REVIEW ON
Glyphosate fate and toxicity to fish with special relevance to salmon and steelhead populations in the Skeena River watershed

Prepared for:

T. Buck Suzuki Environmental Foundation (TBSEF)

Prepared by:

Dr. Chris Kennedy
BioWest Environmental Research Consultants
Burnaby, BC
Canada V5G 1M7

November 21, 2017

Report Priorities:

T. Buck Suzuki Environmental Foundation (TBSEF) requested a summary on glyphosate fate/toxicity to salmon and steelhead which would be relevant to glyphosate entering the lower reaches (about 100 km) of the Skeena and the myriad of small watercourses, streams and rivers entering it near their confluence with the Skeena.

This report was to include simplified descriptions of the main concerns for the benefit of local residents who have a limited scientific vocabulary.

TBSEF is especially interested in regional aspects of spraying glyphosate in the area of the location of the CN railway (which closely follows the Skeena River)—the discussion is to include (including simplified highlights) those aspects which may make glyphosate spraying more or less dangerous for fish than in other areas including: 1) high acidity, 2) high rainfall, 3) temperate climate, 4) proximity of rail line to Skeena and crossing of myriad of small watercourses, streams and rivers, 5) large numbers of returning salmon during the spray period in question, 6) proximity/overlap to/of chum salmon spawning grounds and coho spawning grounds to spray area, and 7) lack of soil, i.e. long stretches of vegetation over rocky ground.

Executive Summary

Glyphosate (*N*–[phosphonomethyl]glycine) is the active ingredient in Roundup® and other broad-spectrum, post-emergence, non-selective herbicides. It is the world's largest-selling chemical used for plant control in agricultural, silvicultural, and urban environments. Commercially available glyphosate-based herbicide formulations are complex mixtures and include various forms of glyphosate, as well as various surfactants and other adjuvants.

Glyphosate is highly soluble and stable in water. Glyphosate's loss from water is through sediment adsorption and microbial degradation and its overall loss from aquatic systems ranges from 27 to 146 days. The strong sorption of glyphosate to soil/sediments translates into a relative immobility (relatively nonleachable) and will be transported to surface waters mainly sorbed to soil particles. Glyphosate contamination of surface water is highly dependent on a water body's proximity to herbicide application, climatic conditions, and foliage and soil characteristics. Direct overspray or drift during herbicide application and surface runoff can result in significant quantities of glyphosate entering aquatic environments.

The vast array of unique commercially available formulas, the proprietary nature of additive formulations, and relative proportion of individual constituents contained therein, make attributing toxicity to individual components of glyphosate-based herbicides or predictions of toxicity difficult. Currently, glyphosate formulations are classified as slightly toxic to fish. There appears to be a discrepancy when the toxicity of the active ingredient, glyphosate is compared with the toxicity of the commercial products and their various constituents.

Numerous studies regarding the toxic effects of glyphosate-based commercial formulations on aquatic animals exist, although fewer are attributed to the acute toxicity of the active ingredient glyphosate. For glyphosate, 96-h LC50 (concentration lethal to 50% of a group of fish) values range from 7 to 4000 mg/L (for rainbow trout, *Oncorhynchus mykiss*: 1.3 to 824 mg/L). The 96-h LC50 of formulated glyphosate (Roundup®) for various fish species ranges from 4.3 to >100 mg/L (for rainbow trout, *Oncorhynchus mykiss*, values ranged from 8.2-27 mg/L). Much of the toxicity of the commercial formulation is attributed to the surfactant portion, particularly polyethoxylated tallow amine (POEA) that has a range of LC50 96-h values between 0.65 and 7.4 mg/L. Glyphosate or glyphosate-containing herbicide exposure can result in the following sublethal effects in various fish species: avoidance behaviour, alterations in olfactory ability, histopathological changes, oxidative stress, genotoxicity, malformations, hematologic changes, alterations of hormone profiles, and decreases in the cell-mediated immune response.

