogeneity was tested using Levene's test. Transformations were used to stabilize the variance in the case of heterogeneity of variance at $p \leq 0.05$. One-way analysis of covariance (ANCOVA) was used to analyze body weights with the initial body weights used as the covariate. In all cases, $p \leq 0.05$ were considered statistically significant.

Results

One-week dermal study in rats

All animals survived until the end of the study. All animals receiving 100 mg/kg/day of AAD-1 appeared normal throughout the study. Dermal irritation, redness, and scabs, were observed at the exposure site of all 300 and 1000 mg/kg/day animals. The scabs were severe in the high-dose animals and statistically significantly decreased food consumption and lower mean body weight was observed in the high-dose males. The no-observable effect level (NOEL) was considered to be 100 mg/kg/day (Bechtel, 1996).

One-month oral (gavage) study in rats

Mortality was observed in the two highest dose-level groups receiving SA (5/5 females and 4/5 males at 2000 mg/kg/day and 2/5 femlaes and 1/5 males at 1000 mg/kg/day) (Reyna, 1982a). Other effects observed in males and females in the three highest dose-level groups included decreased body weight and food intake, salivation, increased urine output, and hyperplasia and keratinization of the forestomach mucosa. These histological findings indicate

irritation of the gastrointestinal system. The NOEL was 100 mg/kg/day. It is important to note that in rodents the stomach is divided into two parts by an elevated fold. The forestomach or non-glandular part of the stomach is continuous with the esophagus and is lined by keratinized, stratified squamous epithelium. The remaining part, or the glandular stomach, connects to the duodenum and is lined by a glandular epithelium. There is no forestomach in humans and the lining of the stomach is completely glandular.

One-month oral (feeding) studies in rats

No mortality or target organ toxicity was observed in any study. Clinical signs and gross lesions indicated local irritation of the gastrointestinal system.

AA treatment-related effects included reduced body weights (compared to controls) throughout the study in mid-dose males (5-7%) and high-dose males (15-19%) and high-dose females (10-13%). Food consumption (gms/day) was reduced for mid-dose males during the first week of the study and high-dose animals throughout the study. Soft stool was observed in high-dose males and females and prominent/enlarged lymphoid aggregates in the colon in 5/10 high-dose females secondary to colonic irritation was noted. The NOELs were 800 ppm in males (51.7 mg/kg/day) and 2000 ppm in females (159.9 mg/kg/day) (Ogrowsky, 1989).

The only effects associated with administration of PE were decreased weight gain and food consumption. Cumulative weight gains were decreased in males and females at all levels except males at 800 ppm in animals feed PE. Due to the relative small magnitude and/or lack of a clear dose response, only the body weight changes at 5000 and 2000 in males and females were considered toxicologically significant. At the end of the study, cumulative weight gains were 53-54% of controls in males and females at 5000 ppm, and cumulative weight gains were 82-87% of controls in males and females at 2000 ppm. Statistically significant decreases in food consumption occurred in males at 5000 ppm and females at all levels throughout the study. Due to the relatively small magnitude of changes in the females at 2000 and 800 ppm only those decreases in the high-dose males and females considered toxicologically significant.. The NOELs were 800 ppm in males (66.2 mg/kg/day) and 2000 ppm in females (172 mg/kg/day) (Stout, 2001).

AAD-2 treatment-related effects included decreased food consumption and body weight in high-dose animals only. Based on the significant decreases in mean body weight gain and food consumption, it was concluded that the high-dose level of 3000 ppm was unpalatable when administered to male and female rats in the diet for 1-month. The NOEL was 500 ppm (42.6/47.8 mg/kg/day, male/female.) (Dudek, 2000).

AAD-1 treatment-related effects included decreased food consumption and body weight loss in high-dose animals and decreased body weight and weight gain in three highest-dosed animal groups. At the end of the study cumulative weight gains in females were <1%, 59%, 75% and 100% of controls and in males 23%, 91%, 95% and 95% of controls for high to low dose levels. These changes were accompanied by decreased food consumption and by clinical changes of decreased defecation and dehydration. The NOELs were 1000 ppm in males (75.6 mg/kg/day) and 100 ppm in females (8.3 mg/kg/day) (Stout, 1997).

Three-month oral (feeding) studies in rats

The only clinical sign of toxicity observed was soft stools which occurred a the high-dsoe level , primarily in females. The food consumption of high-dose males and females dosed with

AA, was dramatically reduced (32% and 28% below controls, respectively on a gm/day basis) particularly during the first week of the study, suggesting some lack of diet palatability. Statistically significant reductions in body weights were noted in high-dose males and females throughout the study; body weights were reduced 19% and 18%, respectively below controls a the end of the study. Various changes in serum hematology/clinical chemistry was observed in the high-dose animals, the changes were not clearly dose-related in males and not significant in females, and may be secondary to decreased food consumption.. Intestinal irritation was noted in the mid- and high-dose animals as evidenced by hypertrophy and/or vacuolation of the histiocytes of the lamina propria of the jejunum and ilieum The NOEL was 500 ppm (33.0 and 39.9 mg/kg/day for males and females, respectively) (Stout, 1990).

Decreased weight gain, accompanied by reduced food consumption, occurred in high-dose animals receiving PE. Group mean body weights and cumulative weight gains were statistically significantly decreased in males and females at 3000 ppm throughout the study. At the end of the study, group mean cumulative weight gains were 81% and 69% of controls for males and females, respectively. .Decreased weight gains, accompanied by decreased food consumption, occurred in males and females at 3000 ppm. However, the most marked effects occurred early in the study, suggesting that the diet may have been unpalatable. Therefore, the decreased body weight gains were not considered toxicologically significant. There were no other findings attributed to the test material and considered toxicologically significant. The NOEL was the highest dose tested, 3000 ppm (181 and 218 mg/kg/day for males and females respectively (Stout and Thake, 2001).

