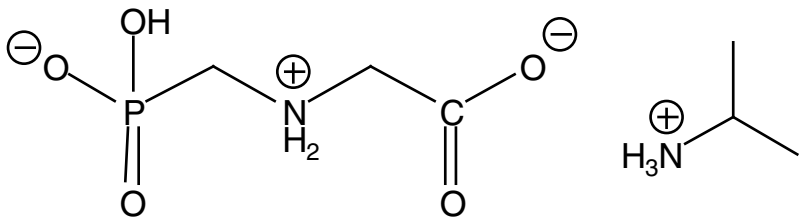
Chapter 3 — Glyphosate



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3.1 Introduction

Glyphosate is a post-emergent, non-selective phosphonoglycine herbicide widely used on crops, in forestry, and in residential settings. Glyphosate kills plants by restricting the shikimic acid pathway that allows plants to synthesize phenolic compounds and amino acids. Disruption of this pathway inhibits photosynthesis, cellular growth, and nucleic acid production. This mechanism of toxicity is operative in plants but not in animals.

Glyphosate was first registered as the isopropylamine salt in 1974. Ninety million pounds of glyphosate were applied to US crops and forests in 2001, making it the most heavily used pesticide in the agricultural sector.[1] An additional 18–23 million pounds were used in home gardens and for governmental uses in 2001. Since 2001, glyphosate use has increased in both annual application rates and in fraction of use compared to other herbicides. In California, where more recent data are available, use of glyphosate and its salts increased 59% between 2001 and 2007, largely due to an increasing number of acres planted in glyphosate-tolerant, genetically modified crops.[2] Glyphosate is currently registered for use in the European Union and was re-evaluated by the EU most recently in 2001.[3]

This chapter focuses on the human toxicity, ecotoxicity, and environmental fate of glyphosate, drawing from the United States Forest Service's Human Health and Ecological Risk Assessment 2003[4] (USFS 2003), US EPA's Re-registration Decision 1993[5] (RED 1993), and the EPA Ecotox database[6] (Terretox for the terrestrial database and AQUIRE for the aquatic database), the World Health Organization (WHO) glyphosate evaluation,[7] and the California Office of Environmental Health Hazard Assessment Public Health Goal evaluation for glyphosate in drinking water.[8] An extensive survey of the peer-reviewed literature was conducted to find additional research results not available in these documents.

3.1.1 Effects of Toxic Surfactants in Formulated Products

Surfactants are often added to herbicide products to increase the absorption of the active ingredient and may contribute to their toxicity.[9] The presence of such "inert" ingredients is especially relevant for glyphosate products, in which one of the commonly used "inerts"—polyoxyethyleneamine (POEA)—has been found to be more toxic than glyphosate itself. Manufacturers are not required to provide the specific identity and concentrations of each "inert" ingredient in a pesticide formulation. Without such information, it is difficult to determine the potential contribution of the inert ingredient to the toxicity of the marketed product.

In an effort to distinguish the relative toxicities of various glyphosate formulations, the USFS compared 35 of the most common glyphosate products (see Appendix 3a in reference 4), many of which contain POEA. Polyoxyethyleneamine (POEA) surfactants are not under consideration for use in this project due to their known high toxicity to aquatic organisms; therefore, this risk assessment does not present a comprehensive discussion of all glyphosate products. Much of the toxicity data, especially the peer-reviewed literature, focuses on commercial formulations of glyphosate, with particular attention given to Roundup. Restricting this report only to products like Aquamaster, which contains only glyphosate and water (the herbicide being considered for use by MMWD), or even technical-grade glyphosate, would discard a wealth of data on glyphosate toxicity. We take an intermediate position in this risk assessment: *focusing* on studies

that use glyphosate with no surfactant, but also including studies that in which products with surfactants are used for perspective. Every attempt is made to be clear about what substance is being referred to in the text and Appendix tables. “Technical grade” glyphosate refers to glyphosate without surfactants.

No surfactants are present in the Aquamaster product, but the label does recommend mixing the product with a surfactant prior to use. MMWD is considering two surfactants—Sylgard 309, a silicone-based non-ionic surfactant—and Competitor, which contains ethyl oleate and alkyl ethoxylates. The use of glyphosate with a surfactant may change the risks associated with glyphosate use, but both Competitor and Sylgard 309 are less acutely toxic to aquatic organisms than POEA and do not contain any known endocrine disrupting compounds. See Chapter 8 for more information about these surfactants.

3.2 Glyphosate Toxicity to Humans and Levels of Concern

The herbicide glyphosate (N-(phosphonomethyl)glycine) is an organophosphorus compound. Unlike the more toxic organophosphorus insecticides, glyphosate is not an inhibitor of cholinesterase. It affects amino acid metabolism in plants and bacteria through a pathway that does not exist in humans or animals.

There are large amounts of data available on potential acute and chronic health effects related to human exposure to glyphosate, in contrast to the less widely used herbicides triclopyr and clopyralid—the other conventional herbicides proposed for use by MMWD. The glyphosate data encompass a much larger variety of exposure scenarios and target populations, including farmers, applicators, and gardeners, and often include women and children. In contrast, triclopyr and clopyralid research focuses on short-term studies of male forestry workers exposed for three months or less per year. Most of the toxicity data used by US EPA for assessing risk to humans is from animal studies, which are summarized in Section 3.3.

In spite of the large quantity of glyphosate used and widespread human exposure, serious poisonings are rare primarily because glyphosate is not well absorbed through the skin or by inhalation, the most common routes of exposure. The low absorption rate also accounts for the low to non-detectable levels found in the urine in biomonitoring studies. Most reported acute illnesses involve irritant contact effects on the skin and the eyes. Glyphosate is well absorbed when swallowed, and all the reports of serious illness and deaths are from accidental or suicidal ingestions.

There are fewer studies available of potential chronic health effects related to glyphosate. Some have shown an increased risk of cancer and adverse reproductive outcome in workers with the highest exposure. These risks are not especially relevant to the general public not involved in conducting herbicide applications. As opposed to workers, the general public will probably be exposed to low levels of glyphosate only a few times per year. Other factors to consider when assessing the risk of cancer and other chronic effects of glyphosate, even in highly exposed workers, are concurrent exposure to other pesticides, lack of direct exposure data, and the influence of other factors known to be related to the disease being studied.

As discussed below, because of the small number of reported cases, the findings in the reported studies do not suggest that glyphosate exposure is a major risk factor for cancer or adverse reproductive outcome. More precise studies and longer terms of exposure with a larger number of cases are needed to determine the risk more reliably.

There is only one epidemiological study of neurological disease and glyphosate, related to Parkinson's disease, in which no increase in risk was found. Since occupational herbicide exposure is a known risk factor for Parkinson's disease,[10] and glyphosate is one of the most widely used herbicides, further study would be necessary to confirm these findings.

The epidemiological studies presented in this section vary in their ability to distinguish statistically significant effects. Those with large study populations like the Agricultural Health Study provide the most definitive results, but even these studies will typically require followup to confirm the findings. Some of the studies presented here are confounded by factors such as exposures to multiple pesticides or suffer from poor study design. Some of the findings are not statistically significant. No definitive conclusions can be drawn from these studies, and further study would be required to clarify the results. These studies are included because even the non-statistically significant results may provide useful information necessary to better understand links between exposures and disease and also provide information on the strength of any associations.

3.2.1 Health Effects

The following health summary of the adverse health effects of glyphosate exposure in humans is based on data from scientific journals, state and federal regulatory agencies and other primary sources.

3.2.1.A Acute Effects—Skin

Exposure to glyphosate formulations can cause skin irritation, and contact or allergic dermatitis. Most skin problems are of the irritant type. Severe skin burns are rare. Surfactants in formulated products, including polyoxyethyleneamine (POEA) and other inert ingredients, may cause or enhance the irritant properties of glyphosate.[11]

SENSOR reports: The following selected incidents are examples of skin injuries implicating glyphosate.

- After a 49-year-old woman was splashed with a glyphosate herbicide, her skin peeled and flaked for over three months. Michigan 2001-2003.
- A 20-year-old male in a warehouse store was stocking shelves when a bag of glyphosate-containing herbicide broke onto his arms, causing redness and irritation. Michigan 2005.
- A 58-year-old female licensed applicator wearing appropriate protective equipment developed redness of her face and neck a few hours after applying glyphosate; it was thought to be an allergic reaction. Washington 2004.
- A three-year-old boy developed skin symptoms while in a churchyard with a worker mowing the grass and applying glyphosate for spot treatment of weeds. The child reportedly followed the applicator around the churchyard. Washington 2004.

- A 23-year-old male landscaper assistant applying a weed control product with a hand sprayer, developed skin symptoms on his extremities. He was diagnosed with an apparent allergic reaction. Washington 2004.

Of 815 glyphosate-related calls to the Cal-EPA PISP over a 15 year period (1982-1997), 250 involved skin irritation (63 percent) without systemic symptoms.[82] In a case report, a 78 year old woman developed extensive chemical burns on her trunk and legs caused by accidental contact with a glyphosate-surfactant formulation. The lesions healed in four weeks without scarring.

A study of 366 banana plantation workers in Panama found two glyphosate positive patch tests (among 37 tested), indicating allergic sensitivity.[12]

A case of occupational allergic photocontact dermatitis was thought to be from the inert ingredient benzisothiazolin-3-one[13, 14] in a glyphosate product.[15]

3.2.1.B Acute Effects—Eyes

Eye injury from glyphosate can result in redness, tearing, burning, conjunctivitis, and damage to the cornea. Spray mist can cause oral or nasal discomfort, an unpleasant taste in the mouth, tingling and throat irritation.

Of 1,513 glyphosate-related calls to a poison control center in the U.S. from 1993 to 1997, transient eye symptoms occurred in 70 percent. Temporary injury occurred in two percent, one of which took more than two weeks to resolve. In no instance did exposure result in permanent change to the structure or function of the eye.[16]

SENSOR reports: The following selected incidents are examples of eye injuries implicating glyphosate.

- A female pesticide applicator for a lawn service company sprayed some glyphosate herbicide onto her face and eyes while fixing a pump. She had burning, and tearing of her eyes which were swollen shut for about a week. The tearing lasted almost a year. Michigan 2006.
- A 21 year-old man fixing a glyphosate sprayer without wearing gloves touched his eye, which became red, and he felt burning, dryness, and itch. He was diagnosed in an ER with a corneal abrasion. Michigan 2004.
- A 25 year-old male landscape employee wearing eye protection got glyphosate in his eye, requiring medical attention for corneal irritation. Washington 2004.
- A 19 year-old man applying glyphosate to the family garden had eye irritation and burning, which began about two hours after wind gusts blew spray onto his face. It resolved in 24 hours. Washington 2005.
- A 2 year-old boy sprayed himself in the face with a "ready to use" herbicide container at home. His mother immediately washed him off and ran water over his eyes for 3 min. His eye irritation resolved before he was taken in for a medical exam the next day. Washington 2002.
- A 45 year-old male home owner splashed a glyphosate product in his eye while applying it to his lawn. He was not wearing eye protection as recommended by the product label. He flushed his eye, but sought medical care for persistent irritation. Washington 2003.

- A 58 year-old male correctional officer was escorting an applicator spraying weeds on prison grounds when spray from the boom hit his face. He sought treatment for eye and respiratory symptoms. Washington 2001.

A study from New Zealand reported periorbital edema and chemosis (swelling of the conjunctiva, the tissue that lines the surface of the eye), and prolonged skin irritation related to glyphosate. [17]

3.2.1.C Acute Effects—Asthma

Exposure to pesticides can trigger or exacerbate asthma, induce bronchospasm, and increase bronchial hyperreactivity. Glyphosate has not been shown to be an independent risk factor for asthma, i.e. to cause asthma. There was one report in the SENSOR data of glyphosate triggering an attack in a woman with a known history of asthma.

- A 44 year old woman was spraying glyphosate in her rose garden when a gust of wind blew into her face. She immediately became symptomatic, was put into a shower by her husband, became worse and was taken to the ER. She had a history of asthma and the label apparently had cautionary comments for people with that condition. Washington 2004.

3.2.1.D Acute Effects—Systemic Poisoning

Systemic poisoning occurs when a toxic chemical enters the blood stream and is carried throughout the body, adversely affecting internal organs and body systems. Since glyphosate is poorly absorbed through the skin and inhalation is not a major route of entry, adverse effects resulting in life-threatening illness and death are related to accidental or suicidal ingestion.

It is not always clear in reported cases whether concentrated or dilute formulations were ingested. Nor is the contribution of inert ingredients in the product usually reported; when inert ingredients are known, POEA and other surfactants are most often implicated. There were no fatalities from accidental ingestion in the reported cases, and all deaths were suicides. [18,19,20,21,22,23,24]

Ingestion of glyphosate products affects the upper gastrointestinal tract, resulting in erosion injury to the esophagus, stomach, and duodenum[25] and can cause severe laryngeal injury.[26] Swallowing more than 85 mL of the concentrated formulation is likely to cause significant toxicity in adults. Moderate symptoms were associated with swallowing 20 to 500 mL, mild symptoms with 5 to 150 mL, and no symptoms with 5 to 50 mL. The mean dose for those who died was 330 mL, and for the survivors 122 mL.[27] People older than 40 years of age who ingest more than 150 mL may be at the greatest risk of a fatal outcome.[28] The minimum lethal dose has not been established.

3.2.1.E Chronic Health Effects—Cancer

The EPA classified glyphosate as 'Group E: Evidence of Non-Carcinogenicity for Humans' in 1991. The 'E' group was used "for agents that show no evidence for carcinogenicity in at least two adequate animal tests in different species or in both adequate epidemiologic and animal studies." [29] This classification system is no longer in use, but glyphosate will retain the older rating until EPA re-evaluates the chemical. See Section 2.2.6.A for more information on cancer classifications.

Since the 1991 EPA classification, there have been several epidemiological studies of glyphosate exposure as a risk factor for cancer in humans. All of the studies are in men who use pesticides regularly—farmers or other applicators. Some of these studies show an increased risk of cancer in those with the highest exposure. These risks are unlikely to be relevant to consumers who are exposed to back yard amounts of glyphosate or less. Because the glyphosate applicators in these studies were also exposed to other pesticides and their direct exposure was not measured, it is uncertain if the cancer found in these workers is due to glyphosate, another pesticide, or perhaps the influence of some other factors related to cancer that were not included in the study.

As discussed below, because of the small number of reported cases, the findings in the reported studies do not mean that glyphosate exposure is a major risk factor for cancer and adverse reproductive outcome, only that more precise studies and longer terms of exposure with a larger number of cases are needed to determine whether or not the results are reliable.

Sweden: A case-control (retrospective) study conducted in Sweden from 1993 to 1995 reported a non-statistically significant 230 percent increase in risk (Odds Ratio, or OR = 2.3, Confidence Interval, or CI = 0.8–2.2) of non-Hodgkin lymphoma (NHL) associated with exposure to glyphosate.[30] See Section 2.2.7.E for more on statistical significance. The data are not particularly robust, with findings based on only four cases and three controls, and the data were not adjusted for concurrent exposure to other pesticides. In a multivariate analysis including exposure to both glyphosate and phenoxyacetic acid herbicides, a non-significant 580 percent increase was found for glyphosate (OR 5.8, CI 0.6–54). The study design and findings were criticized by scientists affiliated with the Monsanto Company, manufacturer of glyphosate.[31]

In 2002, the same Swedish investigators performed a pooled analysis of the NHL study described above, with the results from a previous 1998 case-control study of hairy cell leukemia, thought to be a variant or rare subtype of NHL.[32, 33] In the pooled analysis, a statistically significant 304 percent increase in risk (OR 3.04, CI 1.08–8.52) associated with glyphosate was found on univariate analysis (using only glyphosate exposure as a variable), but not on multivariate analysis (including glyphosate exposure plus other potential contributing factors). Increased risk was also found for a large number of other pesticides and occupational exposures.

Monsanto was also critical of this study, stating “the fact that virtually every tested factor proved positive—inconceivable biologically—speaks to a simpler interpretation, namely differential reporting by cases and controls”. [34] This differential memory of exposure in which people who are ill (cases) are much more likely to remember specific exposures than healthy people (controls) is known as ‘recall bias’.

Canada: In 2001, Canadian investigators reported the findings of a much larger case-control study of 571 new cases of NHL among men in a diversity of occupations, conducted from 1991 to 1994 in six provinces (British Columbia, Manitoba, Ontario, Quebec, and Saskatchewan). Based on “ever” versus “never” exposed to glyphosate, a non-significant 20 percent increase in risk of NHL was found (Risk Ratio, or RR = 1.20, CI 0.83–1.74), based on 51 cases and 133 controls. A significant dose-response was found with a 212 percent increase in risk (RR 2.12, CI

1.20–3.73) among those exposed to glyphosate at least ten days a year compared to those never exposed, based on 23 cases and 36 controls.[35]

NIH: In 2003, pooled data from three National Institutes of Health (NIH) sponsored case-control studies of farmers in Iowa, Kansas, Minnesota and Nebraska, found a significant 210 percent increase in risk (OR 2.1, CI 1.1–4.0) of NHL associated with ever having used glyphosate, after adjusting for exposure to other pesticides.[36]

Agricultural Health Study: In 2001, the results of an investigation of glyphosate and cancer in The Agricultural Health Study (AHS) cohort was reported. The AHS, a prospective study of a 1993 to 1997 cohort of 57,311 private (farmer) and commercial pesticide applicators in Iowa and North Carolina, is sponsored by the Occupational Epidemiology Branch of the NIH's National Cancer Institute.[37]

Among the 40,376 applicators who ever used glyphosate and 13,280 who never used it, 92 cases of NHL were found. No associations were found between NHL and glyphosate exposure, based on cumulative days and intensity of exposure. Nor were any associations found when comparing the highest with the lowest quintile of exposure, or when comparing ever versus never exposed, or by state of residence.

The study did suggest an association between glyphosate and another cancer—multiple myeloma (cancer of plasma cells in the bone marrow). Although the myeloma cases were sparsely distributed among the participants, the highest increased risks were observed at the highest exposure levels. A significant 660 percent increase was found in the upper quartile versus never exposed (RR 6.6, CI 1.4–30.6) with a significant trend across quartiles ($p = 0.01$). Risk ratios were elevated, but not statistically significant, across cumulative days of low (RR 2.3, CI 0.6–8.9) and medium exposure (RR 2.6, CI 0.6–11.5), and borderline significant in the high exposure category (RR 4.4, CI 1.0–20.2), with a non-significant trend ($p = 0.09$). Elevated risks were found in both Iowa (RR 2.6) and North Carolina (RR 2.7).

There were only 19 myeloma cases, and the authors discussed the possibility that the association could be related to bias in the analysis. The number of subjects in the entire group with exposure to glyphosate (unadjusted) was larger than the number of subjects in the group that considered other variables in addition to glyphosate exposure (adjusted). A positive association was found only among the smaller group. Both groups were similar in their use of glyphosate (75.9 percent versus 74.5 percent).

There was no increase in the risk of 'all cancers combined'. The 30 to 60 percent elevated risk for colon, rectal, kidney, and bladder cancer was not statistically significant. Elevated risks of leukemia and pancreatic cancer were observed only for the moderate exposure groups but no increase was found in the highest exposure group.

A significant trend for decreased risk of lung cancer was suggested for the highest exposure group compared to the other exposure, but this trend was not found with comparison to the never exposed group.

In spite of the cohort's size, the large number of participants reporting glyphosate use, and the prospective design, the authors acknowledge their data have limitations. The number of specific cancers occurring during the follow-up period was small; and the analyses provide no information on the timing of pesticide use in relation to disease. This limits defining latency periods (the time from first exposure to the development of cancer) or effects resulting from glyphosate exposure at different ages.[38]

A major difficulty in interpreting results of the available cancer studies is lack of data on direct exposure and concurrent exposure to other pesticides in addition to glyphosate. Without additional better-designed studies and future updates of the important AHS cohort data, whether or not glyphosate poses a significant risk of cancer in humans remains an open question.

3.2.1.F Reproductive Effects

Animal toxicology data do not indicate that glyphosate would be a reproductive toxicant. There are no human studies of glyphosate as an independent risk factor for adverse reproductive health effects. Three reports of reproductive outcome related to glyphosate exposure are from the Ontario Farm Family Health Study (OFFHS), one on spontaneous abortion [39], one on fecundability (fertility) [40], and another on paternally mediated effects.[41] A discussion of these studies in which glyphosate exposure was investigated along with several other pesticides follows.

The OFFHS is a retrospective study of a 1991 to 1992 cohort of farmers and their spouses (women 44 years of age or younger) conducted in Ontario, Canada from 1991 to 1992. Eligible participants lived year round on a farm, and at least one member of the couple worked on the farm. Questionnaire information (by mail and telephone for non-respondents) was collected on current pesticide use and practices on the farm and in the yard for the past five years, and a lifetime reproductive history.

Included in the data collection and analyses were eight other pesticides besides glyphosate (atrazine, captan, carbaryl, cyanazine, 2,4-D, 2,4-DB, dicamba and MCPA); four chemical groups (phenoxy acetic acids, triazines, organophosphates, and thiocarbamates); and four usage groups (herbicides, fungicides, insecticides and miscellaneous). Only results related to glyphosate are discussed in this summary.

Spontaneous Abortions: Women were asked to recall all spontaneous abortions, and the number of weeks into the pregnancy when it occurred (based on the last menstrual period). Glyphosate exposure was asked about in three time periods: pre-conception (three months before and up to the month of conception), early post-conception (less than 12 weeks gestation) and late post-conception (12 to 19 weeks gestation).

In the OFFHS cohort, 395 spontaneous abortions among 2,110 women with 3,936 pregnancies were found (10%). For late abortions, pre-conception exposure to glyphosate was associated with a borderline significant 170 percent increase in risk (OR 1.7, CI 1.0–2). See Section 2.2.7.E for more on statistical significance. Among older women exposed to glyphosate, there was a non-significant 320 percent increase in risk (OR 3.2, CI 0.8–23.0) compared to unexposed women of the same age. The authors did not adjust the data for other factors related to early fetal loss,

including maternal age, smoking, history of previous spontaneous abortion, and exposure to other pesticides.[39]

Fecundability: Fecundibility related to glyphosate exposure was investigated in 2,012 planned pregnancies among 1,048 couples in the OFFHS cohort. Exposure was defined as glyphosate use on the farm during the months of trying to conceive or at any time during the prior two months. Approximately one-third of conceptions had occurred more than ten years before the questionnaire date. The data were adjusted for maternal and paternal age, alcohol use, smoking, and prior oral contraceptive use.

A non-significant 39 percent decrease in conditional fecundability (Fecundity Ratio, or FR = 0.61, CI 0.3–1.26) related to women's glyphosate exposure, regardless of men's was found based on 32 cases. Men's glyphosate exposure showed a significant 30 percent increase in fecundability (FR 1.30, CI 1.07–1.56) based on 175 cases.

In addition to recall bias (*vide supra*) the authors acknowledge that selection bias is a problem with this study, since only women who ultimately became pregnant were included, and "more severely subfecund couples who may have become pregnant by the time of data collection are underrepresented". This would bias the study towards the null, finding no effect, "even if pesticide exposure is associated with sterility or extremely long prolonged time to pregnancy."[40]

Paternally mediated effects: Spontaneous abortion, preterm birth (before 37 weeks), small for gestational age (birth weight below the 10th percentile) and sex ratio (proportion of males among singleton births), were investigated in the offspring of men with direct exposure to pesticides (mixing and applying) in the OFFHS cohort. In a total of 3,984 pregnancies (1,548 live births) there were 375 spontaneous abortions, 276 small for gestational ages, and 128 pre-term births.

Of the small number who had glyphosate exposure, there was a non-significant 50 percent increase in risk of spontaneous abortion (OR 1.5, CI 0.8-2.7) based on 17 cases; a non-significant 240 percent increase in preterm delivery (OR 2.4, CI 0.8-7.9) based on five cases; and a non-significant 20 percent decrease in risk of small for gestation age (OR 0.8, CI 0.2-2.3) based on 5 cases. There was no association with sex ratio.[41]

Birth Defects: An ecological study in California of neural tube defects (NTD) and maternal residence within 1,000 meters of pesticide applications around the month of conception was investigated in two birth cohorts, one in 1987, and another from 1989 to 1991. A geographic model linking California Pesticide Use Reports (PUR) of 59 different pesticides and land-use survey maps was used in the data analysis.[42]

It is noted that the pesticide use reporting system on which the study was based did not require mandatory reporting of all pesticide use until 1990, and the new system was not fully operational until the 1992 reporting year.

Of 315 NTD cases and 652 controls delivered in 1987, and 613 cases and 611 controls delivered between 1989 and 1991 in all California counties, there were 45 cases (4.8 percent) and 33

controls (2.6 percent) exposed to glyphosate. There was a non-significant 40 percent increase in risk (OR 1.4, CI 0.8, 2.5) of NTDs when controlling for exposure to other pesticides.[42]

Neural tube defects (NTDs) include anencephaly in which the infant is born without the forebrain and cranium, and the more common and less severe spina bifida in which herniation of spinal cord contents occurs due to imperfect formation of the vertebrae. A deficiency of the micronutrient folic acid (folate) is the biggest known risk factor for NTDs and perinatal supplementation (including enrichment of flour) has resulted in a marked reduction in this defect in the United States and throughout the world.[43]

In a 2002 Minnesota study of licensed pesticide applicators and their families, a statistically significant association emerged between glyphosate use by parents and neurobehavioral defects (ADD/ADHD) in children, with an odds ratio of 3.6 (CI 1.3–9.6).[44] The authors of this study classify the association as "tentative" and note that:

> *In vitro studies by our group show that this product was not genotoxic in the micronucleus assay and did not have significant pseudoestrogenic effects in MCF-7 cells. In a recent review of the toxicology of glyphosphate, little if any evidence of neurotoxicity was noted other than by intentional ingestion.*

Direct exposure data was not available in any of the above studies, and there was simultaneous exposure to other pesticides in addition to glyphosate. Based on these findings, it is an open question whether or not glyphosate poses a significant potential risk of reproductive harm to humans.

3.2.1.G Chronic Health Effects—Neurological

There are no reports of permanent neurological effects related to glyphosate exposure except for a 2001 case report from Brazil. A 54-year old man with extensive overexposure to a glyphosate product while spraying his garden, developed severe eye effects and skin burns which resolved. One month later he developed hand tremors, and two years later was diagnosed with Parkinsonism, and responded to treatment with Levodopa.[45] It is most likely this was coincidental, and no further cases of Parkinson's or other neurological disease related to glyphosate have been reported.

In a recent study of self-reported Parkinson's disease in the Agricultural Health Study cohort, no associations were found related to glyphosate exposure for either prevalent (OR 1.0, CI 0.6-1.7), or incident cases (OR 1.1, CI 0.6–2.0) of the disease.[46]

3.2.1.H In Vitro Human Data

In addition to the epidemiological studies already described, there are other studies of the effects of glyphosate in human cells, and human cell lines. *In vitro* tests in isolated cell cultures provide information on the potential for adverse effects in the whole organism, although these kinds of tests do not account for the systemic processes that may serve to accentuate or moderate the effect in the whole organism. *In vitro* tests of glyphosate show no significant effects of glyphosate on DNA damage, sister chromatid exchange, and cytokine production, but Roundup was found to alter steroid receptor gene expression.

The comet assay was used to study 24 people (23 females) age 17 to 55 years old (mean age 38 ± SD 12.2) with bystander exposure to aerial spray of a glyphosate product being used in Ecuador for drug crop eradication. The comet assay tests for DNA damage in individual cells by using electrophoresis and fluorescence microscopy. Undamaged DNA retains a compact, highly organized association with matrix proteins in the nucleus. This structure is essential for cell division and proper protein coding. When damage occurs, this configuration is disrupted, creating DNA fragments of varying sizes and structures. These fragments are of non-uniform length and move through an electrically charged gel (technique known as electrophoresis) at varying speeds, creating "lines" or "tails" on the gels . Cells containing greater levels of DNA strand break damage generate comets with more intense 'tails.' Twenty-one unexposed controls were included (18 females) including four teenagers, and one person aged 71 (mean age 33 ± SD 15). The blood samples were not drawn until two weeks to two months after exposure. The authors state that neither the cases nor the controls smoked tobacco, drank alcohol, took non-prescription drugs or had been exposed to other pesticides. Comet length of 35.5 µm in those exposed was greater than the 25.94 µm in the controls.[47] It is difficult to interpret this study, since the authors do not provide sufficient information on how the study participants were selected, and did not apply any test of statistical significance.