In the coastal areas of British Columbia, climatic conditions frequently include high precipitation in autumn and winter with low temperatures; under such conditions, watersheds with areas of high water table, seasonally saturated or coarse-textured mineral soils/substrates low in organic matter, and frequent surface runoff events could lead to appreciable concentrations of chemicals in glyphosate herbicide formulations reaching surface waters following application close to aquatic ecosystems. A significant knowledge gap is understanding the bioavailability of glyphosate, glyphosate metabolites, or other formulation constituents in aquatic environments (either dissolved in water or associated with sediment particles) in these systems. Due to the above information, it is possible that the presence of glyphosate-based herbicides in salmon habitat in the Skeena River watershed may cause adverse effects to Pacific salmonid populations.

- there is potential for glyphosate-based herbicides in salmon habitat in the Skeena River watershed to cause adverse effects to Pacific salmonid populations

GLYPHOSATE FATE AND EFFECTS

Glyphosate and glyphosate-based herbicides

Glyphosate [*N* -(phosphonomethyl)glycine] (CAS no. 1071-83-6), is the active ingredient in weed-killing pesticide formulations which are broad-spectrum, post-emergence, non-selective herbicides. Glyphosate was first identified to have herbicidal action in 1970 and commercial formulations were released by Monsanto Corp. (St. Louis, MO) 4 years later. Glyphosate-based herbicides are currently among the most widely used agricultural chemicals globally. Commonly sold under various trade names (e.g. Roundup®), glyphosate-based herbicides were the 17th most commonly used pesticide in the late 1980s, in terms of active ingredient applied, but by 2002 it was the most commonly used herbicide in the United States (Gianessi and Reigner 2006). It is also the world's largest-selling chemical used for weed control in agricultural, silvicultural, and urban applications (Baylis 2000; Franz et al. 1997; Veiga et al. 2001). Adding to glyphosate popularity was the introduction in 1997 of Roundup Ready® crops, such as cotton, maize and soybeans (Kolpin et al. 2006), which are made glyphosate-resistant by incorporation of a naturally occurring, glyphosate-resistant protein. The impact of this single class of herbicides on modern agriculture practices is undeniable (Gilbert 2013); the combination of the broad-spectrum herbicidal nature of glyphosate and the development of resistant crop varieties has elevated glyphosate-based herbicides to among the most important herbicidal chemicals used to date.

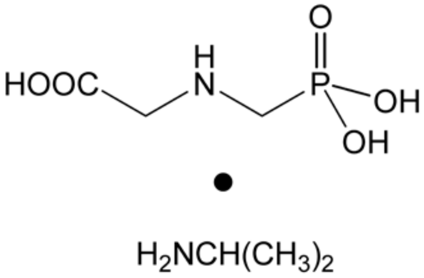
Glyphosate was developed as a non-selective, broad range herbicide with strong herbicidal action. Though sensitivity to glyphosate varies between plant species, all naturally occurring higher plants have some susceptibility to glyphosate. This property limited the historical use of glyphosate to applications where all plants were to be removed, such as prior to seeding as chemical fallow, or on a small scale where targeted application was possible (Duke and Powles 2008). In this traditional setting, the likelihood of contaminating the aquatic environment was relatively low, as applications typically occurred less frequently than in modern operations.

Commercially available glyphosate-based herbicide formulations are complex mixtures in which glyphosate acts as the active ingredient. Glyphosate concentrations are often reported as relative proportion of active ingredient (mg a.i./L), proportion of acid equivalents (mg a.e./L), percent glyphosate, or mass of glyphosate applied per unit area (kg/ha). To understand the relationship between these reporting methods it is necessary to clarify how glyphosate-based herbicides are formulated. The addition of surfactants and other adjuvants is necessary to allow the active ingredient to penetrate the plant surface and translocate to the site of action (Wang and Liu 2007). The versatility of modern glyphosate-based herbicides can be attributed to the wide variety of formulations currently available, each tailored for a specific range of application conditions. Each formula contains a particular concentration of glyphosate with a particular adjuvant profile. Often the identity of these adjuvants remains proprietary, which can make attributing toxicity to individual components of glyphosate-based herbicides difficult. Toxicological research of these products is confounded by the vast array of unique commercially available formulas, a lack of consistency in reporting the exact formula used, and the relative proportion of individual constituents contained therein. These inconsistencies in reporting can lead to incorrect herbicide applications and to an over or underestimation of toxicity. With large toxicity differences between individual formulations, it is critical that the complete name and description of the product being investigated is known.