The primary effects associated with administration of AAD-1 in this study were decreased food consumption and weight gain. No specific target organ was identified.. Cumulative weight gains were decreased in males at 3000 ppm and females at 1000 ppm at the end of the study, cumulative weight gains at these dietary concentrations were 61 and 76% of controls in males and females, respectively. The NOEL was 1000 ppm (58.8 mg/kg) in males and 500 ppm (35.4 mg/kg/day) in females (Stout, 1997).

Three-month oral (gavage) study in rats

Treatment-related changes only occurred at the highest dose level tested with SA (400 mg/kg/day) and included mortality (4/20 females), decreased body weight, changes in the clinical condition of the animals (salivation and breathing difficulties), and hyperplasia and hyperkeratosis involving the squamous epithelium of the nonglandular stomach. These histological findings are relatively common lesions in rats which can be induced by a variety of chemicals, especially when given by gavage and indicate a non-spcific response probably confined to rodents or other speices with squamous epithelium in the cardiac region of the stomach. The NOEL was 100 mg/kg/day (Reyna and Thake, 1983).

One-month oral (capsule) studies in dogs

Administration of SA via capsules to beagle dogs for one month resulted in gastrointestinal irritation as evidenced by loose stools, petechial hemorrhages of the stomach lining, regurgitation and salivation at the 500 mg/kg/day level, similar effects were observed at 150 mg/kg/day with the exception of salivation and animals a the lowest dose of 50 mg/kg/day had loose stools. A no-effect level was not established (Reyna, 1982).

There were no differences in any parameter in dogs dosed at 30 mg/kg/day AAD-1 compared to controls. For the escalating dose group, appearance and behavior, body weight, and

food consumption were unaffected at 30, 48, and 60 mg/kg/day doses. However, marked body weight loss and decreased food consumption occurred when the doses were escalated to 90, 120 and 200 mg/kg/day. At the end of this phase clinical signs of thin appearance, no/few feces, vomitus, and excessive salivation were noted. The NOEL was 30 mg/kg/day (Wolford, 1997).

Three-month oral (capsule) studies in dogs

No relevant toxicological effects were observed when SA was administered at 20 and 80 mg/kg/day. At the highest dose level tested, 300 mg/kg/day, treatment-related changes included emesis, decreased food intake and body weight, and an equivocal decrease in adrenal weights in females. The NOEL was 80 mg/kg/day (Reyna and Thake, 1983).

No effects were observed on survival, general appearance, behavior, neurological function, or cardiac function in rats receiving AA. Changes in the clinical condition of the animals, including emesis, diarrhea, anorexia, and subsequent dehydration were observed in mid- and high-dose animals, indicating significant gastrointestinal irritation. Since body weights were reduced in all dose groups, no clear NOEL was established. Under the conditions of the study the only significant finding was the inability of dogs to tolerate daily ingestion of surfactants due to gastrointestinal irritation (Fillmore, 1973).

Dose-related toxicity observed in the escalating high-dose group receiving AAD-1 (30 to 90 mg/kg/day) included mortality (1/10 animals at 90 mg/kg/day), clinical signs of toxicity that increased in frequency and severity as the dosage was increased (excessive salivation, alterations in feces, cloudy vomitus, retching, ocular/nasal discharge, changes in respiration, pupil dilation, changes in activity), decreases in body weight and body weight gain, decreased food consumption, and mildly reduced albumin at the end of the study. At 30 mg/kg/day, the only biologically significant effects were slight reductions in mean body weight and cumulative body weight gain in males only, and several occurrences of excessive salivation in one female. There were no treatment-related ophthalmic, macroscopic, or microscopic findings or effects on organ weights at any dose level. The 10 mg/kg/day dose level was the NOEL (Wolford, 1997).

Developmental toxicity studies

Material and Methods

Pilot developmental toxicity study in rats

The potential maternal and developmental toxicity of AAD-2 (Stump, 2000) was evaluated in a dose-range finding developmental study in rats. Test material was suspended in Mazola corn oil and administered daily via gavage to five groups of eight rats from gestational days 6 through 15. All rats were observed twice daily for mortality and morbidity. Detailed clinical observations were made at least once each day and body weights and food consumption were recorded on gestation days 0, 6, 9, 12, 15, 16, 18 and 20. Mean body weight changes were calculated for each corresponding interval as well as days 6-16, 16-20, and 0-20. All surviving maternal animals were sacrificed by carbon dioxide inhalation on gestation day 20. The thoracic and abdominal cavities were opened by a midline incision and the contents examined. The liver, kidneys, spleen and thyroid glands from each dam were removed and weighed. The uterus and ovaries were excised. The number of corpora lutea on each ovary was recorded. The trimmed uterus was weighed and opened and the number and location of each fetus, early and late

resorptions, and the total number of implantations was recorded. Each fetus was weighed, sexed, and examined externally for development variations or malformations.

Results

No treatment-related external fetal malformations or variations were noted in any group and developmental toxicity occurred only at maternally toxic doses.

Significant maternal toxicity observed in the three highest dose groups and included mortality (8/8 dams, 1/8 dams, and 1/8 dams in the 450, 250, and 150 mg/kg/day groups, respectively), changes in the clinical condition of the animals (eg. yellow and brown material in the urogenital area, red material around nose and/or mouth, gasping, and rales), mean body weight losses, and reduced food consumption (g/kg/day basis). Reduced body weight gain was observed during the entire treatment period in the 150 mg/kg/day group. Increased mean postimplantation loss in the 250 mg/kg/day group resulted in a decreased mean number of viable fetuses and a decreased mean fetal weight was observed at that dose. The NOELs for maternal and developmental toxicity were 75 and 150 mg/kg/day, respectively.