Chromosome aberrations, sister chromatid exchange (SCE), and mitotic index were studied in peripheral lymphocytes from three healthy donors exposed *in vitro* to different concentrations of glyphosate (atrazine and vinclozolin were also evaluated). Glyphosate produced a dose-related increase in the percent of aberrant cells and an increase of SCE per cell, but did not affect the mitotic index.[48]

In a study of the induction of SCE in human lymphocytes *in vitro*, glyphosate required much higher concentrations to produce an effect, than the other two pesticides studied (the fungicides captan and fenaminosulf).[49]

Human peripheral blood mononuclear cells were exposed to different concentrations of Roundup and technical grade glyphosate for 24, 48, 72, and 96 hours. The Roundup formulation was more cytotoxic (cell damaging) with an LC_{50} (50% lethal concentration) of 56.4 µg/mL at 24 hours, compared to 1,640 µg/mL for technical grade glyphosate. [50]

An investigation of the effects of glyphosate on cytokine production by human peripheral blood mononuclear cells, found only a slight inhibitory effect at the highest concentration (1000 µM). TNF-alpha and IL-1 beta production were not affected. The author concluded that glyphosate "might be a pesticide with only a little damage to the immune system."[51]

Normal human cells (GM38) and human fibrosarcoma (HT1080) cells were exposed *in vitro* to differing concentrations of glyphosate. Cytotoxic effects were found in the normal cells at 4.0-6.5 mM and genotoxic effects at 4.0-6.5 mM. Cytotoxic effects were found in the fibrosarcoma cells at 4.75 -5.75 mM, and genotoxic effects at 4.75 -5.75 mM.[52]

DNA microarray analysis[53] was used to determine a commercial glyphosate product's capacity to alter steroid receptor gene expression in the presence or absence of 17β-estradiol (E2).

Glyphosate plus estrogen altered levels of expression of three genes – EGR1, CXCL12 and HIF1 – out of the entire battery tested.[54]

Oxidative damage to human skin cells from a Roundup formulation was studied in the keratinocyte (epidermal) cell line HaCaT. The antioxidants Vitamins C and E were found to decrease the cytotoxic effect.[55]

Genotoxic effects of oxidative stress induced by a combination of H_2O_2 (hydrogen peroxide) and glyphosate was determined in human fibroblasts. Damage to DNA was found in the presence of low amounts of glyphosate and mild oxidative stress, but glyphosate was not genotoxic when applied separately.[56]

There are two studies of glyphosate effects on aromatase, a cytochrome P450 enzyme that is essential for sex steroid hormone synthesis. (The cytochrome P450 system includes numerous proteins that metabolize xenobiotics).[57] Aromatase catalyzes the conversion of testosterone to estradiol (an estrogen), and is located in estrogen-producing cells in the adrenal glands, ovaries, placenta, testicles, adipose tissue (fat), and brain. Certain aromatase inhibitors are used as anti-estrogen drugs (e.g. Femara®) to treat breast cancer.

The effects of glyphosate and Roundup were studied by application directly on aromatase present in microsomes from human embryonic 293 cells, placental-derived JEG3 cells, and normal human placenta cells at concentrations 100 times lower than in agricultural use. Aromatase activity was enhanced by 40 percent after inclubation with Roundup but the effect was not seen with glyphosate alone.[58, 59]

3.2.1.I Levels of Concern for Humans

The acute and chronic EPA RfD of 2.0 mg/kg-day is used as the toxicity reference value (TRV) for both acute and chronic exposure scenarios. The NOAEL of 175 mg/kg-day is based on a developmental toxicity study in rabbits, where early maternal mortality was observed at the next highest dose of 350 mg/kg-day (see Section 0 below). This NOAEL was adjusted with both intra- and inter-species factors of 10 to give a value of 2.0 mg/kg-day for adults and children.

3.2.2 Routes of Exposure

Potential human exposure to glyphosate is through skin absorption, inhalation, ingestion or the eye. The US Environmental Protection Agency (EPA) classifies glyphosate as moderately acutely toxic (Toxicity Class II), and a mild irritant. The oral reference dose (RfD) determined by the EPA that is not likely to cause harmful effects during a lifetime for both adults and children is 2 mg/kg-day.[5]

Skin: As with most pesticides, the skin is the major route of exposure to glyphosate, but penetration and absorption are low.

In a study using human autopsy samples, Roundup was applied in 1:20 to 1:32 dilutions to thigh skin. Less than two percent of the applied glyphosate penetrated the skin. Twelve hours after the treatment, soap and water removed 89.6 percent of the applied dose, and water alone 83.6 percent.[60]

An *in vitro* study of glyphosate penetration into human skin found only 1.42 percent was absorbed from water. The amount absorbed decreased to 0.74 percent if the glyphosate was added to cotton sheets and immediately placed on the skin. If the cotton sheets were dried for one or two days and then applied to the skin, absorption was much lower—0.08 percent. Chemicals are absorbed more rapidly and in greater amounts if the skin is damp or sweaty. When the investigators wet the two-day dried cotton sheet with water to simulate sweating, the amount absorbed rose from 0.08 to 0.36 percent, an increase of 350 percent.[61]

In a study of 346 human volunteers, 0.1 milliliters (mL) of 41 percent glyphosate was applied to abraded and un-abraded skin of the back, and compared with applications of liquid cleaner, baby shampoo, and liquid dishwashing detergent. In those with un-abraded skin, erythema (redness) was found in only one of 24 subjects (4 percent) after 24 hours. Of 24 subjects with abraded skin, erythema was present in ten (42 percent), absent in ten, and equivocal in four (16 percent). Forty-eight hours after application, glyphosate erythema was similar to that from the liquid cleaner and the dishwashing liquid.

In the same investigation, glyphosate and baby shampoo were less irritating than either the liquid cleaner or dishwashing liquid in a 21 day cumulative irritancy assay. In a photoirritation and photosensitization study, undiluted Roundup was applied to abraded skin of the upper arm for 24 hours with UVA light applied for 45 minutes. No evidence of skin sensitization (allergic reaction) was found.[62]

Eyes: Because blood vessels in the eye are close to the surface, pesticides can enter the bloodstream through the eye and cause poisoning, but this is rare, and is not found with glyphosate. Most cases of eye injuries from glyphosate are from direct contact, or from splashes and spills.

Inhalation: Glyphosate has a very low vapor pressure (1.6×10^{-8} mm Hg), and inhalation is not a major route of exposure. A 1995 California Department of Pesticide Regulation (DPR) study of forestry workers applying glyphosate found that inhalation exposure accounted for less than 0.2 percent of exposure.[63] A study of forest workers in Finland found that exposure to glyphosate through spray droplets in the breathing zone was low, with the highest value being 15.7 micrograms/m^3.[64] No Occupational Health and Safety Administration (OSHA) permissible exposure limit (PEL) or ACGIH threshold limit value (TLV) in air has been set for glyphosate. OSHA has set an arbitrary target level of 1 mg/per cubic meter (m^3).[65]

Ingestion: Glyphosate is absorbed from the gastrointestinal tract. At doses greater than 10 mg/mL, it significantly disrupts the barrier properties of cultured intestinal cells.[66] This route of absorption is important in intentional and accidental ingestions, which are discussed in more detail on page 3-8.

3.2.3 Biomonitoring Studies

Most biomonitoring studies of glyphosate report low to non-detectable levels in the urine of farmers, forestry and landscape workers who handle glyphosate. The highest exposures were less

than 0.02 percent of US EPA's Reference Dose (RfD) and average exposure was less than 0.005 percent of the RfD.

Glyphosate is excreted unchanged in the urine, and can be detected at less than one part per billion (ppb).[67, 68] One ppb is equivalent to one millionth of a gram in one liter of urine, and can also be expressed as nanograms per microliter (ng/µL) or micrograms per liter (µg/L).

Glyphosate does not persist in body tissues and fluids as DDT and other persistent pesticides do. The amounts found are a measure of recent exposure at the time of sampling, and cannot be used to ascertain or predict past or future exposures over time. Testing biological samples for glyphosate is not available in standard medical care facilities.

NIOSH: In 2001, NIOSH sponsored a study investigating take-home pesticide exposure in farm and non-farm families in Iowa. Urine samples were collected from 47 fathers, 48 mothers and 118 children on two occasions approximately one month apart. The adjusted geometric mean (GM) of glyphosate was similar between farm and non-farm groups for the adults. Glyphosate levels in children (66 farm, 52 non-farm) were higher in the non-farm group. The amounts found were similar for boys and girls, with the highest estimated dose of 0.34 µg/kg-day.[69,70] US EPA estimate of NOAEL in laboratory test animals is 175 mg/kg-day, and the oral exposure reference dose (RfD) is 2 mg/kg-day.

Finland: In Finland, five forestry workers and five controls were monitored for urinary glyphosate levels before, during and after clearing trees using brush saws equipped with pressurized sprayers. All samples were below the limit of detection (LOD) of 0.1 ng/µL.[71]

Arkansas: Total urine excreted over a 12 week period was collected and tested for glyphosate in workers employed as applicators, weeders, and scouts at two tree nurseries in Arkansas. No positive urine samples were found (LOD 0.01 µg/mL). High rainfall, irrigation as needed, normal field dissipation and worker training were cited as contributing factors for the low exposure and absorption.[72]

Forestry: Urinary glyphosate levels were measured in 15 forestry workers, the day prior to, the day of and three days following application of the original Roundup. For 11 workers (73 percent), no glyphosate was detected (LOD 10 ppb) on any day; four (27 percent) had detectable levels only on the day of application. The highest individual amount found was 14 ppb. The systemic dose was estimated to be 0.0006 mg/kg body weight.[73]

Farm Family Exposure Study (FFES): The Farm Family Exposure Study is a biomonitoring study of 45 farm families in Minnesota and 50 families in South Carolina, conducted by the University of Minnesota and co-sponsored by CropLife America, a trade association for agricultural chemical companies (Bayer, Dow, DuPont, FMC, Monsanto, and Syngenta) and the American Chemistry Council.

To qualify for inclusion in the study, the families had to live on farms and apply one of the study pesticides (2,4-D, chlorpyrifos (Lorsban™), or glyphosate) to at least 10 acres of farm land within one mile of the family residence. Participating family members had to include the

applicator, spouse and at least one child age 4 to 17. Each participant had to agree to collect urine specimens 24 hours a day for five days. Monsanto's Environmental Sciences Research Laboratory did the glyphosate analyses, which were confirmed by the Centers for Disease Control.[74, ,75]

Glyphosate levels for farmers ranged from < 1 ppb (LOD) to 233 ppb, with a geometric mean of 3.2 ppb. There were large differences between South Carolina (SC) and Minnesota (MN) farmers. Detectable levels of glyphosate were found in 87 percent of SC farmers with a GM of 7.9 ppb, versus 36 percent in MN, with a GM of 1.4 ppb. Only 43 percent of SC farmers wore gloves when handling glyphosate, versus 96 percent in MN.

Spouses had lower urine pesticide levels than applicators or children. There was no correlation between urinary levels for spouses and the distance between the house and the treated field. Of nine children with detectable levels in their urine, eight assisted or were present for the pesticide mixing or application, and all were from SC. The highest level for a child of 29 ppb, was in a teenage boy who actively helped his father with the mixing and application. The boy's father, who did not wear gloves and spent long periods of time repairing a leaking boom sprayer, had the highest level among applicators.

The maximum systemic dose for farmer applicators was estimated to be 0.004 mg/kg, with a geometric mean systemic dose of 0.0001 mg/kg. Maximum systemic dose estimates for spouses and children were 0.00004 mg/kg. [76].

3.2.4 Pesticide Illness Reports

In pesticide poisoning surveillance systems, less than one percent to six percent of reports are related to glyphosate, most of low to mild severity involving the skin and eyes without systemic illness. All serious illness and deaths were from accidental and suicidal ingestion. There are about 100 different formulations of glyphosate, ranging from 41 percent concentrates to less than 1 percent dilute products. In many (perhaps most) incidents, the actual ingredients involved are not known, and it may be difficult to impossible for the treating physician or other health care provider to obtain the required information at the time the patient presents. See Chapter 2, Section 2.2.4 for general information on pesticide poisoning surveillance systems.

Toxic Exposure Surveillance System (TESS): Table 3-1 summarizes glyphosate-related TESS data from 2002 (the first year glyphosate cases were listed separately) to 2006. The data represent contact with a poison control center, usually by telephone, regarding a potential poisoning problem, and does not mean that poisoning or illness definitely occurred. This is reflected in the combined percentage of 'None' and 'Minor' adverse outcomes shown in the table. For glyphosate, major outcomes are those related to accidental and suicidal ingestion. Outcomes are not know for all calls to the center, and the data in the table are based on cases for which follow up information was known, usually because of treatment in a medical care facility.

Table 3-1: Calls to U.S. Poison Control Centers Related to Glyphosate Exposure 2002 to 2006

		Age			Causation			Adverse Outcome					
Year	Total Cases	< 6	6-19	>19	Unintentional	Intentional	Other	None	Minor	None + Minor	Mod	Major	Death
2006	4,496	1,133	321	2,251	3,901	51	30	920	1,111	(45%)	76	9	4
2005	4,679	1,245	378	3,017	4,380	62	24	1,085	1,159	(48%)	126	8	2
2004	4,425	1,162	360	2,872	4,146	59	18	1,024	1,154	(49%)	91	6	1
2003	4,420	1,157	340	2,875	4,109	57	33	1,059	1,167	(50%)	104	5	4
2002	4,472	1,217	397	2,814	4,191	43	20	1,087	1,144	(49%)	104	5	1

Data source: Poison Control Centers, reference 77.

SENSOR: In a summary of SENSOR occupational pesticide cases between 1998 and 1999, glyphosate accounted for 24 reports (2.4 percent), most of which were of low severity.[78] A survey using SENSOR data of young people 15 to 17 years of age, found a total of 531 acute occupational pesticide related illnesses (428 identified by TESS and 103 by eight state agencies). Glyphosate was implicated in 33 cases (6.2 percent).[79]

A survey using SENSOR and Cal-EPA data on retail employees 15 to 64 years old, found a total of 287 cases of acute pesticide related illnesses from 1998 to 2004. Seven were glyphosate-related (2.2 percent), four of low severity and three of moderate severity. The sectors with the highest rates were farm supply, hardware, and garden stores.[80]

California PISP Data: Table 3-2 shows the number of "definite, probable, and possible" glyphosate-related illnesses reported to the PSIP from 1997 (the first year glyphosate cases were listed separately) to 2006. The total number of cases is very small, from 0.93 to 2.3 percent of the total over the 10 year period. Most of the cases were of low to mild severity. There were no fatalities. [81]
A survey of 815 glyphosate related calls to the Cal-EPA PISP over a 15 year period (1982 to 1997), found that 399 (49 percent) involved topical irritation of the eye without systemic symptoms.[82]

Iowa: In Iowa, Roundup was named in pesticide incident reports in 13 of 204 incidents (6.3 percent) in 2002, in 12 of 257 (4.7 percent), in 2004, and in 15 of 471 in 2005 (3.2 percent), of which 87.8 percent were telephone calls from homes.[83]

Washington: In Washington, 28 total incidents were reported in agricultural workers, two from Roundup (7.1 percent) in 2001–2003. Of 36 incidents reported in 2005, six involved Roundup (16.6 percent). In 2004, 32 percent (133) involved exposure to glyphosate.[84]

Michigan: Michigan did not report summary data by specific pesticide. Selected incidents described in the reports are included in the "Acute Effects" section below.[85]

Table 3-2: Glyphosate-related Illnesses Reported by California Physicians To the Pesticide Incident Surveillance Program (PISP), 1997 to 2006

	Total Reported Pesticide Cases		Glyphosate Cases				
			Systemic/Respiratory		Local/Topical		
Year	Definite/Probable	Possible	Definite/Probable	Possible	Definite/Probable	Possible	Total (%)
2006	454	84	0	0	4	1	5 (0.93)
2005	767	144	1	2	5	4	12 (1.3)
2004	552	276	2	7	7	1	17 (2.1)
2003	614	188	0	0	5	5	10 (2.1)
2002	1,025	291	4	8	5	2	19 (1.4)
2001	430	186	0	4	7	2	13 (2.1)
2000	637	256	2	3	11	3	19 (2.1)
1999	830	371	6	3	8	4	21 (1.7)
1998	621	377	2	6	6	9	23 (2.3)
1997	892	427	0	4	11	6	21 (1.6)

Data source: CA DPR Pesticide Illness Surveillance Program, reference 86.

Australia: The Victorian Poisons Information Centre in Australia reported 1,235 glyphosate-related calls among a total of 136,508 (0.9 percent) from 1998 to 2002. Ingestions accounted for 521 (42.2 percent).[87]

Italy: Of 872 agricultural pesticide poisonings referred to the poison control centre of Milan from 2000 to 2001, 53 (6.1 percent) involved glyphosate.[88]

3.3 Glyphosate Toxicity to Animals and Plants and Levels of Concern

This section summarizes glyphosate toxicity to nine taxa groups, including mammals, birds, fish, amphibians, terrestrial and aquatic invertebrates, terrestrial and aquatic plants, and soil microorganisms. Because glyphosate has been used extensively in the United States since 1974, there is a great deal of information about this chemical. The studies most relevant to the MMWD watershed are discussed in detail, and summary data are provided in the Appendices for the remaining studies. This document focuses on the information necessary to assess the potential risks of glyphosate use in the MMWD watershed. Although there are still questions remaining about the sub-lethal effects of glyphosate, the bulk of the data suggests that glyphosate without the POEA surfactant is rarely more than moderately acutely toxic to any of the nine taxa groups.

For glyphosate, there are more studies available than can reasonably be summarized in this risk assessment. In 2003, the USFS performed a risk assessment for forestry use of glyphosate.

Assuming that the USFS report did a thorough job of reporting toxicological studies up to 2003, the studies from that report are included here. A literature search was done for the period between 2003-2008 and additional relevant work was included. The available data from the EPA's Ecotox database were summarized in the text and detailed in the appendices. Studies that were not easily detailed in the appendices tables were described in greater detail in this Chapter. In addition, the records in EPA's Ecotox database were compared to the studies in the combined USFS report and literature search. The goal of the comparison was to assure that no biases were present in the studies that were included in the MMWD risk assessment.

The TRVs used in the MMWD risk assessment were adjusted downward when additional data were available indicating toxicity at concentrations below the USFS TRV, or when only LC_{50} values were available instead of NOECs. This approach uses EPA methodology for assessing effects on endangered species.[89] The adjustment employed was to divide the LC_{50} by six (or 20 in the case of salmonids), based on an extensive review of existing ecotoxicological data on pesticides.[90] When experimental conditions were similar (*e.g.* fraction of the study sample affected, test system, duration, species, and other factors), the data indicated that sublethal effects (LOECs) did not occur at concentrations below one-fourth to one-sixth of an LD_{50} in a variety of fish. This effect is termed the "6x hypothesis." However, it should be noted that this review is almost 30 years out-of-date, and that the factor of six is meant to translate an LC_{50} to a NOEC *of the same species*. The use of a single NOEC for all species in a taxa group suggests that interspecies variability may not be fully accounted for by the factor of six. Unlike human RfDs, wildlife TRVs were not adjusted for inter- and intra-species uncertainty. The factor of six appears to be too low for salmonids, because their olfactory ability seems to be particularly sensitive to pesticide concentrations 20 times lower than the LC_{50}.[91]

Levels of concern for glyphosate are also summarized in this section, with Table 3-7 on page 3-52 presenting the toxicity reference values (TRVs) selected for the MMWD risk assessment and the USFS TRVs for comparison.

Aquatic species have different tolerances for technical grade glyphosate versus formulated products (like Roundup). Although the distinction between glyphosate and formulated products is somewhat less important for terrestrial wildlife, every effort was made to find toxicity endpoints that refer to glyphosate alone.

3.3.1 Mammals

Most information on the toxicity of glyphosate to mammals is derived from studies conducted on laboratory animals for registration purposes. Many of these studies are required by EPA as part of the pesticide registration process, primarily to evaluate the potential toxicity of the chemical to humans. Sources of the data are the CA OEHHA glyphosate review,[8] the US EPA RED,[5] the WHO evaluation,[7] and the USFS risk assessment.[4] Additional recent peer-reviewed studies are also covered in this section. Laboratory animal test data are summarized in Tables 3-3 to 3-6 and mammalian wildlife data from EPA's Ecotox database are summarized in Appendix D, Table D-1.

In general, glyphosate is poorly absorbed by mammals and has low acute toxicity. Glyphosate formulated with surfactants is typically 5–10 times more toxic than glyphosate alone. Long-term

exposures in rats caused salivary gland lesions, reduced body weights and reduced organ weights. There is some conflicting evidence on the occurrence of unilateral renal tubular dilation in male rats on which the older US EPA Integrated Risk Information System (IRIS) reference dose is based, and a single study found reduced sperm quality on exposure to glyphosate. There is no evidence of low-dose endocrine disrupting effects for glyphosate, but some evidence that Roundup (with POEA surfactant) may have endocrine-disrupting effects. Glyphosate was also not found to cause neurotoxicity or immune system toxicity. The chronic reference NOAEL for mammals of 175 mg/kg-day is based on reproductive effects in rabbits, where reduced fetal weights and increased maternal death were noted at the next higher dose. Because of low acute toxicity, no acute NOAEL was established by EPA, so the value of 175 mg/kg-day was used as the comparison toxicity reference value for all glyphosate exposures.

Tumors were observed at various sites in some of the high-dose and intermediate-dose animals in chronic studies. However, results did not always follow a dose-response curve, and/or were not significant relative to historical control data. The weight of the evidence supports US EPA's classification of glyphosate as "E—Evidence of non-carcinogenicity for humans."

3.3.1.A Metabolism and Pharmacokinetics of Glyphosate

Glyphosate is poorly absorbed in mammals and is excreted largely unchanged. The lack of significant absorption is a contributing factor to the low toxicity observed for glyphosate.

When Sprague-Dawley rats were given a single dose of either 10 mg/kg or 1,000 mg/kg of ^{14}C-labeled glyphosate, 97.5% of the administered dose was excreted in the urine and feces as the parent compound.[5] The timing of elimination is biphasic, with an excretion half-life for the first phase of 5.9–6.2 hours and for the second phase 79–106 hours.[92] The degradate aminomethylphosphonic acid (AMPA) was found in the urine at concentrations representing less than 1% of the total glyphosate dose, and less than 1% of the absorbed dose remained in tissues and organs, primarily in bone. A second study with ^{14}C-labeled glyphosate determined that less than 0.01% of the administered dose reaches the bone marrow, with an excretion half-life from bone marrow of 4–8 hours.[5] The magnitude of the dose (10 mg *vs.* 1,000 mg) did not significantly change the metabolism, distribution or excretion of glyphosate.

An NTP study where male rats were dosed by gavage with ^{14}C-labeled glyphosate at 5.6 or 56 mg/kg showed that approximately 50% of the radioactivity was eliminated in the feces in the first 24 hours and >90% of the radioactivity was eliminated within 72 hours.[8] Urinary elimination was complete within 12 hours.

Excretion in milk occurs at a low level in goats, with concentrations of <0.1 ppm observed in whole milk when goats were exposed to 120 ppm glyphosate in diet.[7]

Dermal absorption is also low. Dermal exposure to diluted Roundup in Rhesus monkeys resulted in 3.7–5.5 percent absorption after 12 hours of exposure.[93]

3.3.1.B Acute Toxicity of Glyphosate

Glyphosate has very low acute toxicity by the oral and dermal exposure routes, partly due to its limited absorption (see Section 3.3.1.A). In rats and mice, acute oral LD_{50} values of glyphosate

range from approximately 2,000 to 6,000 mg/kg. It is significantly more toxic by the intraperitoneal (ip) route. It is a moderate eye irritant in pure form, and substantially more irritating when formulated with POEA surfactant.

High doses of glyphosate cause hyperemia (increased blood flow), severe stress, accelerated breathing and occasional asphyxial convulsion.[8] The acute LD_{50} of glyphosate for various species by different routes is given in Table 3-3.

Table 3-3. Acute Toxicity of Glyphosate in Experimental Animals

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

(Adapted from Reference 8.)

Acute toxicity studies reviewed by WHO indicate that the LD_{50} of glyphosate is mostly above 3,000 mg/kg, with a few studies on formulated glyphosate products with LD_{50} values of ~2,000 mg/kg.[7]

Glyphosate is not a strong dermal irritant. Irritation studies conducted by Monsanto using rabbits with intact or abraded skin showed glyphosate produced a relatively low irritant response.[94] Dermal testing of a formulated glyphosate at a concentration five times higher than the normal field application rate resulted in severe local skin reaction, reduced food consumption, body weight loss, mortality, and testicular effects.[95] Eye irritation of glyphosate technical and the formulated product Shackle® was tested in rabbits. Slight irritation was reported in some animals, and the irritation disappeared after a day or more.[96]

Acute toxicity studies (24 h) on the Norway rat (*Rattus norvegicus*) demonstrate the higher toxicity of Roundup compared to glyphosate, with a NOEL of 2,000 mg/kg-day for glyphosate and 200 mg/kg-day for Roundup for diarrhea, near-zero mortality, organ weight in relation to overall weight, and general injury.[97]

3.3.1.C Sub-Chronic Toxicity of Glyphosate

Short-term exposure to glyphosate (a few months to a year), even at high doses, had no effects on the survival of test animals. The most common effect, observed at all dose levels, was salivary gland lesions. Observed effects at the higher doses tested include increased weights of liver, kidney, brain and heart; decreased thymus weight; decreased sperm density, changes in blood chemistry, and decreased pituitary weights (dogs only). See Table 3-4 for a summary of glyphosate toxicity tests.

A review of US EPA's Ecotox database for sub-chronic laboratory studies on Norway rats show

NOELs of 300–560 mg/kg-day for changes in blood chemistry, enzyme levels, and cellular changes, as well as reduced weight gains in studies lasting 2–13 weeks).[6] Slight elevations in liver enzymes and liver tissue changes have been observed at doses of 56 and 560 mg/kg of Roundup (with POEA), including mononuclear cell infiltration, apoptosis of hepatocytes, focal necrosis, and congestion and swelling of hepatocytes.[98]

3.3.1.D Chronic Toxicity and Carcinogenicity of Glyphosate

There are a number of long-term (lifetime) dosing studies for glyphosate. Observed effects at the higher doses (greater than ~2,000 mg/kg) include weight loss, increased liver weights, and inflammation of the gastric mucosa. The cancer data are equivocal. Tumors were observed at various sites in some of the high-dose and intermediate-dose animals. However, results did not always follow a dose-response curve, and/or were not significant relative to historical control data. The weight of the evidence supports US EPA's classification of glyphosate as "E—Evidence of non-carcinogenicity for humans." The available studies are summarized in Table 3-5.

Glyphosate has also been tested in *in vivo* and *in vitro* genetic test systems and was found to be mostly negative for gene mutation, chromosomal aberration and DNA damage. The final assessment of US EPA and CA OEHHA is that the weight of the evidence suggests that glyphosate is neither genotoxic nor clastogenic. However, several studies in which high doses of glyphosate (>1.4 mg/L) were used show positive results for sister chromatid exchange in human lymphocytes,[99] a result supported by two other in vitro studies that also replicated the findings in bovine lymphocytes above 2.9 mg/L.[100] These outcomes may have been a result of oxidative stress at the high concentrations tested. One study where glyphosate was administered to male mice by intraperitoneal (ip) injection (at 2 x 150 mg/kg) resulted in increased micronuclei in bone marrow cells;[101] however, this dose was very high, more than the ip LD_{50} for mice of 130 mg/kg, and the route of exposure is not relevant to exposures to glyphosate from the MMWD vegetation management project. Such results were not observed in other studies where the animals were dosed orally instead.[102]

Table 3-4: Sub-chronic Toxicity of Glyphosate to Mammals

Test animal	Study Duration (days)	Doses Tested (mg/kg-day)	Dose (endpoint) (mg/kg-day)	Observed Effects
CD-1 mice[103] (Monsanto)	90	940, 1,890, 9,710 (M) 1,530, 2,730, 14,860 (F) 98.7% pure glyphosate in diet	1,890 (NOEL)	Liver weights increased. At highest doses, growth retardation and increased organ weights of brain, heart, and kidney
CD-1 mice[5] (Monsanto)	90	250, 500, 2,500	500 (NOEL)	Reduced body weight gains in high-dose mice (M&F)
Sprague-Dawley rats[104] (Monsanto)	90	63, 317, 1,267 (M) 84, 404, 1,623 (F) glyphosate in diet	1,267 (NOEL)	No toxic effects observed. Hematology, blood chemistry, and organ weights not affected by the treatment.
F344N rats[105] (NTP)	91	3,125, 6,250, 12,500, 25,000, 50,000 ppm in diet (M&F)	No NOEL identified	No effect on survival. Reduced weight gain (M at 25,000). Increased organ weights: liver (M at 3,125); kidney & testes (M at 25,000). Decrease in thymus weight (M at 50,000). Changes in blood counts (F at 3,125). Increased alkaline phosphatase (M at 6,250; F, 12,500) and alanine aminotransferase. Decreased sperm density (M at 25,000). Dose-dependent salivary gland lesions at all doses, M & F.
B6C3F1 mice[105] (NTP)	91	3,125, 6,250, 12,500, 25,000, 50,000 ppm in diet (M&F)	507 mg/kg (NOEL) (equivalent to 3,125 ppm in diet)	No effect on survival. Reduced weight gain (M & F at β25,000). Increased organ weights, but not dose-dependent. No effect on sperm motility. Salivary gland lesions, but not at lowest dose.
Beagle dogs[106] (Monsanto)	365	20, 100, 500 orally by capsule	500 (NOAEL)	No observed adverse effects in clinical signs, body weight, eyes, blood chemistry, gross pathology and histopathology. Changes in pituitary weights (M, β500), but significance is not clear since no histological changes.
Rabbits[107]	21	100, 1,000, 5,000 dermally 6 h/d, 5 d/wk	No NOEL identified	No effect on survival and growth. NO evidence of systemic toxicity. Slight erythema and edma in both intact and abraded skin observed at HDT.
Cows[108]	7	400, 500, 630, 790 by nasogastric tube	400 (NOAEL)	At HDT, 1/3 animals died from pneumonia caused by aspiration. Decreased feed intake (β630); diarrhea (β500).
		Sub-chronic RfD	**NA**	**US EPA sub-chronic RfD not defined for glyphosate**

HDT = highest dose tested; NTP = National Toxicology Program; M = male; F = female; RfD = reference dose.