In order to meet solubility requirements for commercial use, currently available glyphosate formulations usually contain the salts of glyphosate as the active ingredient. The identities of these salts are variable and include isopropylamine, diammonium or potassium salt forms, among others.

The most commonly applied form of glyphosate is in the form of its isopropylamine salt (IPA salt) shown in Figure 1. Commercial preparations of glyphosate usually contain three elements: the IPA salt of glyphosate, a surfactant, and water. The most commonly used surfactant is polyethoxylated tallow amine (POEA), which promotes the penetration of glyphosate across the cuticle of target plants.

Figure 1. Structure of glyphosate isopropylamine salt.



Several chemical and physical characteristics for glyphosate are listed in Table 1.

Table 1. Physical and chemical characteristics of glyphosate (Schuette 1998).

Molecular Weight	169.08 g
Water solubility	11,600 ppm (at 25°C)
Vapor pressure	7.5×10^{-8} mm Hg
Henry's law constant	$<1.44 \times 10^{-12}$ atm-m^3/mole
Hydrolysis half-life (average:pH levels/temperature)	>35 days
Soil adsorption coefficient (Kd)	61 g/m^3
Octanol-water coefficient log (Kow)	-3.5
Anaerobic half-life	22.1 days
Aerobic half-life	96.4 days
Field dissipation half-life	44 days

- glyphosate [*N* -(phosphonomethyl)glycine] is the active ingredient in weed-killing pesticide formulations that act as broad-spectrum, post-emergence, non-selective herbicides
- commercially available glyphosate-based herbicide formulations are complex mixtures in which glyphosate acts as the active ingredient
- commercial preparations of glyphosate herbicides usually contain 3 elements: the IPA salt of glyphosate, a surfactant (e.g. polyethoxylated tallow amine [POEA]), and water

Environmental Fate of Glyphosate

Partitioning Behaviour

Air: The vapor pressure of a chemical determines its volatility. Volatilization is the process whereby a chemical moves from a liquid or solid phase to a gas phase. Volatile herbicides (those with high vapor pressures) generally dissipate more rapidly than those with lower vapor pressures. Most herbicides are relatively nonvolatile under normal field-use conditions. The vapor pressure for glyphosate is very low and therefore volatilization of glyphosate into the atmosphere post-treatment will not be significant (Franz et al. 1997). Glyphosate's low Henry's Law Constant indicates that it will tend to partition to water *v*. air. This does not preclude the aerial drift of particles (mists, droplets etc.) of the formulated herbicide and entry to the aquatic environment from the atmosphere, particularly when winds are gusty or when wind velocities are high enough for spray drift to occur.

- glyphosate's tendency to volatilize into the atmosphere is low and it tends to partition to water more so than air
- the atmosphere can be a source of glyphosate through particulate (e.g. mists, droplets) deposition of the herbicide to a water body

Water: Glyphosate is highly soluble in water (11,600 mg/L at 25°C [Segawa 1995]) and has an octanol-water partition coefficient (log Kow) value of -3.3; therefore, glyphosate readily dissolves and disperses in aquatic environments (Mackay et al. 1997). Experiments conducted for the US EPA's re-registration eligibility decision (for glyphosate) indicate that it is stable in water at pH 3, 5, 6, and 9 at 35°C. It is also stable to photo degradation at pH 5, 7 and 9 in a buffered solution under natural sunlight. The hydrolysis half-life is >35 days (Kollman and Segawa 1995). Bronstad and Friestad (1985) also found that glyphosate shows little propensity toward hydrolytic decomposition.