Developmental toxicity studies in rats

Material and Methods

The potential maternal and developmental toxicities of AA (Holson, 1990), PE (Stump, 1999), and AAD-1 (Holson, 1997) were evaluated in developmental toxicity studies in rats. Test materials were suspended in corn oil and administered daily via gavage to three groups of twenty-five rats from gestational days 6 through 15. The dosage levels are indicated in Table 7. All rats were observed at least twice daily for morbidity and mortality. Detailed clinical observations were made least once each day and body weights were recorded on gestation days 0, 6, 9, 12, 16 , 18 and 20. Mean body weight changes were calculated for each corresponding interval as well as days 6-16, 16-20 and 0-20. All surviving dams were sacrificed by carbon dioxide inhalation on gestation day 20. The thoracic and abdominal cavities were opened by a midline incision and contents examined. The liver, kidneys, spleen, and thyroid glands from each dam were removed and weighed (exception: AA, liver only). The uterus and ovaries were excised. The number of corpora lutea on each ovary was recorded. The trimmed uterus was weighed and opened and the number and location of each fetus, early and late resorptions and the total number of implantations was recorded. Each fetus was weighed, sexed, and examined externally, viscerally, and skeletally for development variations or malformations. Heads from approximately one-half of the fetuses from each female were placed in Bouin's fixative for subsequent soft-tissue examination and the heads from the remaining fetuses were examined following a mid-coronal slice. All carcasses were eviscerated and fixed in 100% ethyl alcohol, macerated in potassium hydroxide, and stained with Alizarin Red S for skeletal examination.

Statistical Analyses

One-way analysis of variance (ANOVA) with Dunnett's test was used to analyze maternal body weights and weight changes, cumulative maternal body weight changes, gravid uterine weights, maternal food consumption, organ weights, corpora lutea, total implantations, viable fetuses, and fetal body weights. Kruskal-Wallis test with Mann-Whitney U-test was used to analyze litter proportions of intrauterine data (considering the litter, rather than the fetus, as the experimental unit), litter proportions of fetal malformations, and developmental variations. In the AA study, the Chi-square test with Yates' correction factor was used to analyze fetal sex

ratios, and Fisher's exact test was used to compare for the number of litters with malfunctions and variations.

Results

Significant maternal toxicity was observed at the highest dose tested (300 mg/kg/day) with AA and included mortality (6/25 animals), clinical signs of toxicity (eg. yellow urogenital and anogenital matting, soft stool, diarrhea and mucoid feces in a majority of the animals), and decreased food consumption throughout the treatment period. A significant group mean body weight loss occurred during the first three days of dosing and reduced mean body weight gains throughout gestation (10% below controls at end of study).. At 100 mg/kg/day dose level reduced food consumption and although not reflected in the group mean five animals lost weight during gestational days 6-9. No developmental toxicity was observed at any dose level. The NOELs for maternal and developmental toxicity were 15 and 300 mg/kg/day, respectively (Holson, 1990).

Significant maternal toxicity was observed at the highest dose tested (150 mg/kg/day) with PE and included mortality (1/25), clinical signs of toxicity (rales a the daily examinations and salivation immediately following dosing), and a statistically significant reduced body weight gain (71% of controls) and food consumption (84% of controls on a gm/kg/day) was observed during gestational days 6-16. No developmental toxicity was observed at any dose level. The NOELs for maternal and developmental toxicity were 50 and 150 mg/kg/day, respectively (Stump, 1999).

Maternal toxicity was expressed with AAD-1 by mortality (1/25) at 150 mg/kg/day and clinical signs of toxicity (eg. rales and salivation and clear, brown or tan matting/staining on various body surfaces) and statistically significant inhibition of body weight gain (75 and 20% of controls) and food consumption (85 and 68% of controls on a gm/kg/day basis) at 75 mg/kg/day and 150 mg/kg/day, respectively. Developmental toxicity was only observed at maternally toxic doses and was exhibited by reduced fetal body weights and increased incidences of three skeletal variants. No developmental toxicity was observed at the other dose levels (25 and 75 mg/kg/day). The NOELs for maternal and developmental toxicity were 25 mg/kg/day and 75 mg/kg/day, respectively (Holson, 1997).

Reproduction/developmental toxicity screening study

Material and Methods

The potential effect of AA on male and female reproduction, including gonadal function, mating behavior, conception, parturition, and lactation of F_0 and F_1 generations and development of F_1 and F_2 generations was evaluated (Knapp, 2007). Four groups of male and female Sprague-Dawley rats (20/sex/group – F_0 generation) were administered AA at dietary concentrations of 0, 100, 300, and 1000 ppm (Table 8 provides dosage levels on a mg/kg/day basis) for at least 70 consecutive days prior to mating and continuing throughout the remainder of the study. Offspring (three pups/sex/litter) from the pairing of the F_0 animals were selected on postnatal day (PND) 21 to constitute the F_1 generation and were exposed to the test diet *in utero* and through nursing. Following weaning of the F_1 generation, the test diet was directly administered on a mg/kg/day basis up to PND 70. Beginning on PND 70, the F_1 animals selected from the control and high-dose groups for mating (two pups/sex/litter) were offered control or test diet *ad libitum* at a constant concentration until study termination. The F_2