Table 3-5: Chronic Toxicity of Glyphosate to Mammals—Cancer

Test animal	Study Duration (months)	Doses Tested (mg/kg-day)	Dose (endpoint) (mg/kg-day)	Observed Effects
Sprague-Dawley rats[109] (Monsanto)	24	3.1, 10.3, 31.5 (M) 3.4, 11.2, 34 (F) oral, in diet	31.5 (NOEL)	Survival, hematology, blood chemistry, urinalysis, and organ weights not affected by the treatment. Increase in c-cell thyroid carcinoma in high-dose group, but no dose-response. Significant increase in testicular tumors in high dose males, but age differences could explain this result. This result was not duplicated in a second study.
Sprague-Dawley rats[110] (Monsanto)	24	100, 410, 1,060 oral in diet	410 (NOEL)	No change in survival, appearance, or blood chemistry noted in treated animals. Reduction in body weight gain in high-dose F rats. Increased liver weight in high-dose M rats. Degeneration of lens capsule fibers in eye. Inflammation of gastric mucosa in medium- and high-dose F rats and M rats (but not statistically sigfnificant). Increase in pancreatic islet cell adenomas, but no carcinomas were observed. Increase in adrenal cortical carcinoma at HDT (F).
CD-1 mice[111]	24	157, 814, 4,841 (M) 190, 955, 5,874 (F) oral in diet	814 (NOEL)	Decrease in body weight and adverse liver and kidney effects at HDT. Bronchiolar-alveolar lung tumors, hepatic tumors, and tumors of the lymphoreticular system were observed. No clear dose- response relationships were noted and the tumor incidence was not statistically significant compared to historical controls.
		Chronic RfD	**NA**	**US EPA chronic RfD is defined by a developmental toxicity study. See Table 3-6.**

HDT = highest dose tested; M = male; F = female.

3.3.1.E Reproductive and Developmental Toxicity of Glyphosate

At low or intermediate doses in rats, there were few effects of glyphosate on mating, pregnancy and fertility indices. Effects noted include reduced fetal body weights at the highest doses tested in most studies, and reduction in liver to body weight ratios in second generation offspring in all treatment groups in multi-generation studies. Slightly reduced litter sizes were observed, although this result was not dose-dependent. Glyphosate affects sperm quality at relatively low doses (38 mg/kg-day) in rabbits; a comparable study in rats was not available.

Table 3-6 below provides a summary of reproductive and developmental toxicity of glyphosate in tests with laboratory animals treated with glyphosate alone, without surfactants. There is great variability in the dose ranges tested in the different studies, from 3–30 mg/kg-day (rats) in the lowest-dose study to 300–3,500 mg/kg-day (rats) in the highest-dose study.

An increased incidence of unilateral renal tubular dilation in the male pups of the F3b generation was observed in a 3-generation study with rats dosed at 10–30 mg/kg-day, but this result was not reproducible in a second study in which rats were dosed at much higher levels (150–2,200 mg/kg-day) for two generations. These two multi-generation studies may not be directly comparable, but the null result in the second study has been used by EPA to justify discounting the observed result in the first one.

At very high doses (3,500 mg/kg-day, rats), statistically significant decreases in viable fetuses, mean fetal body weight, and maternal survival have been noted. Reduced maternal survival occurred at much lower doses in rabbits (350 mg/kg-day, Highest Dose Tested, or HDT), with a 62% death rate observed in the dams receiving the HDT.

In the US EPA's 1990 IRIS risk assessment, a chronic RfD for glyphosate of 0.1 mg/kg-day was derived from the NOEL of 10 mg/kg-day from the 3-generation study, based on increased incidence of unilateral renal tubular dilation. However, in the 1993 reregistration decision for glyphosate, US EPA selected a RfD of 2 mg/kg-day from a NOEL of 175 mg/kg-day based on maternal toxicity in the rabbit study. This NOEL is half the dose rate that caused a 62% maternal death rate in the study.

Table 3-6: Reproductive and Developmental Toxicity of Glyphosate to Mammals

Test animal	Study Duration	Doses Tested (mg/kg-day)	Dose (endpoint) (mg/kg-day)	Observed Effects
CD rats[112]	3 generations	3, 10, 30 oral, in diet	10 (NOEL)	No consistent, dose-related effect was seen in mating, fertility or pregnancy indices to indicate an adverse effect of treatment. For all treated groups, liver to body weight ratios of F2b parental females were significantly lower than controls for F2b females and liver to brain weight ratios were also reduced; however, no dose-response releationship observed and effect not observed in treated parents from previous generations. Increased incidence of unilateral renal tubular dilation in the male pups of the F3b generation at HDT, but this was not reproducible in a second study.
CD rats[113]	2 (F0 treated for 11 weeks before mating)	150, 720, 2,200 (oral, in diet)	720 (NOEL)	F0 & F1 adults had reduced body weights (8 to 11 percent) at HDT. Mating, pregnancy, and fertility indices not affected by the treatment in both F0 and F1 animals. The average litter size of high-dose F0 dams was ~2 pups less than controls, and a smaller difference (approximately 1 pup/litter) noted after the first F1 mating, but not statistically significant. No treatment-related decrease in litter size was observed in the F2b generation
Rats[114]	Gestation	0.5%, 1% glyphosate w/v in drinking water	NR	Significant decrease in food & water consumption and maternal body and liver weights at HDT. No differences in fetal body weights. Enzyme effects noted, but dose-response not always observed.
Rabbits[115]	6 weeks	0.01*LD_{50}, 0.1*LD_{50}, but LD_{50} not stated. LD_{50} from a different rabbit study was 3,800 mg/kg-day (see Table 3-3), which translates to doses of 38 and 380 mg/kg-day (oral, in gelatin capsules)	NA	Reduced body weight, ejaculate volume & sperm concentration and increased abnormal & dead sperm at both dose levels. Adverse effects continued into the recovery period.
COBS CD rats[116]	GD 6–19	300, 1,000, 3,500	1,000 (NOAEL)	Statistically significant decrease in viable fetuses and mean fetal body weight at HDT. Reduced maternal body weight gain and early death at HDT.

Table 3-6 (cont.): Reproductive and Developmental Toxicity of Glyphosate to Mammals

Test animal	Study Duration	Doses Tested (mg/kg-day)	Dose (endpoint) (mg/kg-day)	Observed Effects
Rabbits[117]	GD 6–27	75, 175, 350	175 (NOAEL) 350 (LOAEL)	Increase in the incidence of soft stools and diarrhea in middle dose and HDT and nasal discharge at HDT. Statistically significant early maternal mortality at HDT (62%), caused by pneumonia, respiratory disease, enteritis, gastroenteritis, or unknown mechanisms. No biologically meaningful differences in mean number of viable fetuses, early or late resorptions, total implantations, corpora lutea, fetal body weights, fetal sex distribution, or number of fetuses or litters with malformations in any of the treatment groups compared to the controls. Slight decrease in mean fetal body weight of all treated groups, but not significant relative to historical controls.
		Chronic RfD (OPP)	**175/(10*10)[a] = 2**	**EPA OPP's developmental RfD, based on early maternal mortality in rabbits.**
		Chronic RfD (IRIS)	**10/(10*10)[a] = 0.1**	**EPA's chronic RfD, based on increased incidence of renal tubular dilation in F3b offspring.**

NA = not available; NR = not reported; GD = gestation day; HDT = highest dose tested; M = male; F = female.
Shaded rows are the studies on which the RfD is based.
[a]Intraspecies uncertainty factor of 10 and interspecies uncertainty factor of 10.

Reproductive and developmental toxicity studies done with Roundup containing a POEA surfactant are not included in Table 3-6, but much higher developmental toxicity was observed with Roundup compared to glyphosate. In one study, 50% of the dams died between day seven and 14 of pregnancy at a dose of Roundup that was one-third the dose of pure glyphosate.[118] Skeletal alterations such as incomplete skull ossification and enlarged fontanel were also observed with Roundup, but not glyphosate. Another *in vitro* study with mouse Leydig tumor cells demonstrated reduced steroidogenesis in treated cells, which implies potential to impact testosterone production.[119] These effects appear to be due to surfactant exposure or the combined effect of glyphosate and surfactant, since they are not reproducible in studies with glyphosate alone.

3.3.1.F Neurotoxicity

Glyphosate has been tested for neurotoxicity in rats and hens and found to be negative for indications of neurotoxicity. Acute exposures of rats to relatively low doses of glyphosate (one dose of 50, 100 or 200 mg a.e./kg) showed no effects on landing foot splay, sensory perception, muscle strength, or locomotor activity and no abnormal histologic changes in the central or peripheral nervous system tissue over a two-week period.[120] Six hours after dosing, the animals exhibited decreased activity, subdued behavior, and hypothermia, but this was not attributed to glyphosate's effects on the nervous system.

A subchronic study in which rats were dosed with glyphosate in their diets at concentrations of 2,000, 8,000, or 20,000 ppm for 13 weeks showed no neurologic effects, as assessed by changes in locomotor activity, brain weight or dimensions, or damage to nerve tissue (peripheral or central).[121] Effects were noted on growth and food utilization, but again this was not attributed to glyphosate's effects on the nervous system.

Evaluation of delayed neurotoxicity using the standard hen assay[122] showed a slight decrease in brain acetylcholinesterase (AChE) activity after a single gavage dose of glyphosate at 2,000 mg/kg. Delayed locomotor ataxia and neuropathology were not observed.[123]

A dog study conducted by Monsanto is described in the USFS risk assessment.[4]

> *"Williams et al. (2000) also describe a study in which neurological examinations were conducted on dogs that received a single oral dose of 59 or 366 mg/kg of Roundup and the information is attributed to an unpublished study by Monsanto which is cited as Naylor (1988). This study was not identified in a search of the U.S. EPA/CBI files and has not been reviewed in preparation of this risk assessment. According to Williams et al. (2000):*
>
> *'A detailed examination consisting of 12 different measurements of spinal, postural, supporting, and consensual reflexes was performed before treatment, during the postadministration observation period, and again on the following day. Reflexes appeared normal, and there were no clinical signs indicative of neuromuscular abnormalities.' "*

No signs of neurotoxicity were noted in the other sub-chronic studies (see Section 3.3.1.C) where morphological examinations were conducted of the brain (including basal ganglia, a site of injury in Parkinsonism). However, it is unclear from the report whether or not spinal cord and sciatic nerve were examined.[105] There is some speculation that the histological changes observed in salivary glands in both rats and mice may have been caused via an adrenergic mechanism. This hypothesis is based on evidence that the histological changes were less severe in animals treated with drugs that are β-adrenergic neurotransmitter antagonists. Others disagree, since other β-adrenergic effects (e.g., on heart rate and blood pressure) have not been observed when glyphosate was administered intravenously to dogs or rabbits.[124] This debate remains to be settled.

A study evaluating the effect of glyphosate on taste response in gerbils demonstrated that glyphosate decreased taste receptor response.[125] This observation could be explained in one of several ways, including the possibility of a direct neurotoxic effect, biochemical alteration in the epithelial cells on the tongue, or by chemical injury. It is impossible to say definitively whether or not the observed decrease in taste was due to a neurologic effect.

3.3.1.G Immunotoxicity

The powerful protective immune system is highly complex and interacts with all other body systems. The only way to determine potential immunotoxic effects of gyphosate is to directly study its effects on the immune system, including lymphoid tissue (lymphoid nodes, thymus),

bone marrow, lymphocytes (B-cells and T-cells), antibodies, immunoglobulins, among other components.

Glyphosate has been tested specifically for effects on the immune system in both humans and experimental mammals. There is no evidence of an immune response in mammals caused by glyphosate, in either *in vitro* tests with human or sheep cells, or in human dermal exposures, where glyphosate was not found to cause sensitization. The details of these studies may be found in the USFS risk assessment.[4]

3.3.1.H Endocrine Disruption

Glyphosate has been subjected to six *in vitro* assays for estrogenic activity. One of these studies showed cell proliferation effects not deemed to be estrogenic in nature, two studies showed no estrogen receptor binding, and three studies evaluating the effects of both glyphosate alone and Roundup formulations on aromatase activity showed reduced activity at glyphosate concentrations high enough to cause cell death. Aromatase is an enzyme that converts estrogen to testosterone. There was no evidence of the very low-dose effects observed for known endocrine active compounds such as natural estrogens or diethylstilbestrol (DES). No studies were found that explored the androgenic or thyroid effects of glyphosate.

It should be noted that the *in vitro* tests discussed below were conducted in isolated cell cultures. These kinds of tests provide information on the potential for adverse effects in the whole organism, although they do not account for the systemic processes that may serve to accentuate or mitigate an effect in the whole organism. As one author noted:

> *" . . . one of the nagging questions regarding in vitro environmental estrogen evaluation and detection of weak estrogenlike activity is whether these chemicals can have any clinically significant effects in vivo. Cultured cells deprived of background estrogens and nonestrogenic growth factors (CD-treated serum) may be sensitized to estrogenic stimuli. Moreover, the in vivo response to exogenous estrogenic stimuli in a hormonally balanced milieu may well be dampened."* [126]

Concentrations used in *in vitro* tests of endocrine disruption are not directly comparable to doses in humans or experimental animals. *In vitro* studies should instead be compared to "positive controls" (known endocrine disruptors) to which the magnitude of any effects can be compared in the same assay as the test substance. In order to clarify systemic effects and dose/response in whole animals, it is necessary to complement the results of *in vitro* assays with *in vivo* bioassays. For glyphosate, no *in vivo* studies were available. We summarize the results of these studies below.

Lin and Garry studied the potential for estrogenic activity of glyphosate on cell proliferation in MCF-7 human breast cancer cells.[126] Their work showed that cells treated with glyphosate and Roundup proliferated at 135 ± 3.5% and 126 ± 5.1% of the negative control, respectively. The range of glyphosate doses over which effects were observed was 0.228–2.28 mg/L, and the maximum effect was observed at a concentration of 2.28 mg/L (10^{-4} M). For Roundup, the dose range was 1–10 mg/L, with maximum effect observed at 10 mg/L. The concentrations at which glyphosate and Roundup are active are 10,000 times higher than the concentration of DES that

induced maximum proliferation in the same assay at a rate of 238 ± 29.5% of the negative control. For perspective, a concentration of 2.28 mg/L of glyphosate is approximately 3.3 times the glyphosate drinking water standard of 0.7 mg/L. This concentration is comparable to the doses tested in the standard toxicology tests; thus, the result cannot be described as a "low dose" effect. The authors concluded that glyphosate's effects were a result of non-estrogenic induction of cell proliferation.

In a second study, Petit and LeGoff *et al.* investigated estrogen receptor binding relative to the natural estrogen 17β-estradiol using two assays: 1) measurement of the estrogen-dependent activity of β-galactosidase in a recombinant yeast system modified to express the rainbow trout estrogen receptor, and 2) measurement of vitellogenin gene induction in trout hepatocytes.[127] Glyphosate showed no activity in either assay relative to the solvent control.

In a third study by Walsh and McCormick *et al.*, the activity of glyphosate was assessed for its ability to alter steroid hormone biosynthesis in MA-10 mouse Leydig (testicular) tumor cells.[119] Glyphosate alone did not inhibit steroidogenesis (the process of formation of steroid hormone from precursor compounds) or total protein synthesis at any dose tested (0–0.100 mg/L). However, Roundup formulations reduced progesterone synthesis by 94%, with effects noted at concentrations as low as 0.010 mg/L. The authors indicate that this effect was possibly due to the effects of the surfactant on membrane function.

The effects of glyphosate and Roundup on aromatase inhibition in human placental JEG3 cells were evaluated in a 2005 study by the Seralini research group.[128] This study suffers from several major flaws, the most significant one being the lack of incorporation of a positive control substance known to inhibit aromatase in the testing protocols. It is also important to clarify that low-dose effects were not evaluated in this study; instead, the glyphosate concentrations tested in cell cultures were similar to concentrations applied as herbicides—between 0.05 and one *percent* solutions (500 to 10,000 mg/L). For comparison, in order to reach a serum concentration of 10,000 mg/L of glyphosate, a 70 kg adult male with a typical blood volume of five liters would have to drink at least 104 mL (about half a cup) of undiluted Aquamaster containing 480 g/L of glyphosate (this calculation assumes that all of the ingested glyphosate would be circulating in the blood stream and is probably an underestimate). Thus, the doses tested in this study are not representative of any exposure that would occur from normal use of glyphosate. Finally, many of the statements made in this paper are not supported by the data, and some very important points evident in the data were not discussed. The abstract and conclusion are particularly misleading.

The raw data indicate that glyphosate has no effect on JEG3 placental cell viability up to solution concentrations of 0.9% (9,000 mg/L) for a serum-containing medium. Above that concentration, aromatase activity in the cells decreased rapidly, as did cell death. Reduction in cell viability occurred at lower doses in a serum-free medium, between 4,000–8,000 mg/L. No reduction in aromatase activity relative to the negative control was observed at concentrations below 4,000 mg/L. From these data, the authors concluded that:

> *Our studies show that glyphosate acts as a disruptor of mammalian cytochrome P450 aromatase activity from concentrations 100 times lower than the recommended use in agriculture; this is noticeable on human placental cells after only 18 hr, and it can also*

> *affect aromatase gene expression. It also partially disrupts the ubiquitous reductase activity but at higher concentrations. Its effects are allowed and amplified by at least 0.02% of the adjuvants present in Roundup, known to facilitate cell penetration, and this should be carefully taken into account in pesticide evaluation. The dilution of glyphosate in Roundup formulation may multiply its endocrine effect. Roundup may be thus considered as a potential endocrine disruptor. Moreover, at higher doses still below the classical agricultural dilutions, its toxicity on placental cells could induce some reproduction problems.*

This conclusion failed to mention that at high enough concentrations, glyphosate kills JEG3 cells, thereby reducing aromatase activity by virtue of the loss of viable cells that produce it. Thus, another explanation for the observed results is that the "disruption" in aromatase production was a result of cell death caused by the high concentrations of the chemicals to which the cells were exposed. Later work by this same research group also focused on high-dose *in vitro* studies on human placental cells.[129] These high-dose studies are more relevant to the existing animal toxicology studies in the high dose regime and not at all relevant to the study of low-dose estrogenic effects.

More recent work by Seralini *et al.* indicates that the POEA surfactant in formulated Roundup products is much more toxic to placental cells than glyphosate and appears to have some estrogenic activity.[130] The surfactants Competitor and Sylgard 309 that are being considered for use in this project do not contain POEA or the known endocrine disruptors nonylphenol or nonylphenol ethoxylate.

The fact that no low-dose estrogenic effects were observed for glyphosate alone in these *in vitro* studies and that no endocrine effects have been noted in wildlife exposed to glyphosate alone, in spite of its widespread use, strongly suggests, but does not prove, that glyphosate is not an endocrine disruptor. A European Union survey of the scientific literature on endocrine effects of pesticides does not list glyphosate as a chemical of concern,[131] nor do other sources of information on endocrine disrupting effects.[132] However, no comprehensive evaluation of glyphosate or these surfactants has been undertaken, and no final conclusions on the endocrine disrupting ability of these compounds can be drawn at this time. Glyphosate is one of the first set of chemicals slated for testing of endocrine disrupting effects by the EPA, and more information will be available when those tests are completed.

3.3.1.1 Effects on Mammalian Wildlife

US EPA's Ecotox database contains approximately 200 results of field studies on the effects of glyphosate on wild mammals, including mule deer, Norway rats, deer mice, several species of voles and shrews, and chipmunks.[6] A variety of toxicity endpoints were evaluated, primarily changes in species abundance. Survival, reproductive ability, weight loss and general injury were also evaluated. A reduction of populations of small mammals were noted in some field studies, but not others. Table D-1 in Appendix D summarizes these studies. Some of the observed effects are likely due to the secondary, indirect effects of habitat and food loss, not to the toxicity of glyphosate. Section 2.3.5 provides examples of indirect effects due to glyphosate.

The effects of glyphosate treatment on the feeding preferences and daily food consumption of black-tail deer were evaluated.[133] Deer did not avoid eating browse of alder *(Alnus rubra)* and

alfalfa *(Medicago sativa)* that was treated with glyphosate at a rate of 2.2 kg/ha. Sometimes the treated alder browse was even preferred. Effects on deer health were not reported.

A number of studies have been conducted on the effects of glyphosate applications on population, reproductive capacity, and survival of small forest rodents. These animals may come into contact with treated vegetation, eat contaminated food and drink contaminated water from application sites. Indeed, low-level residues of glyphosate were detected in small mammals for a short time after treatment.[134] A Monsanto-funded study of the populations of deer mice (*Peromyscus maniculatus*) and Oregon voles (*Microtus oregoni*) between a control and treated clear-cut area in Oregon over five years had mixed results, with treatment populations, weight and reproductive success exceeding those of control populations in some years, but not in others.[135] Overall, the authors concluded that a single Roundup treatment at 2.2 kg a.i./ha had no significant effects on survival and body mass of the two species.

Insectivorous shrews *(Blarina brivicauda, Sorex cinereus and Sorex hoyi)* and herbivorous voles were less abundant due to a treatment with Roundup at a rate of 1.3 kg a.e./ha.[136] This significant reduction of the number of shrews continued for three years after application, whereas the population of voles recovered. No effects on the populations of the omnivorous deer mice *(Peromyscus maniculatus)* were observed.

In a clear-cut area treated with Roundup at a rate of 1.1 kg a.i./ha, the population density of Southern Redbacked voles *(Clethrionomys gapperi)* was reduced by about 80% in a one-year experiment.[137] In another clear-cut, no adverse effects on deer mice populations were evident after treatment with Roundup at a rate of 1.6 kg a.e./ha.[138]

In contrast, a significant reduction in the population density of deer mice was observed in a treated clear-cut approximately 11 months after treatment with Roundup at 1.2 kg a.e./ha.[139] However, no adverse effects on fertility or fecundity were indicated. The authors speculate that the effect on abundance was due to habitat change with respect to food and cover. At lower application rates of Roundup (0.6 kg a.e./ha), deer mice were not affected.[140]

3.3.1.J Levels of Concern for Mammals

The acute and chronic TRV for glyphosate in mammals is based on a NOAEL in a developmental toxicity study in rabbits of 175 mg/kg-day, where early maternal mortality was observed at the next highest dose of 350 mg/kg-day. No adjustment of this value was performed to make it more protective.

3.3.1.K Data Gaps

Glyphosate is one of the most well-studied herbicides on the market today, and the data set on its toxicity—comprised of many studies by Monsanto and a number of peer-reviewed literature studies—is fairly complete. Nevertheless, the toxicity of glyphosate when used in conjunction with different surfactants (besides POEA) remains largely unexplored. Some aquatic toxicity data exist for glyphosate with non-POEA surfactants; these data show toxicity closer to that of glyphosate alone (see section 3.3.3).

Complete toxicity studies on AMPA, the primary degradation product of glyphosate are also unavailable, although acute toxicity tests indicate that its toxicity is similar to glyphosate. No information is available on AMPA's carcinogenicity, reproductive and developmental toxicity, neurotoxicity, immunotoxicity or endocrine disrupting ability.

3.3.2 Other Terrestrial Organisms

Glyphosate is considered to be not acutely toxic to birds, honeybees and soil microorganisms. Most studies with spiders and other insects suggest that glyphosate is only slightly toxic. Reproductive toxicity in birds has been observed at low doses of Roundup, but not for glyphosate alone. Glyphosate does not appear to do any long-term damage to soil fertility and microorganism communities, but may temporarily increase or decrease populations of certain fungi. There are conflicting studies concerning the toxicity of glyphosate to earthworms.

The TRVs for terrestrial organisms are summarized in Table 3-7. All toxicity studies reported in this document are summarized in Table 3-8. An more detailed presentation of the data in Table 3-8 can be found in Appendix D.

3.3.2.A Birds

Based on the available Monsanto studies submitted to US EPA and approximately a dozen studies in the avian toxicity literature, glyphosate is deemed to be not acutely toxic to slightly acutely toxic to birds. Although the EPA and USFS do not consider glyphosate to cause reproductive toxicity in birds, there is some contradictory evidence for Roundup formulations.

The acute toxicity of glyphosate to the standard avian test species (mallard ducks and bobwhite quail) is generally low. The Terretox database[6] (see Tables D-2 and D-3) and RED 1993[5] show LD_{50} values >2,000 mg/kg of technical grade glyphosate for studies lasting eight days or less (see Section 2.3.4 for toxicity reference tables). An increase in the exposure time to three weeks results in a lowered tolerance for glyphosate exposure, with LD_{50} values dropping to 950 mg/kg. The EPA RED reported a NOEC of 1,000 ppm for long-term reproductive effects in mallards and bobwhite quail. For endpoints expressed as glyphosate concentrations in food, the Terretox database reports LC_{50} values >4,650 mg chemical per kg food for all study species exposed to glyphosate for eight days.

Since the EPA RED was published, more recent studies have shown even higher LC_{50} values of 5,620 mg/kg (five day exposure).[141] This Monsanto study is not publicly available. The USFS reports that this acute toxicity study was actually a NOEC because no mortality was observed.

It is unclear whether glyphosate causes adverse reproductive effects in birds. Two studies[142a,b] report no reproductive effects of glyphosate to bobwhite quail and mallard ducks at concentrations in food of up to 1,000 mg/kg of technical glyphosate (equivalent to a dose of approximately 100 mg/kg body weight). In contrast, a different study using Roundup (presumably with the POEA surfactant) reported statistically significant reduced testosterone production and changes in testicular ducts in mallards exposed to a dose of 3.7 mg a.e./kg body weight.[143] This study also reports reduced weight of male sexual organs; however, this result was not statistically significant. The use of a different chemical formulation, as well as the more

refined reproductive metric (concentration of testosterone) helps explain the difference in endpoint doses between the two studies.

Zebra finches experienced pronounced weight loss after dietary exposures of 5,000 ppm glyphosate.[144] After 3 to 7 days of eating glyphosate-containing food, birds consumed less food and lost an even larger fraction of their original body weight. This is consistent with observations in experimental mammals suggesting that glyphosate may inhibit oxidative phosphorylation and consequently reduce food conversion efficiency.

As reported in USFS 2003, glyphosate does not cause teratogenic activity in birds,[145a–b] nor neurotoxicity in hens.[146]

For a summary of avian toxicity studies on reproductive and sub-lethal effects of glyphosate, see Table D-4 in the Appendix.

Levels of concern for birds: The USFS uses an acute avian NOAEL of 562 mg glyphosate per kg organism body weight and a chronic NOAEL of 100 mg/kg. These values are from the five-day dietary NOAEC of 5,620 ppm (originally an LC_{50} study, but no mortality was observed) reported for mallards and bobwhite quail and the 1,000 ppm reproductive NOAEC for mallards and bobwhite quail.[5] Food intake is typically assumed to be 10% of body weight, giving TRVs of 562 and 100 mg/kg for acute and chronic toxicity respectively. There is a 2007 study in which effects were noted at a dose lower than 100 mg/kg. A LOEL of 74 mg/kg for Roundup was found in a study of male mallards for decreased testosterone and damage to the epididymis (a narrow, tightly coiled tube connecting the testes to the duct which goes to the penis).[143] Because Roundup (with surfactants) was used in this study, the toxicity does not reflect that of Aquamaster. We used the USFS value of 100 mg/kg TRV as a more appropriate chronic TRV for the Aquamaster formulation that is being considered for use in the MMWD watershed.