Studies conducted in Manitoba Canada (Kirkwood 1979) suggest that glyphosate's eventual removal/loss from water is through sediment (particle) adsorption and microbial degradation. Ghassemi et al. (1981) concluded that the rate of degradation in water is generally slower than in soils because there are fewer microorganisms in water than in most soils. Studies conducted in a forest ecosystem (Feng et al. 1990; Goldsbourough et al. 1993; Newton et al. 1994) found that glyphosate dissipated rapidly from surface water ponds high in suspended sediment, with first order half-lives ranging from 1.5-11.2 days. In streams, residue was undetectable in 3-14 days. In USEPA tests using water from natural sources, the half-life of glyphosate ranged from 35 to 63 days (USEPA 1986). For all aquatic systems, sediment appears to be the major sink for glyphosate residue.

In other water/sediment studies, the dissipation half-life of glyphosate from water ranged from 1 to 4 days and from 27 to 146 days for the whole system. Under aerobic conditions, the main degradation pathway leads to the formation of the major breakdown product of glyphosate: aminomethylphosphonic acid (AMPA). AMPA dissipates very rapidly from surface water, with half-lives ranging from 2 to 5 days in water and from 19 to 45 days for the entire aquatic system. Again, the general conclusion here is that the original rapid dissipation of glyphosate/metabolites from water is through adsorption to sediments.

- glyphosate is highly soluble in water and has a very low octanol-water partition coefficient value, therefore glyphosate readily dissolves and disperses in aquatic environments
- glyphosate is stable in water at various pH and temperature values, and is not readily photo degraded
- glyphosate's eventual removal/loss from water is through sediment adsorption and microbial degradation with a dissipation half-life ranging from 27 to 146 d in the whole system

Soil: The mobility, and hence leachability, of a herbicide in soil depends on its sorption characteristics, i.e. strong sorption to soil solids results in relative immobilization, while a weakly sorbed compound can be readily leached. Compared with most other pesticides, glyphosate possesses unique sorption characteristics in soil. Almost all other pesticides are moderately to weakly sorbed in soils, mainly by soil organic matter (SOM), because most of these molecules are dominated by apolar groups (Borggaard and Elberling 2004; Oliveira et al. 2001; Schwarzenbach et al. 1993). In contrast, glyphosate, which is a small molecule with three polar functional groups, is strongly sorbed by soil minerals (Gimsing et al. 2004; Gimsing et al. 2007; Gimsing and Borggaard 2002; Sheals et al. 2002). Accordingly, glyphosate is a polyprotic acid and forms, within the pH range of 4 – 8 found in most soils, mono- and divalent anions with high affinity for trivalent cations such as Al^{3+} and Fe^{3+} (Barja and Afonso 2005; Sheals et al. 2002). The main soil sorption sites of glyphosate are found on surfaces of aluminum and iron oxides, poorly ordered aluminum silicates and edges of layer silicates; accordingly, soils enriched with these variable-charge minerals have been demonstrated to be effective glyphosate sorbents, whereas soils dominated by permanent-charge minerals sorb less glyphosate (De Jonge et al. 2001; Gimsing et al. 2004; Gimsing et al. 2007; Vereecken 2005). In this regard, silicate clays have limited capacity to sorb glyphosate.

In addition to specific surface area and mineral group, glyphosate sorption also depends on pH with the amount of glyphosate sorbed decreasing with increasing pH (Barja and Afonso 2005; Gimsing and Borggaard 2007; Sheals et al 2002). In a study with five soil types, pH was the most important single factor for glyphosate sorption, which was negatively correlated with pH Gimsing et al. 2004; McConnel and Hossner 1985; Wang et al. 2005). The influence of temperature on glyphosate sorption kinetics (and degradation), seems unresolved, but the sorption rate is expected to increase at higher temperature as the rate of interactions between solutes and soil components is expected to double or triple per 10°C increase in temperature (Borggaard and Elberling 2004).

Soil organic matter seems to play a controversial and dual role in soil sorption of glyphosate. On the one hand, investigations have shown that soil sorption of glyphosate is not, or is sometimes negatively, correlated with SOM content (Gerritese et al. 1996; Gimsing and Borggaard 2004; McConnel and Hossner 1989; Vereecken 2005). On the other hand, (Piccolo et al. 1996) reported very high glyphosate sorption by four different purified humus samples.