generation was exposed to the test article *in utero* and through nursing during PND 0 to 4. All animals were observed twice daily for appearance and behavior. Clinical observations, body weights and food consumption were recorded at appropriate intervals for males throughout the study and for females prior to mating and during gestation and lactation. Detail physical examinations were conducted weekly. Vaginal lavages were performed daily for determination of estrous cycles beginning 14 days prior to mating. All F_0 (all groups) and F_1 (control and high-dose groups) females were allowed to deliver and rear their pups until lactation days 21 and 4, respectively. For the F_1 generation, eight pups per litter (four per sex, when possible) were selected on PND 4 to reduce the variability among the litters. Anogenital distance was measured for all F_1 pups on PND 1: thoracic nipple retention was evaluated for all F_1 male pups on PND 11, 12 and 13. F_0 parental animals received a complete detailed gross necropsy following the completion of weaning of the F_1 pups on lactation day 21; selected organs were weighed. Developmental landmarks (balanopreputial separation and vaginal patency) were evaluated for the selected F_1 rats. Nonselected F_1 pups were necropsied on PND 21. F_1 pups not selected for the breeding phase (one/sex/litter if possible) were necropsied on PND 70; selected organs were weighed and blood samples for thyroid and reproductive hormone analyses were collected from the vena cava. Each surviving F_1 parental animal (two/sex/litter from the control and high-dose groups, if available) received a complete detailed gross necropsy after the F_2 pups reached 4 days of age; selected organs were weighed. Spermatogenic endpoints (sperm motility, morphology, and numbers) were recorded for all F_1 males at the scheduled necropsies (PND 70 for males not selected for breeding and after the F_2 pups reached PND 4 fore the males selected for breeding). Designated tissues from all F_0 and F_1 parental animals in the control and high-dose groups were examined microscopically. F_2 pups were necropsied on PND 4.

Statistical analysis

Parental mating, fertility and copulation/conception indices, and F_1 male nipple retention were analyzed using the Chi-square test with Yates' correction factor. Mean parental (weekly, gestation, and lactation) and offspring body weights and body weight changes, parental food consumption and food efficiency data, estrous cycle length, pre-coital intervals, gestation lengths, live litter sizes, former implantation and unaccounted-for sites, numbers of pups born, balanoprepurtial separation data (day of acquisition and body weight), vaginal patency data (day of acquisition and body weight), anogenital distances, absolute and relative organ weights, sperm production rates, epididymal and testicular sperm numbers, and serum hormone concentrations were subjected to a parametric one-way ANOVA followed, if appropriate, by Dunnett's' test to compare the treated groups with the control. Mean litter proportions (percent per litter) of postnatal pup survival, pup sexes at birth (percentage of males per litter), percentages of motile sperm, and percentages of sperm with normal morphology were analyzed using the Kruskal-Wallis nonparametric ANOVA test followed, if appropriate, by the Mann-Whitney U-test. Histopathological findings were analyzed using a two-tailed Fisher's exact test.

Results

No effects were observed on any of the following parameters at any dose level: survival and clinical condition, reproductive performance, body weight and food consumption (pre-mating, gestation and lactation), organ weights and macroscopic and microscopic morphology of the F_0 and F_1 parental generations; developmental landmarks, estrous cyclicity, spermatogenic endpoints and testosterone and thyroid hormone levels of the F_1 generation; the clinical condition

and body weight of the F_1 and F_2 litters; and litter viability and postnatal survival of the F_2 litters. The F_0 generation animals were paired for mating at 21 weeks of age, an older age than typical for reproduction studies at 12-16 weeks, resulting in reductions in reproductive indices. In addition, equivocal findings were observed in three F_0 high-dose females including decreased mean number of pups born, litter size, and postnatal survival. These findings were not statistically significantly from concurrent control findings and were at or slightly below historical control findings. Due to the advanced age of the F_0 high-dose animals it was not possible to re-bred them to determine if the litter effects observed at the high-dose were reproducible therefore the F_1 animals were mated to produce F_2 litters. The litter effects observed in the high-dose F_1 animals were not reproduced in the high-dose F_2 litters A clear NOEL was 300 ppm (16.6 and 14.9 mg/kg/day for the F_0 and F_1 males, respectively, and 19.5 and 18.9 mg/kg/day for the F_0 and F_1 females, respectively)

Genotoxicity studies

A series of Ames, *in vitro* cytogenetics, and *in vivo* mouse micronucleus assays were performed with several of the surfactants.

Material and Methods

Ames assays

The Ames assay (*Salmonella-E. coli* reverse mutation assay) was conducted with each of the surfactants (Stankowski, 1996; Flowers, 1981; Lawlor, 2000; Mecchi, 2000; and Stegeman and Li, 1990). This assay evaluates a test material and/or its metabolites for their ability to induce reverse mutations at the histidine locus in the genome of selected *Salmonella typhimurium* strains and at the tryptophan locus in an *Escherichia coli* strain, both in presence and absence of a metabolic activation system of mammalian microsomal enzymes derived from Aroclor-induced rat liver (S9). The reverse mutations result from DNA frame shifts and/or base substitutions. The experimental procedures were based on the description in Ames *et al* (1975), Maron and Ames (1983), and Maron *et al.* (1981) for *Salmonella* and in Green and Muriel (1976) for *E. coli.* All five surfactants were tested using four strains of *S. typhimurium* (TA98, TA100, TA1535, and TA1537). AAD-1 was also tested with strain TA102. The *E. coli* tester strain WP2*uvr*A was used to assay AAD-1, PE, and AAD-2. Doses were based on the results of dose range finding studies conducted in the presence and absence of S9.

Each Ames assay was conducted with a minimum of six doses of test article in both the presence and absence of S9 along with concurrent vehicle control (DMSO or deionized water) and appropriate positive control substances). The results of the initial mutagenicity assays were confirmed in independent experiments.