3.3.2.B Terrestrial Invertebrates

Although EPA only requires toxicity tests on honeybees as part of chemical registration data, numerous glyphosate toxicity studies have been done on other insects. Based on the EPA RED and the Terretox database, glyphosate does not appear to be acutely toxic to honeybees. It is slightly to moderately toxic to other insects, particularly spiders. There are conflicting studies concerning the toxicity of glyphosate to earthworms.

US EPA's Terretox database[6] and the US EPA glyphosate RED 1993[5] both report a 48-hour oral and contact LD_{50} for bees at doses >100 µg/bee for both Roundup and technical grade glyphosate, a rating of not acutely toxic (see Section 2.3.4 for toxicity ratings). One study reports a 48-hour oral LD_{50} greater than 100 µg/bee and a NOEL of 50 µg/bee.[147] Since all reports suggest that the LD_{50} is not lower than 100 µg/bee, it is worth noting that references within USFS 2003 suggest that the LD_{50} may in fact be much higher. A second study reported 5% mortality for an orally administered dose of 100 µg/bee.[148] This dose was classified as a NOEL because mortality was not significantly different from that in the matched solvent control. The Terretox data suggest that LD_{50} values decrease as study time increases (see Table D-5). For a 96-hour study, the LD_{50} for honeybees is 62 µg/bee, slightly lower than the LD_{50} for the 48-hour exposure period.

Originally tested as a potential insecticide for spider mites, glyphosate has been shown to be practically nontoxic to *Tetranychus urticae*, a pest species on apple trees[149] and *Typhlodromus pyri*, an important predator of spider mites.[150] Direct foliar spray of glyphosate IPA at 0.44 to 3.51 mg ae per kidney bean leaf (assuming a leaf is 25 cm^2, this corresponds to a application rate of 7-56 kg/ha) had no effect on spider mites based on mortality in eggs, larvae, nymphs or adults.[149] An unpublished Monsanto study reports 100% mortality in spider mites in applications of 10 L/ha RoundUp ULTRA (or 3.6 kg a.e./ha) to glass slides.[151] The authors believe that mortality was enhanced due to the "sticky layer" that formed upon application, which may have rendered the insects immobile. The importance of this study in real-world applications is unclear, since insects in natural settings are not usually on surfaces similar to glass slides.

A series of laboratory and field studies[152a-c] on the effects of glyphosate on the spider *Lepthyphantes tenuis* found decreased populations with application rates of 0.36, 0.72, and 1.44 kg a.e./ha. However, mortality was attributed to secondary effects from changes in the vegetation.[152c] In the laboratory, the authors observed 6-12% mortality (statistical significance was not discussed) after three days in *Lepthyphantes tenuis* at the same application rates.[152b]No substantial diversity effects were observed in spider populations at field application rates of 0.09 or 0.18 kg a.e./ha.[152a] However, abundances of all spiders were decreased and weaver spiders (as compared to wandering spiders) were more affected.

Data on other arthropods are less detailed. Leaf litter exposed to application rates of 2.1 kg/ha glyphosate did not report statistically significant toxicity effects in *Philoscia muscorum*.[153] Rove beetles sprayed with 1% Roundup (3.6 g/L) at 6 $\mu L/cm^2$ experienced no measurable mortality or changes in egg-laying.[154] A series of studies evaluating herbicide effects on butterfly populations compared the effects of herbicide use to mechanical removal in rights-of-way maintenance and noted no significant population differences.[155]

The Terretox database reports mainly NOELs and LOELs for a variety of beetles, spiders, hemiptera, diptera, lepidoptera and a few unspecified insects (see Tables D-6 and D-7). The median NOEL for abundance decreases using Roundup is 0.62 kg/ha. The median NOEL for glyphosate is 1.2 kg/ha in ground beetles. The median LOEL for abundance for both glyphosate and Roundup is roughly 0.75 kg/ha. The maximum reported NOEL for any effect is 1.5 kg/ha for application of Vision to ground beetles, a factor of five lower than the maximum application rate on the Aquamaster label.

Three studies report the potential effects of glyphosate on earthworms. In a laboratory study, effects on earthworm cultures treated at levels equivalent to application rates of 0.0007 to 0.0028 kg glyphosate/ha included decreased growth rates and caused early mortality.[156] Although this study would suggest that glyphosate is very toxic to earthworms, the USFS states that the study did not mimic field conditions and is therefore of limited relevance. In another study designed to mimic agricultural use,[157] no effects on earthworms were noted. However, reference 157 does not report exposures in either kg/ha or ppm soil and thus is not particularly useful for this risk assessment. The soil LC_{50} for glyphosate to *Aporrectodea caliginosa*, a worm common in Libya, has been reported to be 246–177 mg glyphosate/kg soil dry weight over exposure periods of 8–37 days.[158]

One study is available on the toxicity of glyphosate to *Helix aspersa*, the brown garden snail. Diets containing 4,994 ppm glyphosate resulted in no mortality over a 14-day exposure period.[159] Assuming a 30% food consumption factor for this species, this corresponds to a dose of about 1,500 mg/kg (4,994 ppm × 0.3 mg/kg bw ppm = 1,498 mg/kg bw).

Levels of concern for bees: The USFS used a 50 µg/bee (540 mg/kg) NOEL as the TRV for honeybees in their risk analysis. We also used this TRV. Most of the other insect and arachnid studies summarized in Appendix D list TRVs in units of kg/ha. These values can be compared directly to the application rates being considered by MMWD to estimate effects on insects or other invertebrates not evaluated in the risk assessment.

3.3.2.C Terrestrial Plants

To be an effective herbicide, glyphosate must be toxic to plants, and any off-target movement of herbicide spray will likely damage non-target vegetation. Table 3-7 on page 3-52 provides a summary of glyphosate toxicity to non-target vegetation. As a broad spectrum herbicide, the toxicity pathway for glyphosate is well understood.[160] Glyphosate kills plants by restricting the shikimic pathway, the pathway that allows plants to synthesize phenolic compounds and aromatic amino acids (phenylalanine, tryptophan, and tyrosine). Disruption of the shikimic pathway inhibits photosynthesis, cellular growth, and nucleic acid production. Depending on the species of plant, weather, and season, glyphosate toxicity occurs within a week of application. Plants wilt and yellow, and eventually die. There is some evidence of a hormetic response (i.e., increased vigor as a result of treatment) at very low concentrations of glyphosate.[4, 161] There is substantial variability in the susceptibility of plants to glyphosate, and this report summarizes a representative sample of the available terrestrial plant studies.

For all herbicides, US EPA requires manufacturers to perform seedling germination and emergence and vegetative vigor studies in non-target plants (including effects on corn and soybean). Seedling germination studies involve submersion of seeds in solution containing the herbicide. Seedling emergence studies involve application of the test compound to soil containing seedlings. Both of these tests simulate the effects of herbicide-contaminated runoff on emergent vegetation. Vegetative vigor studies involve direct foliar applications to young plants and simulate the effects of spray drift.

Most grasses, broadleaf, and woody plants readily absorb glyphosate, distribute it through plant tissues and store the chemical in sensitive roots and stems. The evapotranspiration and growth activity of the plant affects how quickly and thoroughly the chemical is absorbed. Approximately 33% of foliar-applied glyphosate is absorbed within the first few hours. Surfactants also affect absorption: 64% of glyphosate applied without a surfactant washes off plant tissue; 50% washes off with a surfactant.[4] Below ground, glyphosate adsorbs strongly to soils, preventing root uptake.

With the widespread increase in glyphosate use accompanying the advent of Roundup-Ready crops, many wild plants are acquiring glyphosate resistance.[162] About a dozen weed species have developed resistence (including Johnsongrass, rigid ryegrass, and horseweed) and have emerged in China, Brazil, Argentina and the United States. According to a Syngenta survey, 8% of US

farmers say that glyphosate resistence is a problem in the entirety of their acreage.[163] Resistant plants, wild or genetically modified, move glyphosate to the leaf tips protecting sensitive roots and stems. By doing so, these plants sacrifice a small part of the plant to save the rest.[163]

The Terretox database includes more than 1,300 studies on glyphosate toxicity to plants. Studies usually record the percent of a plant damaged or fraction of plants killed as a function of the application rate in kg/ha pesticide. Since glyphosate is an herbicide, and thus we expect mortality and damage upon application, a summary of all the Terretox records seemed unnecessary. The application rates that killed 50% and 100% of experimental plants are the most directly comparable endpoints to LD_{50} values available for other organisms. Glyphosate application rates ranging from 0.21-6.7 kg/ha were capable of killing 100% of a variety of plants including: wheat, oat, barley, purple crabgrass, devilgrass, panic grass cocklebur, thistle, Lamb's quarters, dandelion, meadow garlic and eastern white pine. Application rates ranging from 0.046-4.5 kg/ha were capable of killing 50% of a variety of plants including: ryegrass, Lamb's quarters, potato white fir, blackberry, quackgrass, ivy and sugar pine. Bryophytes and fungi have EC_{50} values as low as 0.8 kg/ha (assumed to be technical grade glyphosate).[164] There do not appear to be patterns in toxicity by plant family or order, consistent with glyphosate's non-selective mechanism of action.

USFS 2003[4] discusses a variety of studies on seedling emergence submitted to the U.S. EPA. Unfortunately these studies are not readily available for review. Therefore, the following summary of the data has been included verbatim from USFS 2003:

> *Since the publication of the RED, additional studies have been submitted to the U. S. EPA or published in the open literature on seedling emergence (Willard 1996; Everett et al. 1996a; Suma et al. 1995) and vegetative vigor (Chetram and Lucash 1994; Everett et al. 1996b). In addition, several studies have been conducted on the effects of drift on nontarget plant species (Al-Khatib and Peterson 1999; Bhatti et al. 1997; De Jong and de Haes 2001; Fletcher et al. 1996; Gilreath et al. 2001; Marrs and Frost 1997; Newmaster et al. 1999).*
>
> *The studies on seedling emergence submitted to the U. S. EPA involve a water dispersible granule formulation (Willard 1996) and a wettable powder formulation (Everett et al. 1996a). Additional details on the formulations cannot be disclosed in this risk assessment. Both studies were consistent with earlier reports indicating no adverse effects in monocots and dicots at application rates of about 4.5 lb a.e./acre. In an open literature publication by Shuma et al. (1995), a Roundup formulation (356 g a.i./L) was associated with complete inhibition of seed viability when applied to oats 15 days after anthesis (flowering or seed formation) at an application rate of 1.76 kg/ha (about 1.6 lb a.i./acre) and applications as low as 0. 44 kg/ha partially inhibited seed germination. This study is not inconsistent with the studies by Everett et al. (1996a) or Willard (1996). These latter studies involved standard test protocols in which seeds were exposed directly rather than harvested from plants after anthesis.*
>
> *The two vegetative vigor studies (Chetram and Lucash 1994; Everett et al. 1996b) both follow similar experimental designs. Various monocots and dicots are grown from seeds to emergence prior to any herbicide application. After the leaves emerge, the plants are sprayed at various rates – expressed in the same units as application rate – to assess the consequences of direct spray and drift. In the study by Everett et al. (1996b) glyphosate was applied as a wettable powder formulation. The most sensitive species was the dicot, oilseed rape (Brassica napus), with an EC25 of 0.083 kg a.e./ha (0. 07 lb a.e./acre) and EC5 of 0. 042 kg a.e. /ha (0. 037 lb a.e.*

/acre). The least sensitive species was the monocot, Purple Nutsedge (Cyperus rotundus), with an EC25 of 0.891 kg a.e./ha (0. 79 lb a.e./acre) and EC5 of 0. 58 kg a.e./ha (0. 52 lb a.e./acre). The EC5 values are essentially NOECs. In the study by Chetram and Lucash (1992), the most sensitive species based on reduced growth were tomato and radish, with a NOEC of 0. 035 lb a.e./acre and the least sensitive species were ryegrass, corn, and onions with a NOEC of 0.56 lb a.e./acre.

Drift studies cited in USFS 2003[4] have measured the extent of damage to non-target plants from glyphosate drift at several different levels. One study simulated drift by applying 1%, 3%, 10%, 33% of the 1.121 kg/ha suggested application rate for glyphosate to soy plants.[165] The authors found that transient damage could be observed at 0.034 kg/hectare (or 3% of suggested application rates). At 33% of the application rate, more than 50% of the plant was damaged seven days after application. After 60 days, only 20% of the plant was damaged, but the height of the plant was stunted by 25%. Agricultural yield was less affected than the plant tissue.

Similar drift studies reported in the USFS 2003 risk assessment simulated drift using the same application rates and found that leaf area, shoot length, and visible damage were all affected by glyphosate applications of 0.34 kg/ha.[166a, b, c]

To quantify how far backpack-sprayer-applied glyphosate traveled, droplet deposition on absorbent paper and plant damage were measured at various distances from the target site.[167] The authors found that damage could be observed in dicotyledonous *Brassica nupus* and monocotyledonous *Poa annua* up to 10 meters away. USFS 2003 reports a study that did a similar experiment involving a larger number of species and found that a buffer of eight meters was sufficient to prevent significant plant mortality to non-target species.[168]

Concerns remain about the potential of glyphosate to affect populations of soil fungi such as *Phytophthera* that may have secondary effects on the incidence of Sudden Oak Death (see the following section), but there is not enough known about these effects to do a quantitative analysis of risk.

Levels of concern for terrestrial plants: There is an abundance of information on the toxicity of glyphosate to plants. We used the USFS TRV for a seed emergence NOEC of 4.5 lb/acre for our analysis of glyphosate hazards to non-target plants (see Table 3-7 on page 3-52). Since glyphosate binds strongly to most soils, there is little uptake by other plants through root absorption,[169] and little threat to seedling germination. For vegetative vigor, we used the USFS TRVs for sensitive species tomato and radish, with a NOEC of 0. 035 lb a.e./acre. For tolerant plants such as ryegrass, corn, and onions, we used the NOEC of 0.56 lb a.e./acre.

3.3.2.D Soil Microbes

The EPA does not require that soil microorganism studies be performed as part of the registration process. There are also no Terretox database records that address soil microorganisms specifically. However, there are numerous soil microbial studies reported by the USFS. Although microbes also have a shikimate pathway that is affected by glyphosate, microbial glyphosate toxicity appears to be low.[4] One long-term study suggests that repeated glyphosate use does not decrease soil fertility.

Glyphosate is readily metabolized by soil bacteria, which are capable of using glyphosate as a source of phosphorous, and in some cases as a source of carbon.[170] In soils, glyphosate is first degraded to AMPA, and then to water, carbon dioxide and phosphate (see Section 3.4.3.A).[171] Glyphosate can both inhibit microbial growth and act as a nutrient for some organisms. In addition, it has also been found to increase carbohydrate and amino acid concentrations in root exudates in soybeans.[172] The combined effect of these two factors influences the populations of soil bacteria and fungi, in some cases promoting microbial growth and in other cases inhibiting it.

There is some evidence that glyphosate may inhibit the growth of microorganisms in artificial media. Direct toxic effects have been observed in fungi at concentrations of 10 ppm[173] and decreased growth in algae and cyanobacteria at concentrations of 800-3,380 mg/L.[174] These effects were consistent with a review[175] suggesting that loss of the shikimic pathway could adversely affect microbes. However, some studies point to other reasons for reduced microbial growth. One study reported decreased growth in the nutrient-absorbing surface (extraradical mycelia) in the fungal symbiont *Glomus intraradices* after 14 days of exposure to 0.5 ppm glyphosate in artificial culture.[176] This decrease in mycorrhizal growth was attributed to the adverse effects of glyphosate on carrot plants grown in soils containing *Glomus intraradices*.

No long-term soil fertility decreases have been reported as a result of glyphosate application. Soil concentrations of 100 ppm of glyphosate or AMPA had no significant effect on soil denitrification.[177] One long term study noted no effects on soil fertility in repeated 1.5 kg/ha, applications over 14 years (although not directly stated, it is implied that this is glyphosate without a surfactant).[178]

In a mini-review from researchers at the Mississippi USDA, glyphosate's effects on biological nitrogen fixation were discussed.[179] The authors concluded that previous laboratory research demonstrated the potential for reduced nitrogen fixation in glyphosate-resistant soybean plants. Although the MMWD will not be treating glyphosate-resistant plants, it is relevant to determine the impact of glyphosate on soil microbes which provide critical ecosystem functions.

The review found that soybean yield reductions due to reduced N_2 fixation have not been demonstrated. The authors focused on the symbiont *Bradyryhizobium japonicum* and mentioned that some bacteria in the nitrogen-fixing family Rhizobiaceae were not affected by glyphosate. In a later paper by the authors,[180] glyphosate-resistant soybean plants were shown to have reduced nitrate assimilation but relatively unchanged nitrogen fixation at application rates of 1.1 and 3.4 kg ae/ha. Reduced assimilation resulted in temporarily lower leaf nitrogen, increased plant protein and decreased plant oils. Although neither study suggests definitive whole plant or whole ecosystem alterations, these studies do suggest that glyphosate affects soil microbes and nutrient cycling.

When fungi are grown in soil media treated with glyphosate, no permanent changes in fungal populations and growth are observed. Application rates of 0.54 kg/ha showed decreased populations of soil fungi after 2 months, but no effect after 6 months.[4] Similarly, at 5 kg/ha of glyphosate, transient decreases in soil microbial activity were noted.[181] Application of 3.23 kg/ha did not affect soil fungi after 10-14 months. Several field studies have reported an increase in

microbial activity after the application of glyphosate.[182] Although not directly stated in USFS 2003, it is implied that these studies used glyphosate without a surfactant.

Glyphosate treatments of soybean seedlings have been observed to temporarily increase the populations of *Pythium* fungi in the soil.[183] Popluations of both *Pythium* and *Fusarium* fungi increased in soils treated with glyphosate or in which plants treated with glyphosate were grown.[184] This effect was attributed to cell leakage caused by glyphosate treatment, which made the root environment more favorable for *Fusarium* and *Pythium* growth. In contrast, laboratory studies with *Phytophthera cinnamomi* showed that glyphosate inhibited fungal growth.[185]

This work may be relevant to the MMWD watershed because of the high levels of Sudden Oak Death (SOD) caused by *Phytophthera ramorum* in the watershed. It is not clear how transferable the results from soybean experiments are to invasive weeds like broom in oak woodland environments. Broom, like soybean, is a legume with root nodules.[186] Whether or not this would translate to any increase or decrease of SOD is unknown at this time. Further research is needed to determine whether glyphosate use on broom and other invasive weeds might affect soil populations of *P. ramorum* and whether application timing might limit either effect.

Levels of concern for soil microbes: There are no soil microbe exposure models available, and since data are sparse and not representative of the full range of soil microorganisms, no TRV was selected. The units for soil microbe toxicity tests reported in Section 3.3.2.D are in kg/ha and can be compared directly to application rates that are being considered by the MMWD to estimate potential effects on soil microorganisms not evaluated in the risk assessment.

3.3.3 Aquatic Organisms

There is a substantial literature on the effects of glyphosate in both pure form and with surfactants on aquatic organisms. The EPA AQUIRE database gives a range of LC_{50} values and subchronic toxcicity levels for aquatic organisms. The use of surfactants in glyphosate products is a topic of particular concern. Many studies report that the surfactants in formulated glyphosate products are as toxic or more toxic than glyphosate alone.[4] The products that are considered most toxic are Credit Systemic, Credit, Glyfos, Glyphosate (a formulated product containing glyphosate and other ingredients), Glyphosate Original, Prosecutor Plus Tracker, Razor SPI, Razor, Roundup Original, Roundup Pro Concentrate, and Roundup UltraMax.[4] Less toxic products include: Aqua Neat, Aquamaster, Debit TMF, Eagre, Foresters' Non-Selective Herbicide, Glyphosate VMF, Roundup Biactive, and Roundup Custom.[4] Aquamaster, the product proposed for use by the MMWD, does not contain any additional surfactants. Additional discussion of surfactants can be found in Chapter 8 of this report.

3.3.3.A Fish

The EPA requires acute fish toxicity studies as part of the chemical registration process. The RED contains roughly 35 fish studies and the AQUIRE database contains over 600 records of studies, only about half of which were complete enough to summarize in this report. Acute toxicity studies indicate that, technical glyphosate ranges from not acutely toxic to slightly toxic to fish, and that glyphosate formulations with POEA surfactant can be slightly to highly acutely toxic.

Glyphosate toxicity can vary substantially depending on the product formulation, water hardness and pH. Fish studies suggest that increasing water hardness ameliorates glyphosate toxicity.[187] The ameliorating effect of water hardness is reversed in formulated glyphosate products; increased hardness makes Roundup slightly *more* toxic than soft water.[187] Amphibian studies indicate that high pH increases the toxicity of glyphosate.[188a, b] Some glyphosate formulations, like Roundup, contain surfactants that are toxic. These solutions have slight to moderate acute toxicity to fish. Tables D-8 and D-9 summarize the available data on glyphosate toxicity to fish; the most relevant studies are discussed below.

As summarized by USFS,[4] the EPA RED[5] reports 96-hour glyphosate LC_{50} values in freshwater fish ranging from 86 (70–106) mg/L in rainbow trout for an 83% pure sample of technical grade glyphosate to 140 mg/L (120–170) mg/L in rainbow trout using 97.6% pure technical grade glyphosate. Cold water fish like trout typically have higher glyphosate tolerances than warm water bluegill sunfish with an LC_{50} of >24 mg/L. Some reports find even lower LC_{50} values for technical grade glyphosate: 10 mg/L for trout in soft water[187] and 3 mg/L for unfed flagfish.[189] Use of formulated glyphosate products generally results in lower LC_{50} values compared to technical grade glyphosate. A large range of values has been reported, in part based on different product formulations and in part based on water hardness and different experimental conditions.

The AQUIRE database gives a range of 96-hour LC_{50} values for different fish. The range and median LC_{50} values in the AQUIRE database differ for technical grade glyphosate and formulated products. For technical grade glyphosate, LC_{50} values range from 8–645 mg a.e./L with a median value of 94 mg a.e./L. For glyphosate in formulated products containing surfactants, LC_{50} values range from 2.3–65.2 mg a.e./L, with a median of 23 mg a.e./L. There appear to be no systematic toxicity differences between species. Table D-8 in Appendix D provides a summary of the available records for LC_{50} values in fish in the AQUIRE database.

There are fewer studies on the sub-lethal effects of glyphosate on fish. The USFS 2003[4] cites six studies reporting acute effects other than mortality in fish.[190a-f] At concentrations that were fatal, Roundup damaged carp liver cells.[190a] Because this study reported effects on fish that were exposed to lethal concentrations of Roundup, this is not a particularly relevant study of the sub-lethal effects of glyphosate. These studies are summarized in Appendix D, Table D-9.

Roundup concentrations of 0.74 and 3.7 mg a.e./kg caused increased micronuclei frequency (the higher the frequency of micronuclei, the higher the likelihood that a chemical is carcinogenic).[190b] As reported in USFS 2003,[4] short-term exposures between 5–85% of the 96-hour LC_{50} values of several glyphosate formulations do not induce changes in biochemical blood parameters (used to indicate physiological stress).[190c] Also reported in USFS 2003, trout do not exhibit avoidance responses to glyphosate formulations at concentrations less than the 96-hour LC_{50}.[190d] However, behavioral changes (*e. g.* coughing and changes in ventilation rates, changes in swimming, loss of equilibrium, and changes in coloration) occurred at 25% of the LC_{50} values over 96-hour exposure periods. Histologic changes in the gills, kidneys, and liver of carp, *Cyprinus carpio*, were observed at concentrations of 2.5, 5 and 10 a.e.mg/L technical grade glyphosate over a period of 14 days.[190d]

The median chronic LOEC observed for rainbow trout exposed to Roundup is 0.07 mg/L. At this concentration, trout had reduced olfactory abilities.[191]

Levels of concern for fish: The USFS evaluated the effects of two formulations of glyphosate on aquatic life—one less toxic containing only glyphosate and one more toxic, representing Roundup containing POEA surfactant—on two surrogate species, a tolerant species and a sensitive species. The USFS also reported an acute and a chronic TRV, resulting in eight total TRVs for toxicity to fish. Since the MMWD will be using the less toxic glyphosate formulation (with no surfactant), only the four less-toxic TRVs are reported. See Table 3-7 on page 3-52 for a summary of these values.

Tolerant fish: For tolerant species and less-toxic glyphosate formulations, the USFS uses an acute TRV of 97 mg/L (LC_{50}) for fathead minnows exposed to glyphosate alone. For chronic exposure, the TRV for fathead minnows is 25.7 mg/L (NOEC) for technical grade glyphosate. We use the chronic NOEC of 25.7 mg/L for both the acute and chronic exposure in the MMWD assessment because 50% mortality of fish is not sufficiently protective.

Sensitive fish: For more-sensitive fish species, USFS used an acute TRV for rainbow trout of 10 mg/L (LC_{50}) for glyphosate alone. Using a ratio method (see below), the USFS estimated a chronic TRV of 2.57 mg/L for trout by multiplying the glyphosate-minnow chronic TRV times the trout to minnow ratio of acute TRVs.

$$\text{Chronic TRV}_{\text{glyphosate-trout}} = \text{Chronic TRV}_{\text{glyphosate-minnows}} \times \frac{\text{Acute TRV (glyphosate - trout)}}{\text{Acute TRV (glyphosate - minnows)}}$$

$$= 25.7 \text{ mg/L} \times \frac{10 \text{ mg/L}}{97 \text{ mg/L}} = 2.57 \text{ mg/L}$$

Because there was only an LC_{50} and no NOEC for the acute toxicity studies for sensitive species, we used the chronic USFS-estimated TRV of 2.57 mg/L for both acute and chronic scenarios in the MMWD assessment.[192]

3.3.3.B Amphibians

Although the EPA does not require amphibian studies as part of the registration process, there is a considerable body of literature on the effects of glyphosate on amphibians. In general, glyphosate is slightly more toxic to most amphibians than to fish. The active ingredient is rated not acutely toxic to slightly acutely toxic. Glyphosate formulated with a surfactant ranges from slightly acutely toxic to highly acutely toxic. Two amphibian studies on a total of four species (*Rana clamitans*, *Rana pipiens*, *Bufo amiercanus*, *Xenopus laevis*) show that higher pH makes glyphosate formulations more toxic,[188a, b] but unlike the fish data, no studies showing the effects of water hardness were encountered. Particular attention should be given to mesocosm studies by Rick Relyea that attempt to mimic field conditions. In several field studies, glyphosate treatment after clear-cutting did not appear to reduce amphibian populations.

Laboratory studies: In one study, the LC_{50} values were compared to LC_5 values for both glyphosate and Roundup for the African clawed frog (*Xenopus laevis*).[193] Glyphosate IPA, in the absence of a surfactant, has an LC_{50} of 5,407 mg a.e./L and an LC_5 of 3,779 mg a.e./L. In contrast, the same endpoints for Roundup are 6.9 mg a.e./L (LC_{50}) and 4.7 mg/L (LC_5) and for the surfactant POEA alone are 2.7 mg/L (LC_{50}) and 2.2 mg/L (LC_5).

Compared to the USFS report, the AQUIRE database includes much lower LC_{50} values for amphibians exposed to glyphosate alone, but similar LC_{50} values for the formulated products (see Tables D-10 and D-11). The median 48-hour LC_{50} value for five glyphosate studies for four amphibian species was 343 mg a.e./L. For formulated products with surfactants, the median value for 40 studies over 10 species was 5.5 mg a.e./L. The lowest LC_{50} for a formulated product was 0.41 mg/L for *Rana sylvatica* (in a tank that also contained predators),[194] lower than the lowest NOEC (0.61 mg/L for abnormal development in *Rana clamitans*).

The western chorus frog (*Pseudacris triseriata)* and the plains leopard frog (*Rana blairi*) are highly sensitive to Kleeraway Grass and Weed Killer RTU (Monsanto Australia), a product containing glyphosate IPA and POEA.[195] Tests exposed the frogs to concentrations of 0.56 mg a.e./L, 5.6 mg a.e./L, 56 mg a.e./L, and 560 mg a.e./L for 24 hours. After exposure, tadpoles were monitored for two weeks. Tadpoles were in Gosner stage 25 (characterized by a fully formed tadpole with no limbs, absent external gills, and fully formed feeding parts and mouth). Complete mortality was observed at all concentrations above 0.56 mg a.e./L, and 55% mortality was observed at this concentration. In a repeated experiment in the same study, tadpoles in Gosner stages 26-30 were heartier: above 0.56 mg a.e./L all tadpoles died, but at 0.56 mg a.e./L all tadpoles survived. *Rana blairi* tadpoles died at all concentrations.[195] Another study in *Rana catesbeiana* found genetic damage at Roundup concentrations greater than 6.7 mg/L.[196] Glyphosate alone was not evaluated in the study.