Studies on the competition between glyphosate and phosphate for soil sorption sites concluded that the phosphate content of soils was the most decisive factor for glyphosate sorption. Several subsequent studies have confirmed the competitive sorption of glyphosate and phosphate as well as its substantial variability in various soils (de Jonge et al. 2001; de Jonge and de Jonge 1999; Dion et al 2001; Hance 1976; Wang et al. 2005). The environmental concern in relation to glyphosate/phosphate competition is attributed to the suppressed glyphosate sorption on phosphate-rich soil because reduced sorption may lead to an increased risk of glyphosate leaching to the aquatic environment.

Summarizing, glyphosate is sorbed in soils rich in aluminum and iron oxides and poorly ordered aluminum silicates, while sorption by permanent-charge layer silicates is limited. Sorption increases with increasing specific surface area of the sorbents but decreases at increasing pH. SOM does not seem to sorb glyphosate but may indirectly affect glyphosate sorption. Competition between glyphosate and phosphate for surface sites indicates that in phosphate-rich soils reduced glyphosate sorption will occur. The determination of glyphosate sorption, and hence leachability, in a certain soil is not simple, as it seems to depend on several soil characteristics such as mineralogical composition, pH, phosphate content, and perhaps SOM.

- glyphosate possesses unique sorption characteristics in soil by binding tightly to most soil types
- in addition to surface area and mineral group, glyphosate sorption also depends on pH with the amount of glyphosate sorbed decreasing with increasing pH
- the sorption rate is expected to decrease at lower temperatures
- glyphosate sorption on phosphate-rich soil is reduced and may lead to an increased risk of leaching to the aquatic environment

Persistence in soil

In general, glyphosate is moderately persistent in soil. Soil studies have determined glyphosate half-lives ranging from 3 to 130 days (USEPA 1990; USDA 1984). Several factors determine the length of time herbicides such as glyphosate persist and include soil factors as well as climatic conditions. In the case of glyphosate, each of these factors affects losses from the two main pathways: leaching and microbial degradation.

Soil composition affects glyphosate persistence through soil-herbicide binding, leaching, and vapor loss (minor). In the soil environment, glyphosate is relatively resistant to chemical degradation, is stable to sunlight, is relatively nonleachable, and has a low tendency to runoff (except as adsorbed to colloidal matter). It is relatively immobile in most soil environments as a result of its strong adsorption to soil particles. The climatic variables involved in glyphosate breakdown are moisture, temperature, and sunlight. Degradation rates generally increase as temperature and soil moisture increase, because of increases in both chemical and microbial degradation.

Leaching is one mechanism responsible for glyphosate dissipation (more later on routes to the aquatic environment). The solubility of glyphosate in water helps determine its leaching potential. Leaching occurs when glyphosate is dissolved in water and moves through the soil profile. Leaching is determined by other factors that include glyphosate-soil binding properties (as discussed previously), soil physical characteristics, rainfall frequency and intensity, chemical concentration, and time of application. In general, herbicides that are less soluble in water and strongly bound to soil particles are less likely to leach. Glyphosate has a high water solubility that tends to enhance leaching, but is strongly bound to soils that decreases it translocation.

Glyphosate's primary route of decomposition in the environment is through microbial degradation in soil (Franz et al. 1997) as practically no degradation occurred in sterile soil, whereas degradation took place in non-sterile soil (Rueppel et al 1977; Sprankle et al. 1975; Strange-Hansen et al. 2004). The herbicide is inactivated and biodegraded by soil microbes at rates of degradation related to microbial activity in the soil and factors that affect this activity (Eriksson 1975). Factors that affect microbial activity are moisture, temperature, pH, oxygen, and mineral nutrient supply. Usually, a warm, well-aerated, fertile soil with a near-neutral pH is most favorable for microbial growth and, hence, for glyphosate breakdown. The biological degradation process is carried out under both aerobic and anaerobic conditions by soil microflora. Rates of decomposition depend on soil and microfloral population types. In non-sterile conditions, as much as 55% of ^{14}C-labeled glyphosate is given off as $^{14}CO_2$ within 4 weeks (Rueppel et al., 1977; USDA, 1984).