In vitro human cytogenetic assays

In vitro cytogenetics assays were conducted with AAD-1 and AAD-2 (Murli, 1997, 2000, respectively). The assay evaluates the test material's potential to induce chromosome aberrations in cultured whole blood human lymphocytes with and without metabolic activation. Chromosomal aberrations are a consequence of failure or mistakes in a cell's repair processes such that treatment-induced breaks in the chromatin do not rejoin or rejoin in abnormal configurations.

The assays were conducted using venous blood drawn from healthy, adult human donors. An *in vitro* metabolic activation system was derived from Aroclor-induced rat liver (S9). The assays were performed without or with the activation system. Vehicle controls were cultures containing the vehicle, sterile deionized water (AAD-1), or DMSO (AAD-2). Negative controls were cultures containing only cells and culture medium. Positive control agents used in the assays were mitomycin C for the series without activation and cyclophosphamide for the series with activation.

In the initial assays, replicate cultures of lymphocytes were incubated with varying doses of the test material, with or without S9, for three hours. The cultures were harvested 22 hours after initiation of treatment and analyzed for chromosomal aberrations, polyploidy, or endoreduplication. .In the confirmatory assays, replicate cultures of lymphocytes were incubated with varying doses of the test materials, with S9 (3-hr treatment) or without S9 (~19-hr treatment), and harvested 22 hours after initiation of treatment. Cultures were analyzed for chromosomal aberrations, polyploidy, or endoreduplication.

In vivo mouse micronucleus assays

Three of the surfactants (PE, AAD-2, and AA)were tested using the *in vivo* mouse micronucleus assay (Myhr, 2000a; Myhr, 2000b; and Stegeman and Kier, 1998, respectively). The assay evaluates the test article for *in vivo* clastogenic activity and/or disruption of the mitotic apparatus by quantifying micronuclei in polychromatic erythrocytes (PCE) in mouse bone marrow.

In two of the micronucleus assays, Crl:CD-1®(ICR)BR mice (Charles River Laboratories, Raleigh, NC, USA) received, via gavage in a corn oil vehicle, PE at dose levels of 0, 375, 750, or 1500 mg/kg (males) and 0, 500, 1000, or 2000 mg/kg (females) (Myhr, 2000a) or AAD-2 at dose levels of 0, 450, 900, or 1750 mg/kg (males only) (Myhr, 2000b). Six animals/sex were dosed per level for each harvest time. The positive control groups consisted of six males and six females dosed by gavage with cyclophosphamide at 80 mg/kg. The bone marrow was harvested from 5 animals/sex from vehicle control and high-dose levels both 24 and 48 hours following dosing. Five animals/sex dosed with test material at the low- and mid-dose groups and five animals/sex dosed with the positive control were euthanized approximately 24 hours after dosing for extraction of bone marrow. Samples of 2000 polychromatic erythrocytes (PCEs) per animal were analyzed for the frequency of micronuclei. Cytotoxicity was assessed for each animal by scoring the number of PCEs and normochromatic erythrocytes (NCEs) in the first 200 erythrocytes.

In a third micronucleus assay (Stegeman and Kier, 1998), male and female CD-1® mice (10/sex) received AA in a corn oil vehicle via a single ip injection at a target dose level of 100 mg/kg. Negative control mice (10/sex) were treated with corn oil only while positive control mice (5/sex) received cyclophosphamide at 60 mg/kg. Bone marrow from the test and vehicle control animals was sampled at approximately 24 and 48 hr after dosing. A single sampling time of 24 hr was used for the positive control group. Five male and five female mice were sampled at each time point. Slides of bone marrow cells were prepared from five animals/time point for each group and scored for the occurrence of micronucleated polychromatic erythrocytes (PCE) and the PCE/total erythrocyte ratio calculated.

Statistical analysis

Ames assays

In the assays with PE and AAD-2, no statistical analysis was conducted; rather, the criteria for assessing the studies' results relied on the magnitude of the response in the test groups relative to the vehicle control group. In the assays with AA and SA, the revertants/plate values were transformed as log10 (revertants/plate) and analyzed using Bartlett's test for homogeneity of variance followed by a one-sided t-test at $p < 0.01$. The dose response was evaluated with regression analysis. The AAD-1 assay was analyzed only when there was a 50% increase in revertant frequency relative to the control by the method developed by.

In vitro human cytogenetic assays

A Cochran-Armitage test for linear trend and Fisher's Exact Test were used to compare the percentage of cells with aberrations in treated cells to the vehicle controls. Cells exhibiting polyploidy and/or endoreduplication were also examined as indicators of possible induction of numerical aberrations. $P \leq 0.01$ was considered significant.

In vivo mouse micronucleus assay

In the gavage studies (Myhr, 2000a, 2000b), an analysis of variance was used to analyze the proportion of cells with micronuclei/animal and the PCE:NCE ratio if the variances were homogeneous. If the variances were heterogeneous, ranked proportions were used. If the analysis of variance was positive, Dunnett's test was used to determine which groups were statistically different from the vehicle control.

In the ip-dosing study (Stegeman and Kier, 1998), the PCE frequency was transformed as the square root prior to analysis. The PCE/total erythrocyte ratio was not transformed. Dunnett's test was used for comparison to vehicle controls.

$P \leq 0.05$ was considered significant in all three studies.

Results

The results of these all of the genotoxicity assays are summarized in Table 9. These uniformly negative results indicate that these surfactants are not mutagenic.