A review of five species of tadpoles exposed to various glyphosate formulations[197] (*Helioporus eyrei*, *Limnodynastes doralis*, *Litoria moorei, Crinia insignifera*) yields 48-hour LC_{50} values of 2.9–51.8 mg a.e./L for Roundup and 9.0–16.1 mg a.e./L for Touchdown. Roundup Biactive, a formulation containing a non-POEA surfactant that is approved for use in water, was much less toxic, with LC_{50} values ranging from 328 to >494 mg a.e./L. In the same study, the LC_{50} values for newly metamorphosed frogs exposed to Roundup ranged from 49.4 to 51. 8 mg a.e./L. These tadpoles were significantly less sensitive to glyphosate compared to *Pseudacris triseriata* and *Rana blairi* tadpoles;[195] however, other studies (discussed below) support the observation of higher sensitivity of most amphibians to glyphosate formulations with surfactants.

In another study, glyphosate concentrations of 1.8 mg/L caused reduced growth and survival in *Rana pipiens*.[198] The same study found that POEA alone and Roundup containing POEA caused significantly higher mortality than glyphosate alone and reduced growth at concentrations of 0.6 mg/L. POEA and Roundup also disrupt sexual development and the ratio of male:female adult frogs, while glyphosate does not.

A study of the toxicity of a POEA-containing Roundup formulation to 13 species of larval amphibians was reported in 2009.[199] Ninety-six hour LC_{50} values for frogs ranged from 0.8 to 2.0 mg a.e./L; for salamanders, values ranged from 2.7 to 3.2 mg a.e./L. Very little difference in sensitivity between species was noted.

Mesocosm and field studies: Rick Relyea performed multiple mesocosm and field studies on the effects of Roundup on amphibians. These studies may not be especially relevant to the MMWD project, since no Roundup or POEA surfactants are being considered for potential use on MMWD lands. However, the studies provide good examples of research that considers ecosystem effects and exemplifies potential controversies associated with glyphosate. Of particular interest are Professor Rick Relyea's "multi-tiered studies" which look at Roundup at a range of concentrations and experimental conditions. Relyea's studies examine: 1) how different Roundup concentrations—alone and in combination with other chemicals—affect five tadpole species' growth and survival in the laboratory;[200] 2) how Roundup affects the ability of six tadpole species to survive in the presence or absence of predator stress;[201] 3) how Roundup affects three tadpole species in artificial "ecosystems", or mesocosms, with invertebrate and vertebrate predators;[202] 4) how tadpole survival is affected by Roundup in different mesocosms which include different types of soil;[194] and 5) how the maximum application rate suggested on the Roundup label affects mesocosms lacking predators.[203]

The first study by Relyea[200] found that Roundup kills 30–60% of *Rana clamitans*, *Rana catesbeiana*, and *Bufo americanus* tadpoles at concentrations of 1.5 mg a.e./L. The particularly insensitive *Rana pipiens* only experiences about 15% mortality at this concentration. If the concentration of Roundup is reduced to 0.74 mg a.e./L, mortality is 5-15%. Mortality is unchanged with an additional 0.74 mg a.e./L of carbaryl or malathion. The presence of diazinon in conjunction with glyphosate does increase mortality because diazinon is more toxic than glyphosate; however, "chemical cocktails" of more than one pesticide examined in this study were no more toxic than their individual constituents. At both 0.74 and 1.5 mg a.e./L of diazinon added to glyphosate there was considerable reduction in tadpole growth. The reduction in growth was comparable to the growth reduction as a result of diazinon alone.

The second study by Relyea found that the ability of six tadpole species (*Rana clamitans*, *Rana sylvatica*, *Rana catesbeiana*, *Rana pipiens*, *Hyla versicolor*, and *Bufo americanus*) to survive Roundup exposure was reduced considerably in the presence of predatory stress from the red-spotted newt, or *Notophthalmus viridescens*.[201] Newts were not in direct contact with the tadpoles, but instead emitted chemical cues into the tadpoles' water. In the absence of the newt signal, Roundup LC_{50} values ranged from 1.1–11.4 mg a.e./L compared to 0.41–1.86 mg a.e./L in the presence of predatory cues.

Relyea's third study created whole-amphibian mesocosms with predators.[202] Mesocosms included *Hyla versicolor, Bufo americanus* and *Rana pipiens* tadpoles, zooplankton and predatory newts (*Notophthalmus viridescens*), predatory beetles (*Dytiscus* sp.) or no predators. The study found that Roundup concentrations of 0.96 mg a.e./L directly decreased survival by 40%, but there were no indirect effects of either predator. The fourth study found that the addition of either sand or loam soil did not affect the toxicity of glyphosate compared to tanks with no soil. This result is contrary to two studies[204] that suggest that sediments make glyphosate and POEA inaccessible, and thus less toxic, to aquatic life. However, the type of sediment matters (see Section 3.4.2).

Controversy surrounds the final Relyea study[203] which looked at the effects of a variety of chemicals applied at the maximum rate to mesocosms that included predators.[205] Three primary criticisms were leveled by Monsanto scientists: (1) the study is not as influential as it claims, (2)

the application rate is too high relative to actual ecosystem concentrations, and (3) there were methodological issues with the study. The first criticism revolves around the fact that Relyea did not reference articles that were conducted by scientists involved with all Roundup risk assessments prior to 2005. Often these risk assessments were published in collaboration with Monsanto. However, the reason these studies were not referenced in Relyea's work is that they had not been published in the peer-reviewed literature at the time of Relyea's initial submission. The second criticism states that the maximum application rate reported on the Roundup label is never applied to water bodies and therefore is not a reasonable concentration for a toxicity study. Numerous studies have reported temporary pulses of chemicals that approach, but rarely exceed, the application rate used by Relyea.[206] Furthermore, as stated in a previous paper by Relyea's critics, "small wetlands occurring within the target area are likely to be directly oversprayed," suggesting that Relyea's estimate of high aquatic concentrations are indeed realistic.[207] The third criticism, that there were methodological errors in the control treatment, was adequately addressed in a rebuttal by Relyea.[205]

Relyea's studies have been compared to a study conducted by Joel Trumbo at the CA Department of Fish and Game where a vegetation-free pond was treated with high concentrations of Rodeo in combination with the surfactant R-11. The R-11 product contains nonylphenolethoxylate (NPE) and nonylphenol (NP).[208] The concentrations of glyphosate, NPE, and NP were monitored in the pond for eight days after treatment, using composite sampling of the surface water in the pond. Surface sampling should provide a worst-case estimate of initial concentration, since pesticide applications to water bodies result in the formation of a concentrated layer of herbicide/surfactant on the surface of the water. This phenomenon would be exacerbated in thermally stratified water bodies or very shallow waters like vernal pools frequently used as breeding habitat by frogs.

Concentrations in the pond peaked in one location at 3.1 mg/L glyphosate (mean 1.83 mg/L), 1.8 mg/L NPE (mean 1.1 mg/L) and 0.03 mg/L NP (mean value) one hour after the applications and declined rapidly within the first 24 hours to 0.3 mg/L glyphosate, 0.4 mg/L NPE and 0.005 mg/L NP. The observed difference between the composite mean and the maximum concentrations suggests that there was some inhomogeneity in the distribution of the chemicals in the pond. Laboratory tests to determine 96-hour LC_{50} values for *Rana pipiens* for the mixture of glyphosate, NPE and NP were conducted separately, and these LC_{50} values (6.5 mg/L glyphosate, 1.7 mg/L NPE) were compared to measured concentrations in the pond. The author concluded that the mix of Rodeo and R-11 surfactant would not pose an acute toxicity hazard to tadpoles, even at the comparatively high application rates used in the study. This conclusion is not inconsistent with Relyea's work; the two studies taken together indicate that sub-lethal effects such as susceptibility to predation may play a more significant role in amphibian mortality than the acute toxicity of the applied chemicals.

Another field study reported the effects of glyphosate application on populations of six species of amphibians.[209] The effects of both clear-cutting and glyphosate were measured by comparing the capture rates of six amphibian species at the different sites. Red alder was removed from two sites in the Oregon coast range. One site received follow-up glyphosate treatment and a third site was kept as an untreated control. Prior to treatments, a survey of the amphibian species in the area was conducted. The survey species included: rough-skin newt, ensatina salamander, Pacific

giant salamander, Dunn's salamander, western redback salamander, and red-legged frog. Clear-cutting reduced amphibian populations regardless of whether glyphosate was used, but there was no difference between glyphosate treated clear-cut sites and untreated clear-cut sites.

Perkins et al 2000[193] looked for teratogenic effects of Roundup and Rodeo on *Xenopus laevis*, or the African clawed frog. Only groups that were exposed to lethal concentrations of glyphosate IPA, Roundup, and the POEA surfactant reported statistically significantly higher levels of abnormalities. The abnormalities linked to exposure include uncoiling of the gut, edema, blistering, abnormal pigmentation, and axial twisting in control embryos. See Table D-12 for a summary of sublethal effects of glyphosate on amphibians.

Studies of Aquamaster combined with the surfactants Competitor and Agri-Dex: The Santa Clara Valley Water District sponsored a study of the toxicity of the product Aquamaster (containing only glyphosate and water) and mixtures of Aquamaster with the surfactants Competitor and Agri-Dex to the California toad, *Bufo boreas halophilus*, a species native to the area.[210] This study is potentially the most relevant to the MMWD project, since Competitor is one of the adjuvants selected for potential use with Aquamaster. In the study, concentrations representing actual tank mixtures of Aquamaster and the two surfactants were tested. Based on four replicates of six concentrations, the 24-hour LC_{50} value for Aquamaster alone was determined to be 8,245 mg a.i./L; the LC_{50} for a 2:1 mixture of Aquamaster and Agri-dex was 5,092 mg a.i./L; and the LC_{50} for a 2:1 mixture of Aquamaster and Competitor was 854 mg a.i./L. Forty-eight hour LC_{50} values were 6,411 mg a.i./L for Aquamaster alone; 4,254 mg a.i./L for the Aquamaster/Agri-Dex mixture; and 711 mg a.i./L for the Aquamaster/Competitor mixture.

The results of this study are muddied by the use of undefined percents as concentration units instead of mg a.i./L, the lack of discussion of how the LC_{50} was derived from the data, and the lack of a control study on the surfactants alone; however, the data do indicate that the toxicity of glyphosate to *B. boreas halophilus* is enhanced by addition of the two surfactants. Mixtures containing Competitor were approximately 10 times more toxic than Aquamaster alone and mixtures containing Agri-Dex were approximately 1.6 times more toxic. The test species *B. boreas halophilus* appears to be one of the least-sensitive amphibians to glyphosate, with the observed LC_{50} higher than any value for amphibians exposed to glyphosate in US EPA's entire AQUIRE database.[6] All of the mixtures tested, including those containing surfactants, are classified as "Not acutely toxic," according to the standard toxicity ranking schema (see Table 2-6 on page 2-19).

Levels of concern for amphibians: The USFS reports neither exposure estimates nor TRVs for tadpoles or other amphibians. The literature on the toxicity of glyphosate to tadpoles is robust; therefore, we developed TRVs for less toxic glyphosate formulations. We only perform a risk assessment for the less toxic formulation, since Aquamaster does not contain a highly toxic surfactant and neither Competitor nor Sylgard 309 are as toxic as the POEA used in the more toxic glyphosate formulations. For acute exposure to glyphosate, the lowest LC_{50} from the AQUIRE database is used: >38.9 mg/L for *Rana clamitans*. This number is divided by six according to the 6x hypothesis[89] to give 6.5 mg/L, which corrects for the use of an LC_{50} instead

of a NOEC. The lowest (and only) chronic NOEC of 1.8 mg/L for *Rana clamitans* was used as the chronic glyphosate TRV.

3.3.3.C Aquatic Invertebrates

The EPA RED 1993 and AQUIRE database summarize multiple aquatic invertebrate toxicity tests. Overall, glyphosate is rated not acutely toxic to moderately acutely toxic for aquatic invertebrates. Field studies suggest that glyphosate is not acutely toxic at normal application rates. Tables D-13, D-14, D-15 and D-16 in the Appendix summarize the available toxicity data and highlights are discussed below.

The EPA RED 1993[5] reports that technical grade glyphosate is not acutely toxic to *Daphnia magna*, with a 48-hour LC_{50} of 780 mg/L. For the common midge, *Chironomus plumosus*, the 48-hour LC_{50} for 96.7% technical grade glyphosate is 55 (31-97) mg/L, which gives it a ranking of slightly toxic.

The toxicity of formulated glyphosate products to aquatic invertebrates can be much greater, with LC_{50} values as low as 3 mg/L (moderately acutely toxic). The AQUIRE database (see Tables D-13 and D-14) and USFS give comparable LC_{50} values for formulated products. For *Ceriodaphnia dubia*, AQUIRE reports an LC_{50} between 4 and 79 mg/L, with a median of 22 mg/L for Roundup. USFS 2003 [211] reports a mortality EC_{50} value for a formulation of glyphosate IPA (48%) and an oxide-coco-amide-propyl dimethyl-amine surfactant (15%) of 66 mg/L in *D. spinulata* and 62 mg/L in *D. magna*.

Glyphosate appears to increase the reproductive capacity of the aquatic snail, *Pseudosuccinea columella*, the intermediate host of the sheep liver fluke.[212] Snails were exposed to concentrations of 0.1, 1, and 10 a.e.mg/L of glyphosate without surfactants for three generations. The resulting effects were not concentration-dependent. The first two generations' maturation times were unaffected. The third generation of snails exposed to 1 mg/L developed much faster than those exposed to 0.1, 10 mg/L or the control group. The proportion of eggs that hatched was inhibited at 10 mg/L and slightly inhibited at 0.1 mg/L. Egg-laying capacity increased at all concentrations. In a follow-up study, snails were found to contain higher concentrations of the amino acids alanine, glycine, glutamic acid and threonine, than the controls. The authors suggest that glyphosate may be serving as an energy source for snails.[213] This study suggests that glyphosate may be beneficial to snails and thus harmful to livestock that are vulnerable to fluke infection.

Various field studies have not noted any remarkable effects of glyphosate on aquatic invertebrates. Application rates of 1 L Rodeo/ha for control of purple loosestrife produced no adverse effects to aquatic invertebrates.[214] USFS reports: (1) no indication of lethality in two water hyacinth weevils, *Neochetin eichhorniae* and *N. bruchi* at application rates of 0.94 or 1.48 kg a.i./ha as glyphosate IPA (Rodeo),[215] (2) no differences in invertebrate survival in a forest mesocosm over an 8-day period after sprays of 2.2 kg/ha, 22 kg/ha and 220 kg/ha of Rodeo,[216] and (3) no indication of short- or long-term (119 days) invertebrate effects after the application of a 4.7 L/ha Rodeo and 1 L/ha X-77 mixture to control of smooth cordgrass in an estuarine mudflats.[217] Glyphosate sprayed to the estuarine mudflat in reference 217 is quickly flushed from

the surrounding tidal waters but remains in sediments (28-59% of applied glyphosate remained after 119 days) and vegetation (1-9% of applied glyphosate remained after 28 days).[218]

As for fish, the same issues of water hardness, pH, and surfactant concentrations affect the toxicity of glyphosate to aquatic invertebrates. The USFS points out that "*the high variability in toxicity of the surfactant formulations to daphnids must be acknowledged*." Indeed, the AQUIRE database reports a range of EC_{50} values from 1 mg/L to greater than 5,000 mg/L for all invertebrates in the database.

Levels of concern for aquatic invertebrates: All aquatic invertebrate LC_{50} values used by the USFS for TRVs for glyphosate are from the EPA RED.[5] The USFS selected an acute TRV of 780 mg/L (LC_{50}) for technical grade glyphosate. This number is divided by six according to the 6x hypothesis[89] to give 130 mg/L, which corrects for the use of an LC_{50} instead of a NOEC. The USFS used a chronic TRV of 50 mg/L (NOEC). We also used this TRV in the MMWD hazard quotient calculations.

3.3.3.D Aquatic Plants

Gyphosate toxicity to plants is reported in both the RED and AQUIRE database. Toxicity to aquatic plants and algae varies considerably. However, aquatic plant toxicity endpoints are in the range of slightly to moderately acutely toxic, with just a few studies suggesting that glyphosate is highly toxic to aquatic plants.

Lemna minor, or duckweed, experiences reduced growth at glyphosate concentrations of 49.6 mg/L of technical grade glyphosate or 10.2 mg/L of formulated product.[219] Another duckweed study reports a NOEC of 3 mg/L for decreased growth rate, a LOEC of 6 mg/L, and an EC_{50} of 12 mg/L.[220] These studies suggest that glyphosate is slightly to moderately toxic to aquatic plants. Although glyphosate degrades rapidly in water, it can remain in water hyacinth tissues at least two weeks (the maximum duration of the study) after the concentration in the surrounding water is no longer measureable.[221]

Skeletonema costatum, a marine diatom, appears to be the most sensitive species reported in the RED. Population growth rates were substantially decreased at concentrations of 0.85 mg/L technical grade glyphosate.[5] The least sensitive species reported in the EPA RED is *Navicula pelliculosa*, another diatom, with an LC_{50} of 39.9 mg/L. The most sensitive species reported in the AQUIRE database was *Pseudokirchneriella subcapitata* which had decreased growth rates at 0.0028 mg/L for Roundup and 5.3 mg/L for technical grade glyphosate. The most tolerant species of algae reported in the AQUIRE and USFS 2003 respectively are *Chlorella fusca*, with an EC_{50} of 377 mg/L[222] or *Chorella pyreniodosa* with an EC_{50} of 1,082 mg/L. The endpoint for both studies was 50% inhibition of growth.[6]

Studies designed to mimic field exposure yielded different results than acute toxicity tests. Periphyton exposed to low glyphosate concentrations (0.0019–0.2874 mg/L) experienced increased growth rates.[223] The authors of the study hypothesize that algae use glyphosate as a phosphorous source, contributing to eutrophication. In a study designed to mimic direct overhead spraying of ponds, phytoplankton and zooplankton experienced no adverse effect after an application of Roundup at a rate of 0.43 kg/ha.[224]

Levels of concern for aquatic plants: USFS selected a TRV of 3 mg/L (NOEC) for duckweed for aquatic plant hazard quotient calculations. We used the same value for the MMWD assessment and did only a single risk assessment of the less-toxic formulations of glyphosate.

3.3.4 Data Gaps

Because of glyphosate's extensive use over the past 34 years, a considerable body of literature has been amassed focusing on potential toxicity to wildlife. Realistically, the toxicity literature on the active ingredient glyphosate is as good as can be expected. However, there is still uncertainty regarding the toxicity of various surfactants used in conjunction with glyphosate. The identity and concentration of surfactants is viewed as proprietary information by herbicide manufacturers. "Inert" ingredients like surfactants are not reported on product labels and toxicity information is not required. Although the USFS 2003[225] has a table outlining the toxicity of various glyphosate products, it is difficult to draw precise conclusions about toxicity without knowing all of the components in the formulated product and their concentrations.

Acute toxicity information was available for multiple surrogate species for all wildlife taxa in the MMWD watershed. Acute toxicity values vary substantially between species and tests. More information describing this variability would better define the range of toxicity. NOELs or LOELs were available for the sub-lethal effects of glyphosate for at least one surrogate species. Some sub-lethal toxicity information was available for all taxa. However fewer replicates were available for sub-lethal effects and rarely were reproductive, carcinogenic, developmental, histological and endocrine toxicity information available for all taxa. Reproductive toxicity was available for mammals, birds, and invertebrates. Carcinogenic and/or genetic toxicity data were available for mammals and fish. Developmental toxicity was available for mammals, birds, and amphibians. Histological information was available for mammals and fish.

Robust field measurements of the effects of glyphosate in natural settings are rare. A few studies addressed issues of competition and trophic interactions to glyphosate.[201, 202, 203] Several others compared species richness and productivity in field sites where glyphosate was applied to sites where glyphosate was not applied.[209] Several studies addressed "chemical cocktails" where species were exposed to more than one herbicide at a time.[200, 222] More field studies like these would help clarify the differences between laboratory and field toxicity of glyphosate.

More research is urgently needed to determine whether glyphosate affects growth of the soil fungus *Phythophthera ramorum* that causes Sudden Oak Death.

Table 3-7: Comparison of Glyphosate Toxicity Reference Values Used in USFS and MMWD Risk Assessments

		USFS				MMWD			
Taxa	**Exposure Type**	**Selected Endpoint**	**Dose**	**Adjustments to Dose**	**TRV Used in USFS Risk Assessment**	**Selected Endpoint**	**Dose**	**Adjustments to Dose**	**TRV Used in MMWD Risk Assessment**
Humans									
	acute RfD	NOAEL (rabbit)	175 mg/kg-day	÷100[a]	2 mg/kg-day	NOAEL (rabbit)	175 mg/kg-day	÷100[a]	2 mg/kg-day
	chronic RfD	NOAEL (rabbit)	175 mg/kg-day	÷100[a]	2 mg/kg-day	NOAEL (rabbit)	175 mg/kg-day	÷100[a]	2 mg/kg-day
Mammals									
	acute	NOAEL (rabbit)	175 mg/kg-day	None	175 mg/kg-day	NOAEL (rabbit)	175 mg/kg-day	None	175 mg/kg-day
	chronic	NOAEL (rabbit)	175 mg/kg-day	None	175 mg/kg-day	NOAEL (rabbit)	175 mg/kg-day	None	175 mg/kg-day
Birds									
	acute	NOEC (diet, mallard)	5,620 mg/kg-day	X 10%[b]	562[b] mg/kg-day	NOEC (diet, mallard)	5,620 mg/kg-day	X 10%[b]	562[b] mg/kg-day
	chronic	NOAEL (diet, bobwhite)[e]	1,000 mg/kg-day	X 10%[b]	100 mg/kg-day	NOAEL (diet, bobwhite)[e]	1,000 mg/kg-day	X 10%[b]	100 mg/kg-day
Insects									
	honeybees	NOEL	50 (µg/bee)	÷0.000093[c]	540 mg/kg-day	NOEL	50 (µg/bee)	÷0.000093[c]	540 mg/kg-day
Plants									
	vegetative vigor, tolerant	NOEC	0.56 lb/acre	None	0.56 lb/acre	NOEC	0.56 lb/acre	None	0.56 lb/acre
	vegetative vigor, sensitive	NOEC	0.035 lb/acre	None	0.035 lb/acre	NOEC	0.035 lb/acre	None	0.035 lb/acre
	seed emergence	NOEC	4.5 lb/acre	None	4.5 lb/acre	NOEC	4.5 lb/acre	None	4.5 lb/acre
Fish									
	acute, a.i., tolerant	LC_{50} (fathead minnow)	97 mg/L	None	97 mg/L	NOEC (fathd. minnow)	25.7 mg/L	None[d]	25.7 mg/L
	chronic, a.i. tolerant	NOEC (fathd. minnow)	25.7 mg/L	None	25.7 mg/L	NOEC (fathd. minnow)	25.7 mg/L	None	25.7 mg/L
	acute, a.i. sensitive	LC_{50} (rainbow trout)	10 mg/L	None	10 mg/L	NOEC (fathd. minnow)	25.7 mg/L	÷10[d, e]	2.57 mg/L
	chronic, a.i. sensitive	NOEC (fathd. minnow)	25.7 mg/L	÷10[e]	2.57 mg/L	NOEC (fathd. minnow)	25.7 mg/L	÷10[e]	2.57 mg/L
Amphibians									
	acute, a.i.	No TRVs were given for amphibians.				LC_{50} (tadpoles)	38.9 mg/L	÷6[f]	6.5 mg/L
	chronic, a.i.					NOEC (tadpoles)	1.8 mg/L	None	1.8 mg/L
Aquatic Invertebrates									
	acute	LC_{50}	780 mg/L	None	780 mg/L	LC_{50}	780 mg/L	÷6[f]	130 mg/L
	chronic	NOEC	50 mg/L	None	50 mg/L	NOEC	50 mg/L	None	50 mg/L
Aquatic Plants									
	acute	NOEC	3 mg/L	None	3 mg/L	NOEC	3 mg/L	None	3 mg/L
	chronic	NOEC	3 mg/L	None	3 mg/L	NOEC	3 mg/L	None	3 mg/L

[a] For the human RfD, the animal NOAEL was divided by an interspecies uncertainty factor of 10 and an intraspecies factor of 10, equivalent to dividing by 100.
[b] The dietary LC_{50} was multiplied by 10%, which is the USFS estimate of the percentage of a bird's body weight it eats per day.
[c] The LC_{50} of 50 µg/bee was converted to a dose in mg/kg by multiplying by the conversion factor between mg and µg, 0.001 mg/ µg and dividing by the original USFS estimate of body weight of a bee: 0.000093 kg.
[d] Because there were no acute NOECs, the chronic NOEC was used. The acute LC_{50} could have been used and divided by 20[f], however this resulted in a TRV that was lower than the chronic NOEC.
[e] No chronic studies on sensitive species using technical-grade glyphosate were available; a study using glyphosate was used instead. The USFS procedure for turning an acute value into a chronic value for sensitive species was to multiply the chronic TRV for the tolerant species by the ratio of the acute TRV for the sensitive species to the acute TRV for the tolerant species.

(chronic sensitive TRV) = (chronic tolerant TRV) x [(acute sensitive TRV)/(acute tolerant TRV)].

[f] The factor of six is used when there is only an LD_{50} or LC_{50} value available, not the preferred NOAEL or NOEC. The factor of six is used by the US EPA in evaluation of endangered species effects and is based on a review of literature studies in which both LD_{50} or LC_{50} and NOAEL or NOEC values were available for comparison. The factor of 20 is used for especially sensitive species such as salmonids.

Table 3-8: Summary of Glyphosate Ecotoxicity Data

Taxa	Endpoint	Formulation[a]	Number of Studies	Dose (mg/kg, or mg/L for aquatic)[b] Min	Median	Max
Birds	8-day LC_{50}	glyphosate	6	>4,640	>5,000	>5,200
	21-day LD_{50}	Roundup	1	[a]	>3,700	[a]
	21-day LD_{50}	NR, glyphosate	3	950	[c]	2,000
	LOEL–testosterone and histological	Roundup	2	3.7	[c]	3.7
	NOEL–reproductive and weight changes	Roundup	5	74	1,000	1,660
Insects	LOEL–abundance	glyphosate	5	0.36	0.72 kg/ha	1.4
	NOEL–abundance, diversity	glyphosate	4	0.36	1.2 kg/ha	1.2
Insects	LOEL–abundance, food consumption, growth	Roundup	7	0.0028	0.36 kg/ha	2.1
	NOEL–abundance, diversity	Roundup, Vision	13	0.09	0.62 kg/ha	1.5 (a.i.)
Honeybees	2-4 day LD_{50}[d]	a.i., NR	3	62	100 µg/bee	100
	NOEL	not reported	1	[a]	50 µg/bee	[a]
Fish	1-4 day LC_{50}[d]	Roundup	184	2.3	19	65
	1-4 day LC_{50}[d]	glyphosate	111	10	24.5	645
	LOEC–physiology and avoidance	Roundup	2	0.74	[c]	7.4
	NOEC–biochemical	glyphosate	1	[a]	0.11	[a]
	NOEC–physiology, avoidance, feeding	Roundup, Vision	5	0.03	0.31	1.7
Amphibians	2-16 day LC_{50}[d]	Roundup Biactive, Glyphos, glyphosate	17	21	328	5,407
	2-4 day LC_{50}[d]	Roundup, Vision	28	0.41	5.1	38
	LC_{100}	Roundup	15	3.7	8.1	133
	LC_{100}	glyphosate	2	135	[c]	180
	LOEC–growth and development	Roundup	10	0.60	0.71	1.8
	NOEC–growth and development	glyphosate	4	1.8	1.8	1.8
	NOEC–growth and development	Roundup, Vision	11	0.60	2.3	18
Aquatic Invertebrate	LC_{50}	Roundup	9	4.4	22	857
	LC_{50}	glyphosate	2	55	[c]	780
	EC_{50}–population decrease	Roundup	16	0.17	5.3	16,000
	LOEC–mortality	glyphosate	2	2,540	[c]	5,070
	LOEC–mortality	Roundup	7	0.75	29	117
	NOEC–mortality	glyphosate	3	50	1,270	2,540
	NOEC–mortality	Roundup	8	0.96	7	57

See Appendix D for more details on the studies reported here.

[a] Only one value was reported.