- glyphosate is moderately persistent in soil with half-lives ranging from 3 to 130 days
- in the soil environment, glyphosate is relatively resistant to chemical degradation, is stable to sunlight, is relatively nonleachable, and has a low tendency to runoff
- glyphosate has a high water solubility which tends to enhance leaching, but is strongly bound to soils which decreases it translocation

Degradation pathways

Microorganisms degrade glyphosate through two pathways (Dick and Quinn 1995; Jacob et al. 1985; Jacob et al. 1988; Kishore and Jacob 1987; Lerbs et al. 1990; Liu et al. 1991). One pathway leads to the intermediate formation of sarcosine and glycine, and the other leads to the formation of AMPA. AMPA is cleaved to produce inorganic phosphate and methylamine, which is ultimately mineralized to CO_2 and NH_3 (Balthazor and Hallas 1986; Franz et al 1997; Pipke and Amrhein 1988). Whether the AMPA or the sarcosine pathway is most common in soil is not known, and it will be difficult to determine. As AMPA is often detected in soil that has received glyphosate (Feng and Thompson 1990; Gimsing et al. 2004; Jacobsen 2003; Landry et al. 2005; Mamy et al. 2005; Moshier and Penner 1978), this degradation pathway must exist in soil microorganisms. Sarcosine, on the other hand, has not been detected in soil, which may simply be attributed to its fast degradation, while the more resistant AMPA is strongly sorbed through the phosphonate group and protected against further microbial degradation (Feng and Thompson 1990; Jacobsen 2003). Degradation in soils is mainly a microbiological process that can be accomplished by different microorganisms, but bacteria, in particular *Pseudomonas* sp., seem the most important. Soils can exhibit great variability in their ability to degrade glyphosate (Aamand and Jacobsen 2001; Carlisle and Trevors 1988; Mamy et al. 2005; Sorensen et al. 2006). Degradation of glyphosate takes place under both aerobic and anaerobic conditions, although the degradation under anaerobic conditions is normally less than under aerobic conditions (Rueppel et al. 1977). Increased temperature also increases glyphosate degradation.

- glyphosate's primary route of decomposition in the environment is through microbial degradation in soil

Case studies on fate:

Goldsborough and Beck 1989. This study found that glyphosate dissipated rapidly in small forest ponds. Roundup® herbicide was applied at a rate of 0.59 lb a.i./acre to the surface of four small boreal forest ponds and to six *in situ* microcosms contained in polyethylene basins. Each microcosm initially contained 40 L of unfiltered water collected from a nearby stream. Three randomly selected microcosms contained 0.01 m^3 of intact sediment collected from a nearby pond. Initial glyphosate concentrations in surface water samples collected within 0.5 to 6 hours after application averaged 53 µg/L. Levels of AMPA did not exceed 2.2 µg/L in any pond water sample and in most cases were at or below the 0.50 µg/L detection limit. Glyphosate levels remained at or above the initial treatment concentration in those microcosms containing only water but decreased rapidly in the three microcosms containing pond sediment. The average estimated half-life for glyphosate dissipation in the microcosms containing pond sediment was 5.8 days. Concentrations of AMPA in microcosms were much lower than the levels of glyphosate and did not exceed 20 µg/L.