Risk Characterization

On the conceptual level, risk characterization is very simple, consisting of bringing together two risk components, hazard and exposure. Risk assessment includes bringing together the probabilities of hazard and exposure to produce an understanding of the likelihood of some type of adverse outcome. The process involves three components: data gathering to estimate the hazard, exposure assessment, and risk characterization (combining the factors) to produce an estimate of the magnitude and probability of the anticipated adverse effect. The method used in this assessment to characterize risk was margin of exposure (MOE). In this process, the lowest dose level from the most appropriate animal toxicity study was selected and compared to a conservative estimate of human exposure. Inasmuch as the findings in the studies with these surfactants were similar, the lowest dose level selected for risk characterization was 10 mg/kg/day from the 3-month dog oral study with AAD-1 (Table 10).

Margin of exposure calculation

In general, surfactant systems used in pesticide formulations are complex mixtures of structurally related molecules differing in size and composition of their substitution groups (difference in chain length, molecular weight, number of ethoxylations, etc.). Given these differences, monitoring the exposure associated with the use of these varied surfactant systems is rather virtually impossible.

A conservative solution to this problem is to monitor exposure data for the active ingredient in a pesticide formulation, then extrapolating this data to the formulation's surfactant system. This extrapolation should be based on the active ingredient-to-surfactant ratio in the pesticide formulation, taking into account the exposure properties of the active ingredient versus the surfactant system (dermal uptake, clothing permeation, etc.). This methodology is used below to evaluate the risk associated with surfactant systems in Roundup-branded herbicides.

Acquavella *et al.* (2004) published a large-scale biomonitoring study that reported measured urinary glyphosate concentrations and estimated systemic doses for farmers, their spouses, and their children. Overall, 40% of the farmers did not have detectable levels of the herbicide in their urine samples despite some of them making applications on up to 100 acres. The maximum systemic doses associated with the monitored application for these farmers, their spouses, and their children were estimated to be, respectively, 0.004, 0.00004 and 0.0008 mg/kg bw/day. The lifetime average daily dose (LADD) for farmers (assuming 20 applications per year and 40 years of professional glyphosate use out of a 70-year lifetime) is estimated to be 0.0001 mg/kg bw/day.

For extrapolation to surfactant systems, it is assumed that (1) the systemic dose is entirely associated with dermal exposure (no inhalation or oral exposure); (2) dermal uptake equals 100% of the dermal dose (versus 3% for glyphosate); (3) similar clothing permeation compared to glyphosate; and (4) a glyphosate:surfactant ratio of 4:1, which is typical for agricultural formulations. An LADD calculation makes sense only for the farmer (and not their spouses or children) and is outlined in Table 12.

To evaluate chronic risk, estimates of human exposure were compared to the lowest dose that produced no adverse effects in repeat-dose animal studies. Based on the lowest relevant NOAEL, 10 mg/kg/day (90-day dog study), a Margin of Exposure (MOE) can be calculated as follows:

[EMBED Equation.3]

Typically, MOEs of 100 or more, based on 10x for intraspecies sensitivity and 10x for interspecies differences, are generally indicative of minimal to no concern for potential adverse effects from exposures to chemicals. The MOEs for the surfactants described in this paper that are used in Roundup-branded herbicides are conservatively two orders of magnitude higher than the usual 100-fold level of minimal toxicological concern. This indicates that these surfactants do not pose a health risk to humans when used according to regulatory labels.

DISCUSSION

Surfactants solubilize components of membranes and this physical interaction can disrupt membrane integrity and function and is the source of many of the observed biological effects of surfactants.. This property causes the irritation seen after exposure to skin, eyes, and mucous membranes. This non-specific surface-activity may also account for the gastrointestinal irritation seen after repeated oral ingestion at relatively high concentrations of surfactant. Secondary effects such as decreased food consumption and decreased body weight gains may be the result of gastrointestinal irritation or palatability issues with the diet. The results of the toxicology studies demonstrate that the surfactants described in this article and that are found in Roundup branded herbicides are no more than slightly toxic after acute oral ingestion, do not target any specific organ system, do not produce malformations, produce some developmental toxicity, but only at maternally toxic doses, are not genotoxic, and may produce eye, skin and gastrointestinal irritation that can be attributed to the non-specific physicochemical properties of surfactants rather than a specific toxicological mode of action.

Similar effects have been observed in rats and rabbits exposed to surfactants used in household products such as liquid detergents and dishwashing soap. Test results for a significant number of detergent products demonstrate that they are irritating to the eyes, skins and esophagus of rabbits (Seabuagh et al., 1977). Benke et al., (1977) found the oral LD_{50} of an alkyl polyethoxylate nonionic surfactant in Wistar rats to be approximately 3300 mg/kg; when applied undiluted to the skin of rabbits it was severely irritating and resulted in a dermal LD50 of approximately 2000 mg/kg; and undiluted it produced severe irritation in rabbits eyes. Brown and Benke (1977) feed Sprague-Dawley rats diets containing 0, 1000, 5000 or 10,000 ppm of an alkyl polyethoxylated nonionic surfactant for 91-days and observed dose-related decreased body weight gains possibly related to a palatability factor and a dose-related liver-to-body weight ratio increase. No toxicological significant changes were seen in clinical chemistry, hematology or urine analysis. No test-related gross lesions were seen at necropsy and histological evaluations including the livers were normal. No teratogenic or reproductive effects were noted when a detergent containing a mixture of surfactants was feed to Sprague-Dawley rats with doses ranging from 80 to 800 mg/kg/day either during organogenesis or continuously throughout reproduction (Nolen et.al., 1975). In the same study no teratogenic effects were observed when New Zealand rabbits received daily doses with the same mixture ranging from 50-300 mg/kg/day via gavage. The results of genotoxicity studies conducted with surfactants are overwhelmingly negative. (Yam et al., 1984; Bizukojc-Liwarska, et al., 2005). The estimates of daily human exposure to surfactants from residues on dishes and eating utensils, toothpastes and contaminated water range from 3 mg/kg/day (Swisher, 1968) to as high as 15-20 mg/kg/day (Moncriedff, 1969).