[b] All terrestrial values (except those for insects) are reported in mg/kg. Insect toxicity is reported in either µg/bee for honeyebees or kg/ha for all other insects.

[c] No median is reported because there were only two studies.

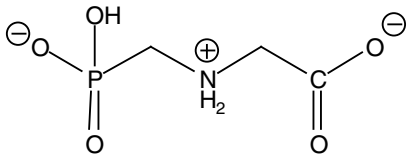
[d] Different study durations (2 versus 4 days) are grouped. See Appendix E for specifics.

3.4 Environmental Fate of Glyphosate

3.4.1 Overview

Glyphosate (CAS number 1017-83-6) is an organophosphonate herbicide, with empirical formula of $C_3H_8NO_5P$ and chemical structure shown below. Glyphosate is usually formulated as a sodium or potassium salt or as an amine or trimethylsulfonium salt of the carboxylic acid. Once in an aqueous environment, salts of glyphosate behave similarly to glyphosate itself, thus this review will report studies that involve both glyphosate and glyphosate salts.

In the most biologically relevant pH range of 5 to 9, the tri-protic weak acid glyphosate exists in the form shown below.



In aqueous solution, the phosphate (-PO3H2) and carboxylate (-COOH) groups in glyphosate participate in acid-base equilibria with water, losing or gaining a proton (H^+) depending on solution pH. The form of glyphosate at a given pH is dictated by the acid dissociation constants (pKa) as shown in Figure 1 below.[7] At different soil pH values, the predominant form of glyphosate will govern the ability of the compound to bind to soils and be transported in water.

Table 3-9: Chemical and Physical Properties of Glyphosate

Property	Glyphosate	Glyphosate IPA salt
CAS number	1071-83-6	38641-94-0
EPA PC code	417300	103601
Molecular weight (g/mol)	169.09	228.22
Water solubility (mg/L at ~25°)	11,600	15,000
Half-life (days)	44–60	44–60
Hydrolysis	35	35
Anaerobic	22	22
Aerobic	96	96
Field dissipation	3–174	3–174
Vapor pressure (mm Hg at ~25°C)	$< 7.5 \times 10^{-8}$	---
K_{oc} (mL/g)	554–34,000	554–34,000
K_{ow}	0.000316	---
K_H (atm-m^3/mol at ~25°C)	$< 1.44 \times 10^{-12}$	$< 1.44 \times 10^{-12}$

Data source: References 226, 227, 228.

3.4.2 Water Solubility and Soil Binding of Glyphosate

The parent acid glyphosate is a solid at room temperature and is highly water soluble (11,600 mg/L at 25°C). The octanol-water partition coefficient, log K_{ow}, is -2.8 to -3.5,[7, 228] indicating high solubility in water relative to organic solvents and low potential for bioaccumulation. The IPA salt of glyphosate has higher water solubility (15,000 mg/L at 25°C).

The organic-carbon-adjusted soil adsorption coefficient (K_{oc}) of glyphosate ranges between 554–34,000 mL/g.[4] The magnitude of this value indicates that, in a mix of soil and water, glyphosate preferentially binds to soils, with little remaining dissolved in solution. Interestingly, the observed range for K_{oc} is quite large compared to other pesticides. A number of studies on the different factors influencing glyphosate binding to soil have been performed to explore this phenomenon. The results indicate that glyphosate adsorption is governed mainly by binding to the mineral phase of soils.[229, 230] Glyphosate sorption increases in soils with low phosphate concentrations, and high availability of phosphate binding sites such as multivalent cations, especially trivalent iron and aluminum (oxides or hydroxides). Glyphosate sorption can occur at both low and high pH. There is only a weak correlation between glyphosate binding and soil organic matter, suggesting that the primary effect of organic matter is blocking of sorption sites on clay minerals. Thus the use of K_{oc} to quantify the adsorption of glyphosate is not an accurate descriptor of the adsorption processes.[229]

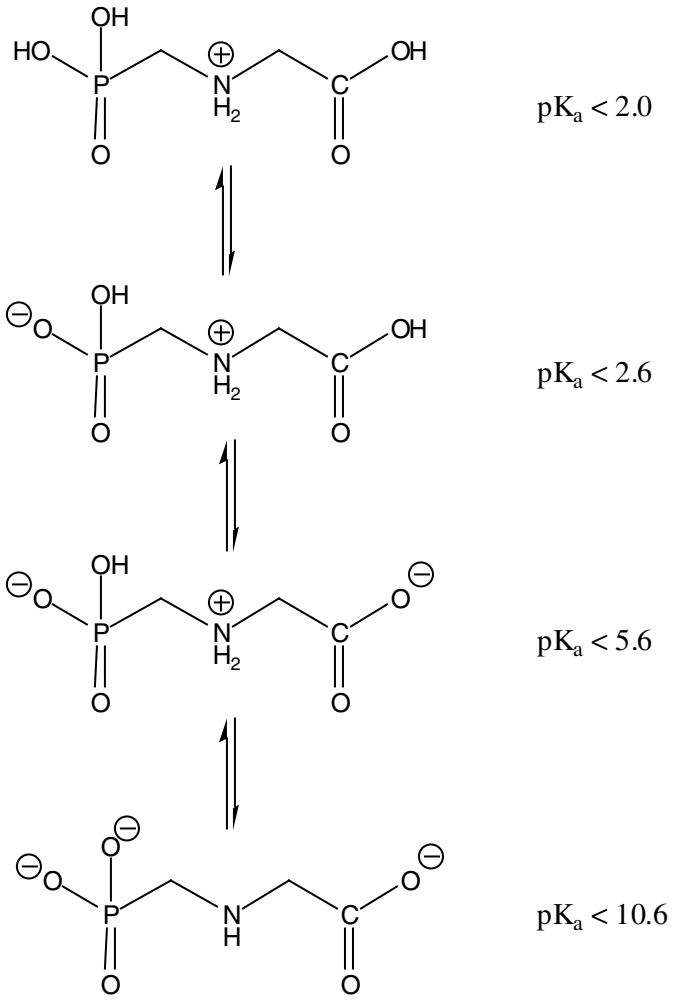


Figure 1: The ionic form of glyphosate is dependent on pH.

The precise relationship between all the pertinent glyphosate binding factors is still an area of active research.[231] However, it is clear that the presence of soil/sediments in aqueous solutions containing glyphosate reduces the effective herbicidal concentration and may also reduce the bioavailability of glyphosate.

3.4.3 Persistence of Glyphosate

Glyphosate and its primary degradation product aminomethylphosphonic acid (AMPA) are moderately persistent in the environment, with measured half-lives ranging from a few days to two years. On average, the half-life is ~1.5–2 months. Table 3-10 provides half-lives for specific studies under a variety of different conditions, and some of the more relevant studies are summarized briefly below.

In sterile, buffered, aqueous solution in the laboratory, glyphosate degrades primarily by hydrolysis, with a chemical half-life of greater than 340 days.[7] The addition of calcium ions to solution increases the photodegradation rate and decreases the half-life of glyphosate to 4–>14 days. UV light does not substantially accelerate the degradation of glyphosate in sterile buffered solution.[7] Photolysis does not seem to be a major degradation pathway for glyphosate in soils.[7]

In natural settings, glyphosate is subject to microbial degradation, adsorption to sediments and dissipation by wind and water. Thus, dissipation half-lives in the field are typically less than those observed under sterile laboratory conditions. Depending on soil type, temperature, microbial populations, and availability of oxygen, half-lives range from 1.5 days to 2 years.[7]

The dissipation half-life of glyphosate and AMPA in water ranges from 7–14 days.[232] In a recent study by the San Francisco Estuary Institute, two low-volume canals were treated with glyphosate. Immediately following the application, glyphosate concentrations were measured at 1.8 mg/L, a concentration above the Levels of Concern (LOCs) for some aquatic species. Concentrations at four monitoring stations ranged from 0.037–0.820 mg/L in the few hours after the application, but were below LOCs for all species within 24 hours. When two sites with larger water volumes were treated, there were no LOC exceedances. No toxicity was found in bioassays with *Ceriodaphnia dubia* and fathead minnows.[233]

Compared to aqueous half-lives, soil half-lives of both glyphosate and AMPA are substantially longer, 2–197 days for glyphosate and 76–240 days for AMPA.[232] In an Oregon study, the dissipation half-life in loamy soils was 29 days with and 40 days without leaf litter.[234] A Swedish study of glyphosate half-life in forest soils gave a value of <50 days.[235] Monsanto studies show half-lives ranging from 3–174 days in a variety of soils at different soil pH and organic matter content.[236] Studies in three forest locations in Oregon, Michigan and Georgia (also by Monsanto) show that most dissipation half-lives are <120 days.[237] Glyphosate concentrations were higher in exposed soils compared to those with undisturbed litter, potentially attributable to higher microbial activity in litter. A summary of existing studies by the CA Department of Pesticide Regulation indicates an average soil field dissipation half-life of 44–60 days.[238]

Table 3-10: Half-Life of Glyphosate Under Different Conditions

Conditions	Half-Life (days)	Type of Half-Life*	Comments	Reference
Water: Glyphosate (CAS 1071-83-6)				
35°C, pH 3, 5, 6, 9	Stable	Hydrolysis		228, 5
pH 5, 7, 9 and buffered	Stable	Photo-degradation under natural sunlight		228
	>35	Hydrolysis		228, 238
Forest ecosystem surface water—ponds high in sediment	1.5–11.2	Field dissipation		228, 239, 240, 234
Natural waters	35–63			228, 5
Pondwater	12–70		Glyphosate is strongly sorbed to suspended organic and mineral matter.	241
5–35°C, pH 5, 7, 9	Stable	Hydrolysis		242
Flooded silty clay	7	Aerobic		242
	5–14	Lab and field	Sediment is the major glyphosate sink	7
BC coastal rainforest—perennial slow flowing stream water	2	Field dissipation		243
BC coastal rainforest—sediment from slow flowing stream	22			243
Non-flowing pondwater and flowing water from 3 forest sites	7	Field dissipation		244
Manitoba forest site spray zones		Field study with 40L polyethylene basins	Study compared glyphosate concentrations in filtered and unfiltered pondwater samples	245
— filtered		Little dissipation after 30 days		
— unfiltered	6			
Pondwater (silty clay loam sediment), 23–25°C, pH 5.9–7.0	14	Aerobic		246
Pondwater (silty clay loam sediment), 20–27°C, pH 5.7–6.5	14	Anaerobic		246

Table 3-10 (cont.): Half-Life of Glyphosate Under Different Conditions

Conditions	Half-Life (days)	Type of Half-Life*	Comments	Reference
Soil: Glyphosate (CAS 1071-83-6)				
20-acre forest sites	100 (118 for AMPA)	Field dissipation	Aerially applied at 3.75 lb ai/acre	247
Canadian forest site, sandy soil	24	Field dissipation	Dead wood, live brush, and vegetation removed with minimal disturbance of the 5-10 cm soil horizon	248
	1–174	Field studies	Strongly adsorbed to most soils, even those with lower organic and clay content	241
25°C, sandy loam	1.85	Lab, aerobic		242
25°C, silt loam	2.06	Lab, aerobic		242
	0.9	Aerobic		227
25°C, pH 7.3, sandy loam	2	Bio-degradation; Lab, aerobic	Technical grade glyphosate	249
25°C, pH 7.5, silt loam	2	Bio-degradation; Lab, aerobic	Technical grade glyphosate	249
32°C, pH 5.7, sandy loam	130	Greenhouse dissipation	Technical grade glyphosate	250
32°C, pH 6.5, silt loam	3	Greenhouse dissipation	Technical grade glyphosate	250
32°C, pH 7.0, silty clay loam	25–27	Greenhouse dissipation	Technical grade glyphosate	250
pH 3.5–3.7, sandy, humo-ferric, boreal soils	20 (approx)	Field dissipation		251
pH 4.2–4.9, sandy loam, sandy clay loam	45–60	Field dissipation		252
pH 4.0–4.7, loam	29–40	Field dissipation		234
Loamy sand	3–4	Field dissipation		253
Sandy clay loam	122–174	Field dissipation		253

Glyphosate can be transported away from an application site or degrade in soil, water and air through a number of different chemical or biological processes. The most important processes for dissipation of glyphosate are microbial biodegradation, complexation in water with ions, e.g., Ca^{2+}, Mg^{2+}, and $Fe^{+2/+3}$, sorption to sediments and soils, uptake by plants, and photodegradation in water. Figure 2 describes the various degradation pathways for glyphosate.

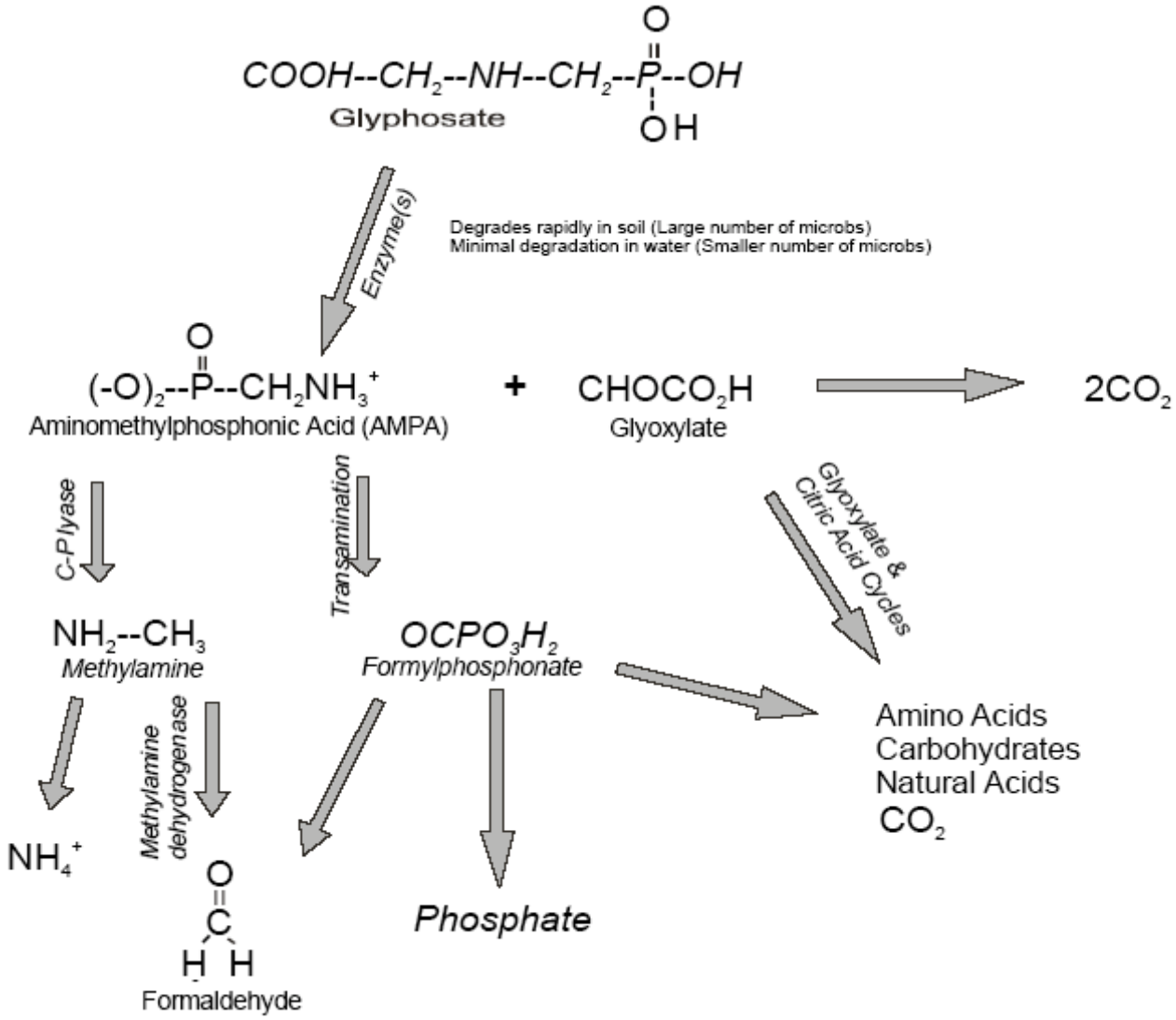


Figure 2: Degradation pathways of glyphosate, excerpted from Reference 228.

3.4.3.A Microbial Degradation

The primary route of glyphosate degradation in the environment is via microbial degradation in soil.[228] Under aerobic conditions, AMPA and glyoxylate are the primary degradation products. Further degradation yields carbon dioxide, phosphate, ammonia, and formaldehyde, where formaldehyde rapidly reacts with water and/or hydroxyl radicals to form methanol. AMPA degrades more slowly than glyphosate, and may also adsorb more strongly to soils.

Typically, the initial degradation rate is rapid, followed by a slower breakdown of the remaining glyphosate.[254] This observation may indicate rapid metabolism of free glyphosate, followed by slower degradation of the less-available, soil-bound glyphosate. The degradation rate is generally correlated with the microbial activity of soils, with microbes degrading glyphosate faster under aerobic conditions and at higher temperatures. Recent work in Denmark indicates that degradation rates correlate most strongly with the population size of *Pseudomonas* spp.bacteria in the soil.[255]

3.4.3.B Transport by Air

Air transport of glyphosate away from the application site can occur through spray drift during and for a short time after an application. Spray drift can contaminate soil and surface waters, damage non-target plants, and expose humans and wildlife through inhalation and dermal exposure. Post-application volatilization drift is not a significant source of off-site transport for glyphosate and its salts because of their low vapor pressures ($< 7 \times 10^{-9}$ mm Hg at 25° C). When dissolved in water, glyphosate does not appreciably escape to the air, as indicated by its very low Henry's law constant of $<1.44 \times 10^{-12}$ atm-m^3/mol.

3.4.3.C Transport by Water

In most agricultural settings, glyphosate does not leach more than six inches into soils. EPA concludes that glyphosate does not typically reach groundwater.[5] Laboratory experiments show that <0.1–11 percent of the applied herbicidal activity (glyphosate not bound to soil) is recovered when water is leached through soil columns under conditions simulating an extremely high rainfall.[7] Other work indicates that there are conditions under which substantial leaching of glyphosate to groundwater can still occur—when soil quality is poor, containing a high percentage of gravel and a low organic matter,[256] or in the presence of fractured soils.[257]

Surface waters may become contaminated when glyphosate-containing herbicides are applied over or near waterways, or during heavy rains when chemicals could runoff into lakes, streams and reservoirs.[258] Factors affecting the amount of glyphosate in runoff are rainfall intensity, soil composition, slope characteristics and vegetation cover.[230] Dissipation of glyphosate in surface waters is fairly rapid and occurs through dilution, downstream transport, or sorption to sediments.[259]

A Danish study measured concentrations of glyphosate in groundwater of <0.1–4.7 µg/L underlying agricultural application sites with subsurface tile drains.[257] Pesticides with strong soil-sorbing characteristics are generally not expected to contaminate groundwater, but preferential transport to groundwater can occur through flow pathways such as sand, gravel or soil fractures. This type of transport is enhanced by high post-application rainfall intensities on saturated soils and/or by structural characteristics that provide flow channels into groundwater.[229]

The discovery of glyphosate in field drain water in Denmark above the country's limit for all chemical contaminants of 0.1 µg/L led to a ban in 2003 of its use on certain clay soils in the fall rainy season. This ban was overturned in 2004 after glyphosate product manufacturers Monsanto, Cheminova and Syngenta objected that the finding of glyphosate and AMPA in subsurface drains did not equate to the presence of glyphosate in the deeper groundwater used

for drinking water.[260] After additional study, the Danish EPA agreed and revoked the ban on autumn uses in December 2004, stating:

> *" . . . the Danish Environmental Protection Agency believes that no unacceptable risk of pollution of the groundwater is associated with the currently approved agricultural use of glyphosate. the Agency thus does not consider that the updated state of our knowledge provides any technical grounds for the imposition of restrictions on the autumn application of glyphosate."* (translated from the Danish by Monsanto[260b])

3.4.3.D Uptake by Plants

Plants treated with glyphosate absorb the chemical though foliage or cut stems of a plant; root systems play a relatively minor role. Surfactants increase the rate of uptake of glyphosate from foliage and stems. Glyphosate is not metabolized by plants, but is translocated to all parts of the plant through the phloem and may accumulate in roots and nodules and eventually be released to the rhizosphere.[261]

3.4.3.E Field Studies on the Environmental Fate of Glyphosate

We evaluated several recent, large-scale monitoring studies to obtain an estimate of the potential for actual off-site transport under field conditions. Glyphosate and its primary degradation product AMPA were monitored in the Midwest US, Argentina, and Canada. Observed concentrations were below drinking water quality standards (US) and water quality criteria (Canada) in the relatively flat landscapes and agricultural soils of the Midwest and Canada. Higher concentrations were observed in the Argentina study.

In 2002, the USGS measured the concentrations of glyphosate, AMPA and several other herbicides in runoff from 51 Midwestern streams at three times during the growing season: 1) after the application of pre-emergence herbicides, May-June, 2) after the application of post-emergence herbicides, June-July, and 3) during harvest season, September-November.[262] Thirty-one to 40 percent of the samples contained glyphosate over the method reporting limit of 0.1 µg/L. The highest concentration detected was 9 µg/L measured during harvest season—well below the US EPA Maximum Contaminant Level (MCL) set for drinking water of 700 µg/L and the California Public Health Goal of 900 µg/L.[8] Most samples were in the range of 0.1–1.0 µg/L. AMPA was detected in 53–83% of samples, with a high concentration of 1.3 µg/L and most samples in the range of 0.1–0.5 µg/L. In spite of the fact that estimated use of glyphosate (50 million lbs) exceeded that of atrazine (42 million lbs) in the Midwest in 2002, observed glyphosate concentrations were substantially lower than those of atrazine. [262] Atrazine concentrations averaged 4 µg/L, with a maximum of 50 µg/L.

An academic Argentinian study evaluated glyphosate runoff in an area of intensive soybean cultivation.[263] Concentrations of glyphosate were much higher than those observed in the USGS study, ranging from 100 to 700 µg/L, with the highest concentrations observed immediately adjacent to the treated field. Concentrations were typically three to five times lower 1.5 kilometers downstream of the application site.

Environment Canada monitored glyphosate in the surface waters of an agricultural area in southern Ontario over a 2-year period.[264] Sampling was conducted bi-weekly from April to

November, but was not explicitly correlated with runoff events. Twenty-one percent of the samples contained glyphosate at concentrations higher than the method detection limit of 5 μg/L and 3.4% were above the method reporting limit of 17 μg/L. The highest concentration measured was 41 μg/L. None of the samples contained glyphosate at levels greater than 65 μg/L, the Canadian Water Quality Guideline for protection of aquatic life.

The USGS and Canadian studies showed fairly low surface water glyphosate concentrations in regions of very high glyphosate use, while Argentinian studies showed substantially higher surface water glyphosate concentrations. The differences observed between the Midwest US and Canadian studies and the Argentinian study may be a result of closer proximity of the Argentinian site to waterways, differences in terrain and soil types, and the contributions of overspray. The conflicting data indicate how important careful application is to avoiding contamination of nearby waterways.

Region 5 (Pacific Southwest) of the USFS has conducted water quality monitoring after herbicide use in reforestation or invasive plant management in the Eldorado, Stanislaus, Sierra, and Angeles National Forests between 1991 and 1999.[265] Glyphosate was monitored in eight of these studies, both immediately after application to detect any spray drift into waterways and during the first runoff event, typically 60–90 days after the application. A buffer zone of at least 10 feet between streams and waterways was utilized in all but one case, where spraying was actually conducted in the waterway for *Arundo donax.* Out of 104 total water and sediment samples taken for the eight projects, only two detections of glyphosate were noted—88 μg/kg in sediment (this result was attributed by USFS to either contamination of the sample or private land contribution) and 15 μg/L for one sample from the *Arundo donax* treatment area.

3.4.4 Aquamaster Product Profile

The Aquamaster™ product has been selected as one of the herbicides to be considered by MMWD for possible use in its Vegetation Management Plan (VMP). Aquamaster™ (US EPA reg # 524-343) contains the isopropylamine (IPA) salt of glyphosate as the active ingredient (a.i.) at 53.8 weight percent, with the remaining 46.2 percent made up with water.[266] It is approved for direct application to water bodies. The product contains 648 g/L (5.4 lbs/gal) of the a.i., which is equivalent to 480 g/L (4.0 lbs/gal) of the acid equivalent (a.e.) of glyphosate.[267] When applied as a foliar spray, Aquamaster is mixed at 0.5–8.0% a.i. in aqueous solution with a surfactant added to aid penetration of the a.i. through the waxy cuticle of the plant surface. The maximum label application rate to brush and forests is two gallons of product per acre per year and 8.44 kg/ha, and 4.22 kg/ha for perennial weeds. MMWD uses would require application rates of 0.5–2.0 lbs/acre. The label recommends use of no more than one quart of surfactant per acre. When applied as a cut-stump treatment, Aquamaster is used full-strength or diluted 1:1 with water and surfactant.

EPA has given this product an acute hazard warning label of CAUTION, placing it in Category IV.[268] This rating means that it is considered to be “Practically non-toxic.” Exposure to skin or eyes may cause skin irritation and eye irritation.[269]

3.4.5 Known Impurities

Technical grade glyphosate contains an impurity, N-nitrosoglyphosate (NNG).Specific information on nitrosamine concentrations in glyphosate formulations have been submitted to U.S.EPA, but are not available for public review.[270] However, the U.S.EPA has determined that 92% of technical grade glyphosate contains NNG at less than one part per million (<1 mg/L) and concludes that this amount is toxicologically insignificant.[4]

The POEA surfactant used in Roundup, another glyphosate-containing product, contains 1,4-dioxane as an impurity, a compound that is rated as a B2, Probable Human Carcinogen by the US EPA. The upper limit of this compound in Roundup is about 0.03%.[4] However, because MMWD has selected Aquamaster as the glyphosate-containing herbicide product, this impurity is not relevant to this risk assessment.

3.5 Exposure Assessment and Risk Characterization for Glyphosate

Assessment of risk requires knowledge of both the inherent toxicity of a chemical and the amount of exposure that is anticipated based on intended uses. Risk characterization combines the hazard and exposure data to provide a picture of risks associated with herbicide use.

This exposure analysis is divided into four categories: workers, general public, terrestrial wildlife, and aquatic life. Two types of applications are modeled: cut stump and foliar (with two types of foliar applications: backpack and ground spray). Higher concentrations are used for cut-stump and basal bark applications than for foliar applications. We assumed that the foliar application rate of glyphosate would be 1.0, 2.0, and 3.0 pounds per acre for Lower, Central and Upper estimates. The application rate would be 0.5, 1.0, and 2.0 pounds per acre for cut-stump or basal bark applications. The Central exposure estimate provides the most likely exposure scenario and the Upper estimate represents a low-probability, worst-case event. More information about the types of exposure scenarios considered in this risk assessment is available in section 2.5. Toxicity reference values for glyphosate used in the analysis are discussed in Section 3.2.1.I (humans) and Section 3.3 (animals and other organisms).

The worksheets created by the Syracuse Environmental Research Associates (SERA) for the USFS were used to calculate estimated glyphosate exposures for workers, the general public, and terrestrial and aquatic wildlife.[271] The details of how the exposure calculations were done are discussed in Section 2.5. Several additional exposure scenarios that were not in the SERA/USFS worksheets were added for the MMWD analysis, including drinking water exposure for birds and large mammals, all exposures for a large carnivore, and a TRV comparison for tadpoles. For water contamination, scenarios for accidental spills of concentrated and diluted glyphosate products, peak runoff and long-term runoff were evaluated to estimate herbicide concentrations in a small, thermally stratified pond and Bon Tempe Reservoir.

Finally, an additional worksheet was developed to sum the dermal and food exposures for wildlife to estimate aggregate doses. Aggregate worker exposures from multiple exposure events were also calculated. No aggregate exposures were estimated for the general public because of the low probability of multiple exposures.

Exposure scenarios were categorized as "**Highly Probable**," "**Probable**," "**Possible**," "**Improbable**" and "**Highly Improbable.**" These five categories are used throughout the exposure estimates to designate the qualitative likelihood of each scenario occurring. Common scenarios and their probabilities are summarized in Tables 2-8 through 2-11. Assigned probabilities are based on the assumption that the application guidelines are followed.

For all of the different exposure scenarios, **Lower**, **Central** and **Upper** estimates were calculated. Upper exposure estimates were calculated by changing all parameters to values that increase estimates; Lower estimates were obtained by changing all parameters to values that decrease estimates; and Central estimates used parameter values that are perceived as most realistic. See Section 2.5 for a complete description of parameter values used in the calculations.

Exposure estimates for humans and wildlife are presented and compared to human reference doses (RfDs) and wildlife toxicity reference values (TRVs) to give hazard quotients (HQs) that provide an estimate of risk from different exposure scenarios. RfDs differ from TRVs by inclusion of uncertainty factors to account for inter- and intra-species variation. Hazard quotients above one indicate that exposure exceeds the level of concern, and humans or wildlife may be at risk of adverse effects. These scenarios are flagged as potentially problematic and recommendations are made for how to avoid them. Hazard quotients between 0.1 and 1.0 suggest that there may be particularly sensitive individuals or species that may be affected. Hazard quotients below 0.1 indicate low levels of risk for the effects that have been studied and are represented by the TRVs. In this document, hazard quotients less than one are reported as a percent of the TRV; HQs greater than one are reported as a multiplier of the TRV, e.g. "the HQ was 2.4 times the TRV."

There are no added "inert" ingredients in Aquamaster, with the only ingredients being glyphosate and water; however, Aquamaster is usually mixed with a surfactant prior to use. The toxicity of mixtures of glyphosate with each of the surfactants being considered for use by MMWD—Competitor and Sylgard 309—is not fully known, but is anticipated to be much lower than that of glyphosate mixed with the POEA surfactant. See Chapter 8 for more discussion of surfactants.

3.5.1 Chemical-Specific Exposure Parameters for Glyphosate

Many of the parameters used to estimate exposure are constant from chemical to chemical, e.g., typical amounts of food consumed, surface area of a child and body weight, among others. These parameters and the values used in the exposure models are discussed in Section 2.5. Other parameters, such as absorption coefficients and water contamination rates, are chemical-specific and are based on experimental data and/or physical properties such as water solubility, K_{ow}, vapor pressure, K_{oc} and half-life.

Table 3-11 presents the glyphosate-specific parameters used in the calculations, including dermal absorption rates, bioconcentration factors, and glyphosate runoff rates. As discussed in Section 2.5.3A, USFS/SERA developed an estimate of dermal absorption rates based on K_{ow} and molecular weight.[271] These values were compared to studies of glyphosate absorption through the skin of monkeys, human cadavers[272a, b] and human volunteers.[273] Estimated dermal absorption was within a factor of four of the empirically measured rates. Given the agreement between the estimates and the empirical values, it would make little difference which absorption

rates were used. The slightly smaller empirical values from the human cadaver studies were used in the worksheets. The bioconcentration factor used by the USFS is derived from experimental data that validate model results.[274, 275] Glyphosate runoff rates are discussed in more detail in Section 3.5.3 on Water Contamination Estimates below.

Table 3-11: Glyphosate-Specific Exposure Parameters

Parameter	Lower Value	Central Value	Upper Value
First-order dermal absorption rate (h^{-1})	0.00013	0.00041	0.001
Dermal permeability (cm/hr)	$1.5x10^{-7}$	$3.7x10^{-7}$	$6.3x10^{-5}$
Water contamination rate, acute (mg/L per lb/acre)	0.001	0.02	0.4
Water contamination rate, chronic (mg/L per lb/acre)	0.0001	0.001	0.008
Bioconcentration (L/kg fish)	0.38	0.38	0.52
Half-life as residue in soil and on food (days)	34	46	58

Data source: Reference 271.

Brenton VMS listed the following techniques as potential strategies for MMWD for controlling invasive species with glyphosate:

- Low volume foliar applications to control broom at 1-2 pounds per acre
- Low volume foliar applications to control broom seedlings at 2 pounds per acre
- Spot foliar applications to control thistle at 1 pound per acre
- Foliar application to annual grasses at 1 pound per acre
- Foliar application to perennial grass at 2 pounds per acre
- Cut stump applications at 1 pound per acre

The application rates and volumes listed in Table 3-12 were used to calculate Lower, Central and Upper exposure estimates for workers, the general public, and terrestrial and aquatic wildlife. Glyphosate will be used in low-volume (10–25 gallons per acre) foliar applications with concentrations of 1–8% Aquamaster (by volume). The cut-stump/basal bark application scenario assumes that Aquamaster is 100% pure product not diluted with surfactant. We designate solutions designed for cut-stump applications "concentrated" and solutions for foliar application "diluted." To obtain the concentration in the Upper estimate, the Upper application rate is divided by the Lower volume. For Lower estimates, the Lower rate is divided by the Upper volume.

Table 3-12: Application Rate and Application Volume Model Inputs

Scenario	Parameter	Lower	Central	Upper
Foliar application	Application rate (lb a.e./acre)	1	2	3
	Percent a.i. (volume %)	1	3	8
	Application volume (gallons)	10	16.7	25
Cut-stump treatments	Application rate (lb a.e./acre)	0.5	1	2
	Percent a.i. (volume %)	100	100	100
	Application volume (gallons)	0.5	0.25	0.125

3.5.2 Application Methods for Glyphosate

Application methods that may be used on MMWD lands for glyphosate include directed foliar or cut-stump, or basal bark (thin-line) methods. In directed foliar applications, the herbicide sprayer

or container is carried by backpack and the herbicide is applied to selected target vegetation. Chemical contact with the arms, hands, or face is Highly Improbable because of the low height of the vegetation treated. In the rare cases where vegetation exceeds a height of 100 cm, one of two additional mitigations is possible: mowing the vegetation before treatment and application methods that target the base or trunk of the plant (e.g. basal bark). To reduce the likelihood of significant exposure, application crews should not walk through treated vegetation. Usually, a worker treats approximately 0.625 acre/hour with a plausible range of 0.25–1.0 acre/hour. The number of acres treated per hour or the number of hours worked per day will likely vary amongst different MMWD application sites. Variation in these numbers will change worker exposure estimates.

The cut-stump application involves cutting the stem, and then spraying or painting Aquamaster at a relatively high concentration (100% of the pure product) on the cut stump surface. Basal bark or thin-line application may also be used. Basal bark or thin-line involves applying a thin band of herbicide to the lower trunk of a target plant. Basal bark applications are less precise and use more dilute product than cut-stump applications but are more precise and concentrated than foliar applications.

3.5.3 Water Contamination Estimates

Concentration estimates for six different water contamination scenarios were calculated for glyphosate, each with Central, Lower and Upper values: four accidental spill scenarios, a peak runoff scenario and a long-term runoff scenario. Only the long-term runoff is considered Probable if the applications guidelines are adhered to. The four spill scenarios included two spill volumes (one and 20 gallons) each for a spill of the diluted product (used for foliar applications) and the concentrated product (used for cut-stump applications) to a thermally-stratified small pond and Bon Tempe reservoir. See Section 2.5.2 for a detailed discussion of these scenarios. Results are shown in Table 3-13.

Throughout this document, the word “contaminated” is used to mean that any amount of a chemical residue is present. “Contaminated” does not necessarily equate to hazardous, but indicates only that the compound is present at some level.

Predicted glyphosate water contamination rates were derived by USFS/SERA using GLEAMS modeling (see Section 2.5.2). The simulation assumes application to a 10-acre area (presumably forested). For the peak runoff scenario, the model assumes runoff into a small stream, which provides an estimate of the maximum concentration that might be observed over a very short period of time. Stream flow dilutes these concentrations rapidly. For long-term runoff, the model assumes runoff into a small pond 1,000 m^2 and one meter deep, which provides an estimate of the maximum concentration that might be observed over a longer period of time. The model results were validated using data from empirical studies where glyphosate application occurred directly adjacent to, or directly over, water bodies in a location where rain is frequent.[276a-d] The Upper value obtained for water contamination rates based on monitoring data is 0.28 mg/L per pound of glyphosate applied.[276c] The Lower range of values from the monitoring data is 0.003 to 0.007 mg/L per pound applied.[276d] The USFS/SERA worksheets provided similar values to bracket the Upper and Lower range of possible water concentrations in the model. As seen in Table 3-11, the acute runoff scenario gives contamination rates of 0.001 to 0.4 mg/L per lb/acre

for Lower and Upper scenarios, respectively. The Lower and Upper long-term contamination rates are 0.0001 and 0.008 mg/L per lb/acre.

Because the USFS model does not account for seasonally strategic vegetation management that avoids the most runoff-prone conditions, the calculations for long-term runoff scenarios were adjusted for MMWD local conditions. Starting with the USFS water contamination rates, the chemical half-life (see Table 3-9) was used to calculate a fraction of the chemical degraded for the 30-120 day window before the rainy season begins. This method is probably still an overestimate of water contamination rates because soils must be saturated before runoff can occur. Saturation typically occurs in November or December in Northern California, which would provide an additional 30–90 days for the chemical to degrade. See Section 2.4.2 for a detailed discussion of these and other limitations of methods used to estimate water contamination rates used in the USFS/SERA worksheets.

The only Probable water contamination scenario is the long-term runoff scenario. Peak runoff is Highly Improbable because applications would be conducted only in the dry season. Large volume accidental spills are also considered Highly Improbable.

Table 3-13: Calculated Glyphosate Concentrations for Water Contamination Scenarios

Scenario		Concentration (mg/L) Central	Lower	Upper
Thermally-stratified pond				
Accidental spill of diluted product	1 gal	0.21	0.073	0.55
	20 gal	4.2	1.5	11
Accidental spill of concentrated product	1 gal	7.3	[a]	[a]
	20 gal	150	[a]	[a]
Well-mixed reservoir				
Accidental spill of diluted product	1 gal	0.000011	3.7×10^{-6}	0.000028
	20 gal	0.00021	0.000074	0.00055
Accidental spill of concentrated product	1 gal	0.00037	[a]	[a]
	20 gal	0.0074	[a]	[a]
Rainfall runoff				
Peak runoff		0.040	0.0010	1.2
Long-term runoff		0.00081	8.7×10^{-6}	0.017

[a] Only a single, worst-case estimate of concentration was calculated for spills of concentrated Aquamaster product.

A final calculation, described in detail in Section 2.5.2, was performed to determine the maximum volume of Aquamaster that could be used in the MMWD watershed without exceeding glyphosate concentrations that produce an HQ > 0.1, 0.5 and 1.0 for a child drinking water from the reservoir, assuming 5% and 100% runoff of applied herbicide shown for the MMWD project Calculations were performed for both Phoenix Lake (well-mixed and thermally stratified) and Bon Tempe Reservoir to provide a range of estimates for the most broom-infested areas on MMWD lands. The results of these calculations for Phoenix Lake are presented in Table 3-14.

In the Phoenix Lake watershed, all 214 acres of broom (excluding broom in the buffer zone[277]) could be treated with Aquamaster and 100% of the applied herbicide could run off into the reservoir without exceeding an HQ of 0.02 for the more likely well-mixed water body (since runoff occurs in the winter and the lake is no longer thermally stratified then). Restrictions on the

volume of Aquamaster that could be used in the watershed would be needed for the two 100% runoff scenarios into a thermally-stratified water body to ensure that HQs not exceed 0.1 for water consumption. If it is determined that higher HQs are acceptable, more acres could be treated (see Table 3-14). An alternative to limiting the acres treated would be to reduce the application rate of the herbicide, as long as efficacy is maintained. All acres of broom could be treated without exceeding an HQ of for the 5% runoff scenario into a thermally stratified Phoenix Lake.

Results for Bon Tempe Reservoir are not presented, because there is less broom in the Bon Tempe watershed than the Phoenix Lake watershed and the volume of the reservoir is 9.75 times larger than Phoenix Lake. The entire broom acreage at Bon Tempe could be treated seven times in a season without exceeding an HQ of 0.1 for even the worst-case 100% runoff scenario.

Table 3-14: Maximum Volume of Aquamaster that Could Be Applied in Phoenix Lake Watershed without Exceeding Hazard Quotients of 0.1, 0.5, and 1.0

	Volume of Aquamaster (gal)		Maximum Area Treated at 2 lb/acre (acres)	
Scenario	Well-Mixed Water Body	Stratified Water Body	Well-Mixed Water Body	Stratified Water Body
HQ = 0.1				
100% runoff, no degradation	530	16	1,100	32
100% runoff, degradation for 60 days (half-life of 46 days)	1,300	39	2,600	78
5% runoff, degradation for 60 days (half-life of 46 days)	26,000	780	52,000	1,600
HQ = 0.5				
100% runoff, no degradation	2,600	80	5,300	160
100% runoff, degradation for 60 days (half-life of 46 days)	6,500	200	13,080	390
5% runoff, degradation for 60 days (half-life of 46 days)	130,000	3900	261,670	7,800
HQ = 1				
100% runoff, no degradation	5,300	160	10,600	320
100% runoff, degradation for 60 days (half-life of 46 days)	13,000	390	26,160	780
5% runoff, degradation for 60 days (half-life of 46 days)	260,000	7800	523,340	16,000

The use of 100% as the percentage of applied herbicide that runs off after an application is an overestimate. Runoff rates for glyphosate calculated by the USFS/SERA worksheets are typically much lower, ranging from four to 20 percent for average rainfall in Marin County. Table 3-15 shows estimated glyphosate loss through runoff as a fraction of the application rate for various soil types. Since site-specific parameters are needed for such modeling, Table 3-15 serves as only a rough estimate of runoff rates for MMWD lands. The USFS/SERA worksheets (and hence the water contamination rates for the exposure estimates) do not incorporate site-specific characteristics like distance between the treatment site and the water body, volume of the water body receiving runoff, seasonality of rain, and acres treated.

Table 3-15: Fraction of Glyphosate Lost as a Function of Annual Rainfall

Annual Rainfall (inches)	Fraction of Herbicide Lost on Soil Type		
	Clay	Loam	Sand
5	0.00	0.00	0.00
10	0.00	0.00	0.00
15	0.01	0.01	0.01
20	0.03	0.07	0.08
25	0.04	0.10	0.09
50	0.10	0.20	0.13

Data source: Reference USFS/SERA worksheet G04.

3.5.4 Risks to Humans

Exposure estimates were performed for both workers and members of the general public. Accidental/incidental and general handling exposures were considered for herbicide applicators for ground spray, backpack spraying and cut-stump applications. Public exposure estimates were developed for the scenarios of people contacting contaminated vegetation on or near an application site, eating contaminated fruit or fish, or drinking contaminated water. Acute and chronic exposure scenarios were evaluated to obtain a range of exposure estimates for both worst-case and more probable scenarios.

3.5.4.A Workers

Risks from accidental and general exposure scenarios were calculated for workers. Accidental exposures include wearing contaminated gloves for one minute and one hour, direct spray onto hands, and direct spray to lower legs. General exposures for backpack spraying and ground spraying were calculated.

None of the Central estimates for general worker exposure exceeded 10% of the RfD. Upper exposures with hazard quotients above 0.1 included general exposure for backpack spraying (12% of the RfD) and ground spraying (23% of the RfD). Aggregate exposure estimates were also below the RfD. The highest exposure estimate for the Improbable scenarios was an accidental spill to the lower legs during a cut stump application and left unwashed for one hour, resulting in an Upper exposure estimate that is 5.7% of the RfD. Doses and hazard quotients for all worker exposure scenarios can be found in Table 3-16.

Exposure estimates from the scenarios that are the most likely to occur for workers are highlighted below:

1. **General exposure due to backpack spraying (Highly Probable)**. The Central dose estimates for general backpack spraying exposure are 1.3% of the RfD for foliar applicators and 0.66% of the RfD for cut-stump applicators. Upper estimates are 12% and 8% of the RfD, respectively.
2. **General exposure due to ground spraying (Highly Probable)**. The Central dose estimate for general ground spraying exposure is 2.2% of the RfD. The Upper estimate is 23% of the RfD.

3. **Wearing contaminated gloves for one minute (Probable)**. The Upper dose estimate for wearing contaminated gloves for one minute for foliar applicators is 0.0023% of the RfD and 0.03% of the RfD for cut-stump applicators. Central estimates are substantially lower.
4. **Wearing contaminated gloves without washing for one hour (Improbable)**. The Upper dose estimates for wearing contaminated gloves without washing for one hour are 0.14% of the RfD for foliar applications and 1.8% of the RfD for cut-stump applicators. The Central estimates are substantially lower.
5. **Accidental spill to the hands that is left unwashed for one hour (Improbable)**. The Upper dose estimates for a spill on workers' hands that is left unwashed for one hour are 0.17% of the RfD for foliar applicators and 2.3% of the RfD for cut-stump applicators. The Central estimates are substantially lower.
6. **Accidental spill to the lower legs that is left unwashed for one hour (Improbable)**. The Upper dose estimate for a spill on workers' lower legs that is left unwashed for one hour is 0.4% of the RfD for foliar applicators and 5.7% of the RfD for cut-stump applicators. The Central estimates are substantially lower.

If accidental worker exposures occur, the dose from that scenario must be added to the general exposure to obtain an aggregate dose. For example, if a worker sprays vegetation with a backpack sprayer for 8 hours and also wears a contaminated glove for one hour, the combined Upper exposure estimate is 0.24 + 0.036 = 0.276 mg/kg-day. For glyphosate, the general exposure estimates are so much higher than the accidental exposures that aggregate exposures are not significantly different from general exposure. A worker would have to spill chemical on his or her hands seven times and leave it there for an hour before he or she would achieve an Upper estimated dose equivalent to a day of general exposure from applying herbicide.

These exposure estimates do not include splashes into the eyes, as there are no quantitative exposure estimates for this situation. Glyphosate and glyphosate formulations are mild to moderate skin and eye irritants,[266] but little systemic absorption would be expected from such an event.

Confidence in these exposure assessments is reasonably high because of the availability of dermal absorption data in humans as well as worker exposure studies. All estimates assume workers wear personal protective equipment. Doses and hazard quotients for all worker exposure scenarios can be found in Table 3-16.

The risk characterizations for both ground spray and cut-stump workers suggest that careful workers would be at minimal risk of exceeding the RfD. Precautions should be taken to avoid spills to unprotected skin and eyes including the use of goggles, gloves, long-sleeved clothing and closed shoes. Applicators should have extra gloves, soap and water for washing off spills, and an eyewash bottle in their vehicle at all times.

Table 3-16: Estimated Glyphosate Exposures and Hazard Quotients for Workers

Scenario	Calculated Dose (mg/kg-event)			RfD (mg/kg-day)	Hazard Quotient (HQ)		
	Central	Lower	Upper		Central	Lower	Upper
Foliar Worker Accidental/Incidental Exposures (dose in mg/kg-event)							
Contaminated gloves, 1 min	$4.2x10^{-6}$	$3.6x10^{-7}$	0.000045	2	$2.1x10^{-6}$	$1.8x10^{-7}$	0.000023
Contaminated gloves, 1 h	0.00025	0.000021	0.0027	2	0.00013	0.000011	0.0014
Spill on hands, 1 h	0.00055	0.000060	0.0035	2	0.00028	0.000030	0.0017
Spill on lower legs, 1 h	0.0014	0.00015	0.0085	2	0.00068	0.000074	0.0043
Cut Stump Worker Accidental/Incidental Exposures (dose in mg/kg-event)							
Contaminated gloves, 1 min	0.00014	0.000036	0.00060	2	0.000072	0.000018	0.00030
Contaminated gloves, 1 h	0.0086	0.0021	0.036	2	0.0043	0.0011	0.018
Spill on hands, 1 h	0.019	0.0060	0.046	2	0.0094	0.0030	0.023
Spill on lower legs, 1 h	0.047	0.015	0.11	2	0.023	0.0074	0.057
Foliar Worker General Exposures (mg/kg-day)							
General exposure, backpack spraying	0.026	0.00045	0.24	2	0.013	0.00023	0.12
General exposure, ground spraying	0.045	0.00066	0.45	2	0.022	0.00033	0.23
Cut Stump Worker General Exposures (mg/kg-day)							
General exposure, backpack spraying	0.013	0.00023	0.16	2	0.0066	0.00011	0.080

RfD = Reference dose. Hazard Quotients above 0.1 are shaded. Hazard Quotients greater than one are **bolded.**

3.5.4.B General Public

Acute and chronic glyphosate exposure scenarios for the general public were evaluated for direct spray onto a person, contact with contaminated vegetation, and consumption of contaminated fruit, fish and water. The general public may be at greater risk than workers because they lack protective gear and may engage in riskier behaviors. Estimated exposures are summarized for different scenarios for the general public in Table 3-17 below.

Only two exposures are considered to be Probable or Possible for the general public:

1. **A man consuming contaminated fish after long-term runoff (Possible).** The Upper chronic dose estimate for a man eating contaminated fish is 0.00035% of the RfD. The Central estimate is substantially lower, indicating that this scenario would not be a major contributor to overall exposure.
2. **A child consuming contaminated water after long-term runoff (Probable).** The Central estimated dose is less than 0.0006% of the RfD. The Upper estimate is 0.019% of the RfD. See below for more details on this calculation.

It is also useful to consider the scenarios that yield the highest exposures, regardless of their probability, to evaluate the potential need for additional precautions to protect the public. For glyphosate, only the Highly Improbable scenario of a child drinking out of a small pond into which 20 gallons of concentrated product had been spilled exceeded an HQ of one for Lower, Central, and Upper estimates. Three additional exposure scenarios resulted in Upper HQs greater than 0.1.

1. **A child drinking from a thermally stratified, small pond contaminated with a 20-gallon spill (Highly Improbable).** The Upper dose estimates for a 20 gallon spill of

diluted and concentrated glyphosate product to a pond are 61% of the RfD and 7.9 times the RfD, respectively. The Upper dose estimates for a one gallon spill of concentrated glyphosate product is 41% of the RfD.

2. **Direct spray of a child over its entire body (Highly Improbable).** The Upper dose estimates for diluted and concentrated glyphosate product are 6.5% of the RfD and 87% of the RfD, respectively.
3. **A woman consuming contaminated berries (Improbable).** The Central chronic dose estimates for eating berries contaminated by diluted and concentrated glyphosate product are 0.64% of the RfD and 0.32% of the RfD, respectively. The Upper chronic HQs exceed 0.1, at 17% and 11% of the RfD, respectively. Foliar applications lead to higher HQs than cut-stump applications because foliar applications have a higher application rate. Exposure calculations do not account for uneven spray of herbicide.
4. **A woman brushing against vegetation contaminated with diluted or concentrated product (Improbable)**. The Central dose estimate for a woman brushing against contaminated vegetation is 0.11% of the RfD for concentrated solutions and 0.051% for dilute solutions. The Upper estimate is 0.4% of the RfD, indicating that this scenario would not be a major contributor to overall exposure.

The scenario of eating contaminated berries is Improbable if the application guidelines are followed. In order to reduce the probability of exposures, the public should be made aware of application timing and locations, and berry bushes or other edible plants should be trimmed or mowed before herbicide treatments. Conducting applications during the week instead of on the weekend, limiting access to application sites, and avoiding off-target direct sprays to blackberry, blueberry (huckleberry), thimbleberry, hazelnut, and manzanita plants will help ensure public safety.

The likelihood of exposures from brushing against contaminated vegetation can be reduced to Improbable by trimming or mowing vegetation prior to treatment.

Water Consumption Scenarios: Only the Highly Improbable scenario in which a child drinks from a thermally stratified pond contaminated with concentrated product resulted in HQs greater than 1.0 (20-gallon spill, HQ=5.5 times the RfD) and greater than 0.1 (1-gallon spill, HQ= 27% of the RfD).

Concentrations of glyphosate from spills into a reservoir like Bon Tempe were lower than those for spills into a small pond by a factor of 20,000, and HQs are substantially less than one—0.04% of the RfD for the Upper exposure estimate for a child drinking out of Bon Tempe reservoir after a 20-gallon spill of concentrated product. Adherence to the MMWD application guidelines would make a high-volume spill of concentrated product into a reservoir Highly Improbable. With a plan in place to notify water treatment plants if such a spill were to occur, we conclude that it is Highly Improbable that drinking water quality in MMWD reservoirs will be compromised by spills of glyphosate into the reservoirs.

Contamination by long-term runoff is Probable if many acres are treated in a single year. The Central estimated concentration is less than 0.0006% of the RfD. The Upper estimate is 0.019% of the RfD. This calculation provides an estimate of water contamination based on application

rate and may *underestimate* concentrations if more than 10 acres are treated. The calculation may *overestimate* concentrations for runoff into water bodies larger than a small pond (i.e., all of the the MMWD reservoirs are much larger than the small pond used in the calculation), and for applications in which a buffer zone is used. See Section 3.5.3 and Section 2.5.2 for a more detailed discussion.

As a check on the USFS worksheet number, calculations were performed to estimate concentrations for a worst-case scenario (Section 3.5.3), assuming that all 214 acres of broom in the Phoenix Lake watershed were treated with Aquamaster at 2.0 lbs/acre. If 100% of the applied herbicide ran off into Phoenix Lake during the winter rainy season, the HQ for a child drinking water from the reservoir would be 2% of the RfD. This scenario that is unlikely to occur, since 100% of the herbicide will not run off (5–20% is a more realistic estimate, see Table 3-15), and Phoenix Lake is not currently used as a water supply. The larger Bon Tempe reservoir would dilute any runoff by a factor of 9.75 compared to Phoenix Lake, and there is slightly less broom in the Bon Tempe watershed. Adjusting the parameters to a more realistic, but still high-end scenario (20% runoff into Bon Tempe Reservoir, 214 acres treated) gives an HQ of 0.04% of the RfD. Treatment of fewer acres each year and use of buffer zones around water bodies (as the MMWD application guidelines require) would reduce HQs further.