In summary, surfactants have a history of safe use and the surfactants discussed in this paper do not pose any hazard distinct from that of any commonly-used surfactants in household products, and as used in formulated Roundup branded products are unlikely to produce significant adverse effects to humans, animals, or the environment under normal conditions of exposure.

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TABLE 1
Summary of Acute Toxicology and Irritation Studies Performed with Surfactants

Test Substance[a]	Oral LD_{50}, mg/kg bw (95% CI)	Dermal LD_{50} (mg/kg bw) (95% CI)	Eye Irritation	Skin Irritation (PII)	Dermal Sensitization
AA	1200 (1100 – 1310)	1580 (1370 – 1820)	Corrosive	Severe (5.3)	Slight potential
AAD-1	1516 (1154 – 1993)	> 2000	Moderate	Severe (5.4)	Negative
AAD-2	1100 (860 – 1410)	—	—	Severe (5.3)	—
SA	3098 (2776-3549)	5496 (3888-infinity)	Corrosive	Corrosive	—

[a] No acute toxicology studies were conducted with PE

TABLE 2
Exposure Concentration and Dose Levels in One-Month Feeding Studies in Rats with Surfactants

	AA		PE		AAD-1		AAD-2	
#/sex	10		5		5		10	
Target	Males	Females	Males	Females	Males	Females	Males	Females
(ppm)	(mg/kg bw/day)		(mg/kg bw/day)		(mg/kg bw/day)		(mg/kg bw/day)	
0	0	0	0	0	0	0	0	0
20							1.7	2.0
100					7.7	8.3	8.4	9.5
500					39.0	41.6	42.6	47.8
800	51.7	63.2	66.2	71.6				
1000					75.6	76.4		
2000	122.8	159.9	163	172				
3000							229	260
5000	268.7	324.8	346	374	260	256		

TABLE 3
Hematology, Blood Chemistry, and Clotting Potential Determinations[a] with Surfactants in Rat and Dog Subchronic Toxicity Studies

Analysis	AA		SA		PE	AAD-1		AAD-2
	3-Month Rat	3-Month Dog	3-Month Rat	3-Month Dog	3-Month Rat	3-Month Rat	3-Month Dog	1-Month Rat
Hematocrit (HCT)	X	X	X		X	X	X	X
Hemoglobin (HGB)	X	X	X		X	X	X	X
Mean corpuscular hemoglobin (MCH)	X		X		X	X	X	X
Mean corpuscular hemoglobin concentration (MCHC)	X		X		X	X	X	X
Mean corpuscular volume (MCV)	X		X		X	X	X	X
Platelet count	X		X		X	X	X	X
Red blood cell count (RBC)	X		X		X	X	X	X
Reticulocyte count	X		X		X	X	X	
White blood cell count (total and differential) (WBC)	X	X	X		X	X	X	X
Albumin (ALB)	X		X		X	X	X	X
Alkaline phosphatase (ALK PHOS/AMP)	X	X	X		X	X	X	X
Blood urea nitrogen (BUN)	X		X		X	X	X	X
Calcium (CA)	X	X	X		X	X	X	X
Carbon Dioxide		X						
Chloride (CL)	X	X			X	X	X	X
Cholesterol (CHOL)	X		X		X	X	X	
Creatine phosphokinase (CPK)					X	X	X	
Creatinine (CREA)	X				X	X	X	
Direct bilirubin (DBIL)	X	X	X		X	X		X
Gamma glutamyl transpeptidase (GGT)	X				X	X	X	X
Globulin (GLOB)	X		X		X	X	X	X
Glucose (GLU)	X	X	X		X	X	X	X
Lactate dehydrogenase (LDH)			X					
Phosphorus (PHOS)	X				X	X	X	X
Potassium (K)	X	X	X		X	X	X	X
Serum glutamic oxaloacetic transminase (SGOT/ AST)	X		X		X	X	X	X
Serum glutamic pyruvic transaminase (SGPT/ ALT)	X		X		X	X	X	X

Sodium (NA)	X	X	X		X	X	X	X
Total bilirubin (TBIL)	X	X	X		X	X	X	X
Total protein (TP)	X	X	X		X	X	X	X
Activated partial thromboplastin time (APTT)		X			X	X	X	X
Prothrombin time (PT)		X			X		X	X
Fibrinogen					X			

[a] "X" indicates parameters that were evaluated.

TABLE 4

Retained Tissue List for Subchronic Toxicity Studies with Surfactants in Rats and Dogs[a]

Tissue	AA			PE	AAD-1		SA		AAD-2
	3-Month Rat	3-Month Dog	1-Gen Repro Rat	3-Month Rat	3-Month Rat	3-Month Dog	3-Month Rat	3-Month Dog	1-Month Rat
Adrenals	X	X		x	X	X	x		x
Aorta	x	x		x	x	x	x		x
Bone and marrow	x	x		x	x	x	x		x
Brain	x	X	X	x	x	X	X		x
Caecum	x	x	x	x	x	x			x
Coagulating glands			x						
Colon	x	x	x	x	x	x	x		x
Duodenum	x	x	x	x	x	x	x		x
Epididymides	X	x	X	x	x	x			x
Esophagus	x	x	x	x	x	x	x		x
Eyes	x	x		x	xx	x	x		x
Gall bladder		x				x			
Heart	x	X		x	X	x	XX		x
Ileum	xx	x	x	x	x	x	x		x
Jejunum	xx	x	x	x	x	x			x
Kidneys	X	XX	X	XX	XX	X	XX		X
Liver	X	XX	X	XX	XX	X	XX		X
Lungs (with mainstem bronchi)	x	x		xx	xx	x	x		x
Lymph nodes (mesenteric, submandibular)	xx	x		x	x	x	x		x
Mammary gland		x	x			x			
Muscle	x	x		x	x	x	x		x
Ovaries	x	X	X	xx	x	X	x		X
Oviduct			x						
Pancreas	x	x		x	x	x	x		x
Pituitary	x	X	X	x	x	x	x		x
Prostate	x	Xx	X	x	x	x	x		x
Rectum	x	x	x	x	x	x			x
Salivary gland	x	x		x	x	x	x		x
Sciatic nerve	x	x		x	x	x			x
Seminal vesicles	x	x	x	x	x				x
Skin with mammary tissue	x	x		xx	x	x	x		X
Spinal cord	x	x		x	x	x			x
Spleen	x	x		X	X	x	x		X
Stomach	x	x		x	x	xx	x		x
Testes	X	X	X	X	X	X	X		X