Table 3-17: Estimated Glyphosate Exposures and Hazard Quotients for the General Public

Scenario	Receptor	Calculated Dose (mg/kg-event)			RfD (mg/kg-day)	Hazard Quotient (HQ)		
		Central	Lower	Upper		Central	Lower	Upper
Acute exposure estimates for diluted glyphosate product (foliar treatment)								
Direct spray of child, whole body	Child	0.021	0.0023	0.13	2	0.010	0.0011	0.065
Direct spray of woman, feet, lower legs	Adult female	0.0021	0.00023	0.013	2	0.0010	0.00011	0.0066
Vegetation contact, shorts and T-shirt	Adult female	0.0022	0.00033	0.0083	2	0.0011	0.00016	0.0041
Contaminated fruit consumption	Adult female	0.024	0.0034	0.56	2	0.012	0.0017	0.28
Water consumption (pond) after 1 gal spill	Child	0.016	0.0033	0.061	2	0.0080	0.0017	0.031
20 gal spill	Child	0.32	0.067	1.23	2	0.16	0.033	0.61
Water consumption (reservoir) after 1 gal spill	Child	$8.1x10^{-7}$	$1.7x10^{-7}$	$3.1x10^{-6}$	2	$4.1x10^{-7}$	$7.9x10^{-8}$	$1.6x10^{-6}$
20 gal spill	Child	0.000016	$3.4x10^{-6}$	0.000062	2	$8.2x10^{-6}$	$1.7x10^{-6}$	0.000031
Water consumption after peak runoff	Child	0.0030	0.000046	0.14	2	0.0015	0.000023	0.068
Fish consumption (pond) after 1 gal spill	Adult male	0.000088	0.000030	0.00022	2	0.000044	0.000015	0.00011
20 gal spill	Adult male	0.0017	0.00060	0.0045	2	0.00087	0.00030	0.0022
Fish consumption (reservoir) after 1 gal spill	Adult male	4.4×10^{-9}	$1.5x10^{-9}$	$1.1x10^{-8}$	2	$2.2x10^{-9}$	$7.5x10^{-10}$	$5.5x10^{-9}$
20 gal spill	Adult male	$8.8x10^{-8}$	$3.0x10^{-8}$	2.2×10^{-7}	2	4.4×10^{-8}	$1.5x10^{-10}$	$1.1x10^{-8}$
Fish consumption (pond) after 1 gal spill	Subsistence male	0.00089	0.00030	0.0023	2	0.00044	0.00015	0.0011
20 gal spill	Subsistence male	0.018	0.0061	0.046	2	0.0089	0.0030	0.023
Fish consumption (reservoir) after 1 gal spill	Subsistence male	$4.5x10^{-8}$	$1.5x10^{-8}$	$1.2x10^{-6}$	2	2.3×10^{-8}	$7.8x10^{-9}$	$5.9x10^{-8}$
20 gal spill	Subsistence male	$9.0x10^{-7}$	$3.1x10^{-7}$	$2.3x10^{-6}$	2	$4.5x10^{-7}$	$1.6x10^{-7}$	$1.2x10^{-6}$
Acute exposure estimates for concentrated glyphosate product (cut-stump treatment)								
Direct spray of child, whole body	Child	0.71	0.23	1.74	2	0.36	0.11	0.87
Direct spray of woman, feet, lower legs	Adult female	0.072	0.023	0.17	2	0.036	0.011	0.087
Vegetation contact, shorts and T-shirt	Adult female	0.0010	0.00015	0.0053	2	0.00051	0.000077	0.0027
Contaminated fruit consumption	Adult female	0.012	0.0017	0.37	2	0.0060	0.00084	0.19
Water consumption (pond) after : 1 gal spill	Child	0.55	0.33	0.82	2	0.27	0.17	0.41
20 gal spill	Child	11	6.67	16	2	**5.5**	**3.33**	**7.9**
Water consumption (reservoir) after 1 gal spill	Child	0.000028	0.000017	0.000042	2	0.000014	$8.6x10^{-6}$	0.000021
20 gal spill	Child	0.00055	0.00034	0.00083	2	0.00028	0.00017	0.00042
Water consumption after peak runoff	Child	0.0015	0.000023	0.090	2	0.00075	0.000011	0.045
Fish consumption (pond) after 1 gal spill	Adult male	0.0030	[a]	[a]	2	0.0015	[a]	[a]
20 gal spill	Adult male	0.060	[a]	[a]	2	0.030	[a]	[a]
Fish consumption (reservoir) after 1 gal spill	Adult male	$1.5x10^{-7}$	[a]	[a]	2	$7.6x10^{-8}$	[a]	[a]
20 gal spill	Adult male	$3.0x10^{-6}$	[a]	[a]	2	$1.5x10^{-6}$	[a]	[a]
Fish consumption (pond) after 1 gal spill	Subsistence male	0.030	[a]	[a]	2	0.015	[a]	[a]
20 gal spill	Subsistence male	0.61	[a]	[a]	2	0.31	[a]	[a]
Fish consumption (reservoir) after 1 gal spill	Subsistence male	$1.5x10^{-6}$	[a]	[a]	2	$7.6x10^{-7}$	[a]	[a]
20 gal spill	Subsistence male	0.000031	[a]	[a]	2	0.000015	[a]	[a]

Table 3-17 (cont.): Estimated Glyphosate Exposures and Hazard Quotients for the General Public

Scenario	Receptor	Calculated Dose (mg/kg-event)			RfD (mg/kg-day)	Hazard Quotient (HQ)		
		Central	Lower	Upper		Central	Lower	Upper
Chronic exposure estimates for diluted glyphosate product (foliar treatment)								
Fruit consumption	Adult female	0.013	0.0015	0.34	2	0.0064	0.00075	0.17
Water consumption[b]	Adult male	0.000023	$1.73x10^{-7}$	0.00057	2	0.000012	$8.66x10^{-8}$	0.00029
Fish consumption[b]	Adult male	$3.3x10^{-7}$	$3.6x10^{-9}$	$6.9x10^{-6}$	2	$1.7x10^{-7}$	$1.8x10^{-9}$	$3.5x10^{-6}$
Fish consumption[b]	Subsistence male	$3.6x10^{-7}$	$3.8x10^{-9}$	0.000070	2	$1.8x10^{-7}$	$1.9x10^{-9}$	0.000035
Chronic exposure estimates for concentrated glyphosate product (cut-stump treatment)								
Fruit consumption	Adult female	0.0064	0.0027	0.23	2	0.0032	0.0013	0.11
Water consumption[b]	Adult male	0.000012	$8.66x10^{-8}$	0.000384	2	$6.0x10^{-6}$	$4.3x10^{-8}$	0.00019
Fish consumption[b]	Adult male	$1.7x10^{-7}$	$1.8x10^{-9}$	$4.6x10^{-6}$	2	$8.4x10^{-8}$	$8.9x10^{-10}$	$2.3x10^{-6}$
Fish consumption[b]	Subsistence male	$1.8x10^{-7}$	$1.9x10^{-9}$	$4.9x10^{-6}$	2	$8.9x10^{-8}$	$1.0x10^{-10}$	$2.5x10^{-6}$

RfD = Reference Dose. Hazard Quotients above 0.1 are shaded. Hazard Quotients greater than one are **bolded.**

[a] Only one exposure estimate was evaluated for consumption of contaminated fish, using a single bioconcentration factor and a serving size of fish that would not exceed the recommended consumption limits based on mercury contamination.

[b] Long-term runoff was estimated using the USFS worksheets based on application rate [(mg/L)/(lb/acre)] and is not specific to a particular water body. See Section 2.5.2 for more discussion of this topic.

3.5.5 Risks to Wildlife

The wildlife risk assessment is divided into two parts, terrestrial and aquatic. Aquatic wildlife are at much greater risk from glyphosate exposure compared to terrestrial wildlife. Only the insectivorous small mammal scenario produced HQs greater than one for a single terrestrial exposure. The remainder of the single-exposure terrestrial wildlife exposure scenarios produced HQs less than one. Aggregate doses from dermal exposure and food consumption for insectivorous and herbivorous small mammals are not substantially different than the single-exposure scenarios. The only aquatic scenario considered Probable is the long-term runoff scenario. Exposures from this scenario did not exceed TRVs for glyphosate for any of the species in this risk assessment, with the Upper HQs ranging from 0.017% of the TRV (tolerant fish) to 0.91% of the TRV (tadpoles).

3.5.5.A Terrestrial Wildlife

As of 1993, the MMWD had cataloged 287 vertebrate species in the watershed, 54 of which are mammals and 202 are birds. The wildlife scenarios developed in the SERA worksheets are representative of MMWD wildlife. Tables 3-18, 3-19, and 3-20 show the acute, chronic and aggregate glyphosate exposure estimates and hazard quotients for terrestrial wildlife. See Section 2.5.5 for a discussion of the methods used to estimate wildlife exposures. Section 3.3.2 on page 3-35 contains a summary of glyphosate toxicity studies on terrestrial organisms and a discussion of specific TRVs used for wildlife exposure to glyphosate.

Exposures of highest concern are for insectivorous mammals and birds, but no Central exposure estimates exceeded the TRVs for Probable or Possible exposures. One Upper hazard quotient exceeded one—for insectivorous birds.

1. **A small mammal eating contaminated insects, acute (Probable)**. The Central dose estimate for a small mammal eating contaminated insects is 26% of the TRV. The Upper dose estimate is 1.2 times the TRV, and the Lower estimate is 13% of the TRV.
2. **A small bird eating contaminated insects, acute (Probable)**. The Central dose estimate for a small bird eating contaminated insects is 13% of the TRV. The Upper estimate is 60% of the TRV.
3. **Consumption of contaminated prey by carnivorous mammals or birds, acute (Possible)**. Central estimates of exposures for all carnivorous mammals and birds are less than 2.4% of the TRVs, and the Upper estimates do not exceed 3.6% of the TRVs.
4. **Drinking water contaminated by long-term runoff, chronic (Possible)**. None of the hazard quotients for Upper estimates of long-term runoff scenarios exceed 0.0045% of any TRV. These estimates account only for the application rate used and not the acres treated, the effect of buffer zones, or the volume of the water body.

Of the Improbable scenarios, 100% absorption of direct spray to 50% of the body emerges as a potentially problematic scenario for bees and small mammals, as HQs approach one. Large mammals and birds eating contaminated vegetation (acute) results in a Central HQ of 19% of the TRV for large mammals and 9.6% of the TRV for large birds. Upper HQs for large mammals and large birds eating contaminated vegetation are 83% and 41% of the TRVs, respectively. Central exposure estimates for chronic exposures for large mammals and birds are below 9% of

the TRV, but the Upper estimate for herbivorous birds is 1.4 times the TRV. Upper estimates of dermal exposures to honeybees and small mammals are 89% of the TRV and 42% of the TRV, respectively. Consumption of water after a 20-gallon spill of concentrated Aquamaster into a pond produced Upper hazard quotients ranging from 0.97% of the TRV to 12% of the TRV for terrestrial wildlife.

Tables 3-18 and 3-19 summarize the acute and chronic exposure estimates and hazard quotients for terrestrial wildlife exposure scenarios.

Unlike human RfDs, wildlife TRVs do not include uncertainty factors. If uncertainty factors were applied to the TRVs, hazard quotients would frequently exceed one. However, the estimate uses a modeled application rate that exceeds the intended application rate, therefore Upper estimates should be viewed as improbable worst-case scenarios. These exposure estimates highlight the importance of careful adherence to an application limit of two pounds per acre.

Aggregate exposure estimates are the sum of dermal and food exposures. Water consumption was not included in aggregate exposure estimates because the only Probable water contamination scenario was long-term runoff, which is anticipated to occur at least several months after the day of a direct spray or consumption of contaminated insects or vegetation. USFS/SERA did not calculate aggregate exposures; we added this calculation for insectivorous and herbivorous small mammals because of their vulnerability to direct sprays and eating contaminated food in a single day. The results are presented in Table 3-20. The highest aggregate exposure produced a hazard quotient that is 1.2 times the TRV for the Upper exposure estimate for insectivorous small mammals. No Central estimates exceeded an HQ of 0.83% of the RfD. The contribution from contaminated insects dominates the estimate, with the glyphosate dose from direct spray less than 2% of the aggregate dose.

Table 3-18: Estimated Acute Glyphosate Exposures and Hazard Quotients for Terrestrial Wildlife

Scenario	Receptor	Calculated Dose mg/kg-day or mg/kg/event			TRV (mg/kg)	Hazard Quotient (HQ)		
		Central	Lower	Upper		Central	Lower	Upper
Direct Spray								
First-order absorption	Small mammal	0.47	0.076	1.7	175	0.0027	0.00043	0.0098
100% absorption of direct spray to 50% of body	Small mammal	48	24	73	175	0.27	0.14	0.42
100% absorption of direct spray to 50% of body	Honeybee	320	160	480	540	0.59	0.30	0.89
Consumption of contaminated fruit and vegetation								
Fruit	Small mammal	2.5	0.36	8.0	175	0.014	0.0020	0.046
Grass	Large mammal	34	17	150	175	0.19	0.097	0.83
Grass	Large bird	54	27	230	562	0.096	0.048	0.41
Consumption of contaminated water								
20 gal spill of diluted product into pond	Small mammal	0.62	0.21	1.60	175	0.0036	0.0012	0.0091
	Large mammal	0.27	0.094	0.71	175	0.0016	0.00054	0.0041
	Small bird	1.14	0.39	2.94	562	0.0020	0.00070	0.0052
	Large bird	0.16	0.054	0.41	562	0.00028	0.000097	0.00073
20 gal spill of diluted product into reservoir	Small mammal	0.000031	0.000011	0.000081	175	1.8×10^{-7}	6.2×10^{-8}	4.6×10^{-7}
	Large mammal	0.000014	4.8×10^{-6}	0.000036	175	8.0×10^{-8}	2.7×10^{-8}	2.0×10^{-7}
	Small bird	0.000058	0.000020	0.00015	562	1.0×10^{-7}	3.5×10^{-8}	2.7×10^{-7}
	Large bird	8.0×10^{-6}	2.8×10^{-6}	0.000021	562	1.4×10^{-8}	4.9×10^{-9}	3.7×10^{-8}
20 gal spill of conc. product into pond	Small mammal	21	[a]	[a]	175	0.12	[a]	[a]
	Large mammal	9.4	[a]	[a]	175	0.054	[a]	[a]
	Small bird	39	[a]	[a]	562	0.069	[a]	[a]
	Large bird	5.4	[a]	[a]	562	0.0097	[a]	[a]
20 gal spill of conc. product into reservoir	Small mammal	0.0011	[a]	[a]	175	6.2×10^{-6}	[a]	[a]
	Large mammal	0.00048	[a]	[a]	175	2.7×10^{-6}	[a]	[a]
	Small bird	0.0020	[a]	[a]	562	3.5×10^{-6}	[a]	[a]
	Large bird	0.00027	[a]	[a]	562	4.9×10^{-7}	[a]	[a]
Peak runoff[b]	Small mammal	0.0059	0.00015	0.18	175	0.000034	8.4×10^{-7}	0.0010
	Large mammal	0.0026	0.000065	0.078	175	0.000015	3.7×10^{-7}	0.00044
	Small bird	0.011	0.00027	0.32	562	0.000020	4.8×10^{-7}	0.00058
	Large bird	0.0015	0.000037	0.045	562	2.7×10^{-5}	6.6×10^{-8}	0.000080
Consumption of contaminated insects								
	Small mammal	46	23	208	175	0.26	0.13	**1.2**
	Small bird	75	38	338	562	0.13	0.067	0.60

Table 3-18 (cont.): Estimated Acute Glyphosate Exposures and Hazard Quotients for Terrestrial Wildlife

Scenario	Receptor	Calculated Dose mg/kg-day or mg/kg/event			TRV (mg/kg)	Hazard Quotient (HQ)		
		Central	Lower	Upper		Central	Lower	Upper
Consumption of contaminated fish								
20 gal spill of diluted product into pond	Fish-eating bird	0.22	0.038	0.85	562	0.00039	0.000067	0.0015
20 gal spill of diluted product into reservoir	Fish-eating bird	0.000011	$1.9x10^{-6}$	0.000043	562	$2.0x10^{-8}$	$3.4x10^{-9}$	$7.7x10^{-8}$
20 gal spill of concentrated product into pond	Fish-eating bird	7.56	3.78	11.34	562	0.013	0.0067	0.020
20 gal spill of concentrated product into reservoir	Fish-eating bird	0.00038	0.00019	0.00057	562	$6.8x10^{-7}$	$3.4x10^{-7}$	$1.0x10^{-6}$
Consumption of contaminated small mammal								
	Carnivorous small mammal	4.2	2.1	6.3	175	0.024	0.012	0.036
	Carnivorous large mammal	2.3	1.1	3.4	175	0.013	0.0064	0.019
	Carnivorous bird	6.5	3.2	9.7	562	0.012	0.0058	0.017

TRV = Toxicity Reference Value. Hazard Quotients above 0.1 are shaded. Hazard Quotients greater than one are **bolded.**

[a] Only a single, worst-case estimate of concentration was calculated for spills of concentrated Aquamaster product.

[b] Long-term runoff was estimated using the USFS worksheets based on application rate [(mg/L)/(lb/acre)] and is not specific to a particular water body. See Section 2.5.2.

Table 3-19: Estimated Chronic Glyphosate Exposures and Hazard Quotients for Terrestrial Wildlife

Scenario	Receptor	Calculated Dose mg/kg-day or mg/kg-event			TRV (mg/kg)	Hazard Quotient (HQ)		
		Central	Lower	Upper		Central	Lower	Upper
Consumption of contaminated fruit and vegetation								
On-site, fruit	Small mammal	0.14	0.0082	0.98	175	0.00080	0.000047	0.0056
Off-site, fruit		0.0014	0.000047	0.018	175	$8.0x10^{-6}$	$2.7x10^{-7}$	0.00011
On-site, vegetation	Large mammal	5.6	0.79	89	175	0.032	0.0045	0.51
Off-site, vegetation		0.19	0.046	1.7	175	0.0011	0.00026	0.0095
On-site, vegetation	Large bird	8.8	1.2	140	100	0.088	0.012	**1.4**
Off-site, vegetation		0.30	0.072	2.6	100	0.0030	0.00072	0.026
Consumption of contaminated water								
Long-term runoff[a]	Small mammal	0.00012	$1.3x10^{-6}$	0.0025	175	$6.7x10^{-7}$	$7.4 x10^{-9}$	0.000014
Long-term runoff[a]	Large mammal	0.000052	$5.63 x10^{-7}$	0.0012	175	$3.0 x10^{-7}$	$3.2 x10^{-9}$	$6.2x10^{-6}$
Long-term runoff[a]	Small bird	0.00022	$2.3 x10^{-6}$	0.0045	100	$2.2x10^{-6}$	$2.3 x10^{-8}$	0.000045
Long-term runoff[a]	Large bird	0.000030	$3.2x10^{-7}$	0.00063	100	$3.0 x10^{-7}$	$3.2 x10^{-9}$	$6.3x10^{-6}$
Consumption of contaminated fish								
Chronic	Fish-eating bird	0.000042	$2.3x10^{-9}$	0.0013	100	$4.2x10^{-7}$	$2.3x10^{-9}$	0.000013

TRV = Toxicity Reference Value. Hazard Quotients above 0.1 are shaded. Hazard Quotients greater than one are **bolded.**

[a] Long-term runoff was estimated using the USFS worksheets based on application rate [(mg/L)/(lb/acre)] and is not specific to a particular water body. See Section 2.5.2 for more discussion of this topic.

Table 3-20: Glyphosate Aggregate Exposures and Hazard Quotients for Terrestrial Wildlife

Animal	Scenario	Exposure Estimates (mg/kg) Central	Lower	Upper
Herbivorous small mammal eating fruit (TRV = 175 mg/kg)				
	Direct spray, first-order absorption	0.47	0.076	1.72
	Eating fruit	2.55	0.36	8.04
	Sum	3.02	0.43	9.76
	HQ	0.017	0.0025	0.056
Insectivorous small mammal (TRV = 175 mg/kg)				
	Direct spray, first-order absorption	0.47	0.076	1.72
	Eating insects	46.3	23.1	208.2
	Sum	46.8	23.2	210
	HQ	0.027	0.13	1.2

TRV = Toxicity Reference Value. Hazard Quotients above 0.1 are shaded. Hazard Quotients greater than one are **bolded.**
The values summed in this table are from Table 3-18.

3.5.5.B Model Validation

For most wildlife exposure estimates there are no empirical studies for comparison. For glyphosate, however, there is a study discussing the concentrations in the bodies of mammals after aerial spraying of a deciduous (Douglas fir before logging in 1955) red alder and bitter cherry forest in Oregon.[276c] The study recorded glyphosate residues in the upper tree canopy, understory canopy, soil, leaf litter, and stream water following the application of glyphosate at 3.3 kg/ha. Periodic rainfall occurred throughout the 55-day sampling period, with more than 10 mm of rain (but less than 20 mm) falling on days 2, 45, 46, 51 and 55. Glyphosate residues declined throughout the study period. Table 3-21 presents concentrations observed in the environment following aerial spraying.

Table 3-22 shows the concentrations of glyphosate measured in the bodies of small mammals from the application site. The bodies of the animals were divided into two different sources: the viscera (or internal organs, roughly 30% of body) and the remainder. The total body concentration in the far right column can be compared to the exposure estimates from Tables 3-18 and 3-19. The observed concentrations are much lower than the exposure estimates. This is not surprising given that there was a dense canopy over the animals sprayed in the experiment and no such protection was assumed for the conservative exposure estimates in the risk assessment.

Each value in Table 3-22 is the average of one to seven animals. The minimum, maximum and standard deviation for each time period was not recorded; therefore, no information on individual variation in exposure is available. In this experiment, exposures to carnivores, omnivores and herbivores were not substantially different, suggesting that feeding habits do not strongly influence small mammal exposures. However, reliable inferences are difficult given the experimental design. Animals were killed to measure their bodily concentrations, thus eliminating the possibility of measuring glyphosate concentrations over time. In the case of herbivores, different species were measured at different time points, confounding any inferences

on the importance of behavior. Herbivores were the only group that did not experience consistent decreases in body concentrations from the beginning of the study to the end. However, this could be due to the fact that different species were measured at different points in time.

Table 3-21: Empirical Environmental Exposures Following Direct Spray

Matrix	Glyphosate Residues (ppm)	Half-life (days)
Above canopy	489 ± 127[a]	--
Mid-canopy	84.0 ± 27.8	14.4
Shrub	89.0 ± 30.4	26.6
Ground Cover	20.4 ± 5.3	10.4
Litter	5.0 ± 3.1	14
Soil	0.8-1.5	29.2-40.2
Stream	0.27 mg/L[b]	< 1
Stream sediment	0.5 mg/L	--

Data source: Reference 276c.

[a] Residues above the canopy were measured with mylar ballons. Measurements were not highly correlated to samples of upper canopy level foliage, suggesting that this technique may not be appropriate for measuring residues.

[b] This value is the maximum value observed shortly after direct aerial spray. After three hours, the glyphosate concentration in the stream had dropped to 0.1 mg/L. By day two, the concentration had dropped to undetectable levels. There were no peaks in glyphosate stream concentrations associated with rain events.

Table 3-22: Empirical Wildlife Exposure Analysis for Glyphosate

		Glyphosate Concentration (ppm)			
Feeding Group	Days Since Treatment	Viscera	Eviserated Body	Whole Body	Central Exposure Estimates (mg/kg)
Carnivores -					Acute small mammal eating contaminated prey + first-order dermal absorption: 4.7
shrew	pretreatment	< 0.10	< 0.10	< 0.10	
	0	1.69	0.41	0.794	
	1	0.45	0.37	0.394	
	3	0.19	0.14	0.155	
	7	0.19	< 0.10	0.127	
	14	0.26	< 0.10	0.148	
	55	< 0.10	< 0.10	< 0.10	No long term small mammal carnivore scenario
weasel	14	< 0.10	< 0.10	< 0.10	
Omnivores –					Acute small insectivore eating contaminated prey + first-order dermal absorption: 46
deer mice	pretreatment	< 0.10	< 0.10	< 0.10	
	0	5.08	0.35	1.769	
	1	1	0.4	0.58	
	3	0.37	< 0.10	0.181	
	7	0.33	0.15	0.204	
	14	0.17	< 0.10	0.121	
	28	< 0.10	< 0.10	< 0.10	No long term small mammal omnivore scenario
	55	< 0.10	< 0.10	< 0.10	
Herbivores -					Acute small mammal eating contaminated fruit + first-order dermal absoprtion: 2.97
woodrat	pretreatment	< 0.10	< 0.10	< 0.10	
squirrel	1	0.37	0.13	0.202	

vole	3	1.42	0.25	0.601	Chronic aggregate small mammal eating fruit + drinking contaminated water: 0.14
vole	7	1.7	< 0.10	0.58	
vole	14	1.54	< 0.10	0.532	
chipmunk	28	0.44	0.23	0.293	
chipmunk	55	0.12	< 0.10	0.106	

Data source: Reference 276c.

3.5.5.C Terrestrial Plants

For terrestrial plants, unintended direct spray will result in an exposure equivalent to the application rate. Most plants that are sprayed directly with glyphosate at or near the recommended range of application rates will be damaged. Buffer zones of 100 feet or use of protective barriers to prevent spray drift are recommended to protect sensitive plants. Glyphosate is unlikely to have significant residual herbicidal activity, since it adsorbs to soils and is poorly absorbed through plant roots.

3.5.5.D Aquatic Wildlife

The calculated water concentrations of glyphosate for aquatic life are the same as those used in the human and terrestrial exposure estimates for drinking water (see Table 3-13). Exposure estimates are compared to TRVs for tolerant and sensitive species for less-toxic glyphosate formulations that do not contain the toxic POEA surfactant. Although amphibians were not explicitly considered in the SERA/USFS worksheets, tadpoles were added to the risk characterizations and considered with aquatic wildlife. Hazard quotients are summarized in Table 3-23.

The only aquatic scenario considered Probable is the long-term runoff scenario. Exposures from this scenario do not exceed TRVs for glyphosate for any of the species in this risk assessment, with the Upper HQs ranging from 0.093% of the TRV for tolerant fish to 0.37% of the TRV for tadpoles. The long-term runoff estimates calculated by the USFS methodology account only for the application rate used and not the acres treated, the effect of buffer zones, or the volume of the water body.

The highest hazard quotients calculated are for the Highly Improbable spills into a small, thermally stratified pond, with Central HQs for spills of concentrated glyphosate products ranging from 5.6% of the TRV for aquatic invertebrates (1 gallon spill) to 48 times the TRV for aquatic plants (20 gallon spill). Other Central HQs for the 20 gallon spill scenario are: 22.4 times the TRV for tadpoles; 56 times the TRV for sensitive fish; and 5.6 times the TRV for tolerant fish. MMWD should do everything possible to minimize the potential for an acute spill of glyphosate near water bodies.

All scenarios for acute spills into Bon Tempe Reservoir produced HQs less than 0.25% of any TRV.

Because only the long-term runoff scenario is likely and all HQs for this scenario are substantially lower than the TRVs, we conclude that use of glyphosate in the MMWD watershed is unlikely to adversely affect aquatic life.

Table 3-23: Estimated Glyphosate Hazard Quotients for Aquatic Wildlife

Receptor	Scenario	Hazard Quotients Central	Lower	Upper	TRV (mg/L)
Sensitive Fish					
Spill of diluted product into pond	1 gal	0.083	0.028	0.21	2.57
	20 gal	1.6	0.57	**4.2**	2.57
Spill of diluted product into reservoir	1 gal	$4.2x10^{-6}$	$1.4x10^{-6}$	0.000011	2.57
	20 gal	0.000084	0.000029	0.00021	2.57
Spill of concentrated product into pond	1 gal	**2.8**	[a]	[a]	2.57
	20 gal	**57**	[a]	[a]	2.57
Spill of concentrated product into reservoir	1 gal	0.00014	[a]	[a]	2.57
	20 gal	0.0029	[a]	[a]	2.57
Peak runoff[b]		0.016	0.00039	0.47	2.57
Long-term runoff[b]		0.00032	$3.4x10^{-6}$	0.0065	2.57
Tolerant Fish					
Spill of diluted product into pond	1 gal	0.0083	0.0028	0.021	25.7
	20 gal	0.16	0.057	0.42	25.7
Spill of diluted product into reservoir	1 gal	4.2×10^{-7}	1.4×10^{-7}	1.1×10^{-6}	25.7
	20 gal	$8.4x10^{-6}$	$2.9x10^{-6}$	0.000021	25.7
Spill of concentrated product into pond	1 gal	0.28	[a]	[a]	25.7
	20 gal	**5.7**	[a]	[a]	25.7
Spill of concentrated product into reservoir	1 gal	0.000014	[a]	[a]	25.7
	20 gal	0.00029	[a]	[a]	25.7
Peak runoff [b]		0.0016	0.000039	0.047	25.7
Long-term runoff[b]		0.000032	3.4×10^{-7}	0.00065	25.7
Amphibians (Tadpoles)					
Spill of diluted product into pond	1 gal	0.033	0.011	0.084	6.48
	20 gal	0.65	0.22	**1.7**	6.48
Spill of diluted product into reservoir	1 gal	1.7×10^{-6}	5.6×10^{-7}	4.3×10^{-6}	6.48
	20 gal	0.000033	0.000011	0.000085	6.48
Spill of concentrated product into pond	1 gal	**1.1**	[a]	[a]	6.48
	20 gal	**22**	[a]	[a]	6.48
Spill of concentrated product into reservoir	1 gal	0.000057	[a]	[a]	6.48
	20 gal	0.0011	[a]	[a]	6.48
Peak runoff[b]		0.0062	0.00015	0.19	6.48
Long-term runoff[b]		0.00045	$4.8x10^{-6}$	0.0093	1.8
Aquatic Invertebrates					
Spill of diluted product into pond	1 gal	0.0016	0.00056	0.0043	130
	20 gal	0.033	0.011	0.084	130
Spill of diluted product into reservoir	1 gal	8.3×10^{-8}	2.8×10^{-8}	2.1×10^{-7}	130
	20 gal	1.7×10^{-6}	5.7×10^{-7}	4.3×10^{-6}	130
Spill of concentrated product into pond	1 gal	0.056	[a]	[a]	130
	20 gal	**1.1**	[a]	[a]	130
Spill of concentrated product into reservoir	1 gal	$2.8x10^{-6}$	[a]	[a]	130
	20 gal	0.000057	[a]	[a]	130
Peak runoff[b]		0.00031	$7.7x10^{-6}$	0.009	130
Long-term runoff[b]		0.000016	$1.7x10^{-7}$	0.00034	50

Table 3-23 (cont): Estimated Glyphosate Hazard Quotients for Aquatic Wildlife

Receptor	Scenario	Hazard Quotients Central	Lower	Upper	TRV (mg/L)
Aquatic Plants					
Spill of diluted product into pond:	1 gal	0.071	0.024	0.18	3
	20 gal	**1.4**	0.48	**3.6**	3
Spill of diluted product into reservoir:	1 gal	$3.6x10^{-6}$	$1.2x10^{-6}$	$9.2x10^{-6}$	3
	20 gal	0.00007	0.000025	0.00018	3
Spill of concentrated product into pond:	1 gal	**2.4**	[a]	[a]	3
	20 gal	**48**	[a]	[a]	3
Spill of concentrated product into reservoir:	1 gal	0.00012	[a]	[a]	3
	20 gal	0.0025	[a]	[a]	3
Peak runoff[b]		0.013	0.00033	0.4	3
Long-term runoff[b]		0.00027	$2.7x10^{-6}$	0.0056	3

TRV = Toxicity Reference Value. Hazard Quotients above 0.1 are shaded. HQs greater than one are **bolded.**

[a] Only a single, worst-case estimate of concentration was calculated for spills of concentrated Aquamaster product.

[b] Peak and long-term runoff were estimated using the USFS worksheets based on application rate [(mg/L)/(lb/acre)] and are not specific to a particular water body. See Section 2.5.2 for more discussion of this topic.

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[277] Approximately 70 acres (or 25% of the total 284 acres) of broom in the Phoenix watershed would not be treated because they lie in the Phoenix Lake buffer zone.