Thymus (if available)	x	x	X	x	x	x	x		X
Thyroids/parathyroids	x	X	X	X	X	X	x		x
Trachea	x	x		x	x	x	x		x
Urinary bladder	x	x		x	x	x	x		x
Uterus (corpus and cervix)	x	x	X	xx	x	x	x		x
Vagina			x	xx		x			
Vas deferens			x						
Gross lesions	x	xx	x	x	x	xx			x

[a] All tissues from the control and high doses that were examined microscopically are denoted by an "x.". A "xx" indicates that tissues from intermediate dose levels that were examined. Capital letters ("X" or "XX") indicate that organs were weighed in addition to being examined microscopically.

TABLE 5
Exposure Concentrations and Dose Levels in Three-Month Rat Feeding Studies with Surfactants

	AA		PE		AAD-1	
#/sex	10		10		10	
Target	Males	Females	Males	Females	Males	Females
(ppm)	(mg/kg bw/day)		(mg/kg bw/day)		(mg/kg bw/day)	
0	0	0	0	0	0	0
20					1.18	1.45
100					5.84	7.44
250			15.7	18.9		
500	33.0	39.9				35.4
800						
1000			63.0	77.7	58.9	68.9
1500	99.3	123.1				
2000						
3000			181	218	174	
4500	292	257				
5000						

TABLE 6
Three-Month Dog Studies with AA and AAD-1 – Urinalysis Determinations[a]

	AA	AAD-1
Volume	x	x
Specific gravity		x
pH	x	x
Protein	x	x
Glucose		x
Ketones		x
Bilirubin	x	x
Blood	x	x
Urobilinogen		x
Appearance	x	x
Albumin	x	
Color	x	
Refractive indices	x	
Creatinine and creatinine clearance	x	
Microscopic examination of sediment		x

[a] An "x" indicates a parameter that was evaluated.

TABLE 7
Dosage Levels (mg/kg/day) Used in the Rat Developmental Toxicity Studies with Three Surfactants

AA	PE	AAD-1
0	0	0
15	15	
		25
	50	
		75
100		
	150	150
300		

TABLE 8
Exposure Concentrations and Dosage Levels Used in the One-generation Rat Reproduction Study with AA

	F_0		F_1	
Target Dose (ppm)	Males	Females	Males	Females
0	0	0	0	0
100	5.5	6.7	5.0	6.0
300	16.6	19.5	14.9	18.9
1000	56.1	66.6	52.8	64.9

TABLE 9
Summary of Results from the Genotoxicity Assays Conducted with Surfactants

Test Substance	Ames	*In vitro* cytogenetics	*In vivo* mouse micronucleus
AA	Not genotoxic		Not genotoxic
PE	Not genotoxic	Not genotoxic	Not genotoxic
AAD-1	Not genotoxic	Not genotoxic	
AAD-2	Not genotoxic	Not genotoxic	Not genotoxic
SA	Not genotoxic		

TABLE 10
No-Observable Adverse Effect Levels (mg/kg/day) for Toxicological Endpoints in Studies with Surfactants

	AA		PE		AAD-1		AAD-2		SA
Type of Study and Species tested	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Males**	**Females**	**Males and Females**
Rat, 1-month feeding	51.7	159.9	71.6	172	75.6	8.3	42.6	47.8	
Rat, 3-month feeding	33	39.9	63	77.7	58.9	35.4			
Rat, 1-month gavage									100
Rat, 3-month gavage									100
Dog, 1-month capsule					30				< 50
Dog, 3-month capsule	<30				10				80
Rat Maternal Toxicity	15		50						
Rat Developmental Toxicity	300		150						

TABLE 11
Systemic Dose and Lifetime Average Daily Dose Calculations for Surfactant Systems Based on an Extrapolation from Acquavella *et al.*, 2004

Glyphosate			Surfactant		
Maximum systemic dose (mg/kg bw)		Maximum actual dermal dose (mg/kg bw)[1]	Extrapolated maximum actual dermal dose (mg/kg bw)[2]	Extrapolated maximum systemic dose (mg/kg bw)[3]	LADD[4] (mg/kg bw)
Farmers	0.004	0.133	0.033	0.033	0.001
Spouses	0.00004	0.00133	0.00033	0.00033	
Children	0.0008	0.02667	0.00677	0.00677	

[1] Maximum systemic dose/3% (glyphosate dermal uptake = 3%)
[2] Maximum actual dermal dose (glyphosate) / 4 (glyphosate :surfactant ratio = 4 :1 in formulation)
[3] Extrapolated maximum dermal dose (surfactant) x 100% (dermal uptake surfactant = 100%)
[4] Lifetime Average Daily Dose - extrapolated maximum systemic dose (surfactant) x 20 x 40/ (365 * 70)