Feng and Thompson 1990. In a glyphosate herbicide fate study comparing well drained and seasonally flooded areas, (initial aerial application of Roundup® (nominal rate 2.0 kg/ha AI) to the Carnation Creek watershed of Vancouver Island, British Columbia) the initial impingement of the active ingredient on target foliage was high, however the resultant leaf litter residues were 10-fold lower and dissipated rapidly, thus representing an insignificant, transient source of residue input to other ecosystem compartments. Initial soil residues of glyphosate in well-drained sites were high 31.4 -39.8 µg/g, while in a seasonally-flooded location initial soil residues were lower (8.16 pg/g) owing to interception of the chemical by surface water present on-site at the time of application. Little evidence of leaching was observed in either well-drained or seasonally flooded soils. Glyphosate soil residues were non-persistent, dissipating to 13-18% of initial levels within 360 days post-application, with an estimated time to 50% dissipation of 45 - 60 days. The behavior of glyphosate soil residues, as observed in this study, is in agreement with the general literature indicating that glyphosate is nonmobile in and relatively nonpersistent in soils.

Horner 1990. In a dissipation study, glyphosate (Accord®) was aerially applied at the maximum label rate of 3.75 lb a.i./acre to three, 20-acre forestry sites representative of areas of normal silviculture practice in the U.S. The distribution, mobility, and dissipation of glyphosate in the soil were determined. The results of this study found that when used under normal silviculture practices according to label directions, the maximum combined glyphosate and AMPA residue level in soil is less than 5 ppm. The average half-life for the dissipation of glyphosate was 100 days and 118 days for AMPA. It was also determined that under conditions of high rainfall, glyphosate and AMPA are tightly bound to the soil and do not move vertically in the soil profile.

Roy et al. 1989. Roy et al. investigated the persistence, movement, and degradation of glyphosate in selected Canadian forest soils. One sandy soil site was selected for persistence and leaching studies and one clay soil site was selected for a mobility study. All dead wood, live brush, and as much vegetation as possible were manually removed from sandy soil site with minimal disturbance of the 5 - 10 cm soil horizon. For the clay soil test site, dead wood and other matter thought to have a potential for runoff channeling were removed, an application strip at the top of the slope was cleared, and a trench was prepared at the bottom of the slope for the collection of runoff water. For the sandy soil site, the half-life for glyphosate dissipation was 24 days, and the time required for 90% dissipation was 78 days. With the exception of the 14-day after-treatment sampling event, glyphosate was found only in the upper organic layer of the soil. There was no evidence of lateral movement of glyphosate down the slope at the clay soil site. No glyphosate residues greater than or equal to 0.1 ppm were detected at any of the sampling stations located down slope from the application zone or in the runoff water collected in the trench.

Glyphosate in the Aquatic Environment

The contamination of surface and groundwater by glyphosate-based herbicides and glyphosate metabolites is possible *via* a number of different pathways classified as either diffuse or point sources. Point sources are the easiest to define and mitigate, as they most often correspond to hard surfaces or locations where chemical handling and application equipment, tanks, or pails are cleaned or stored (Carter 2000). Mitigation for these types of point sources primarily involves proper handling education and the introduction of best management practices for handling all agricultural chemicals. More difficult to identify and mitigate, and by far the largest proportion of glyphosate contamination, arises from non-point or diffuse sources (Reichenberger et al. 2007).

Multiple routes exist for contamination of surface water by glyphosate; primarily through drift during application or as surface and subsurface runoff following application (Borggaard and Gimsing 2008). Predicted worst-case scenarios for glyphosate concentrations in surface waters have been reported to range from 1.7 to 5.2 mg a.e./L, although environmental levels in this range are unlikely to occur except in incidents of accidental spills or direct overwater application (Giesy et al. 2000; Glozier et al. 2012).

The chemical properties of glyphosate suggest that the likelihood of surface or groundwater contamination should be relatively low. Glyphosate alone has the potential to bind tightly to soil particles depending on pH, soil texture and phosphate levels (Sprankle et al. 1975). Transport of glyphosate into surface waters is highly variable and mainly depends on the level of soil particle adsorption that can be highly variable based on soil chemistry and physical characteristics. Sediment glyphosate concentrations are directly influenced by proximity to application sites, a relationship that has been linked to rainfall events, responsible for the transport of glyphosate from the site of application to surface water *via* surface erosion from treated areas (Peruzzo et al. 2008).

The transport of potentially sorbable compounds such as glyphosate from terrestrial to aquatic environments can occur in solution and in suspension, i.e. the compounds can be transported as solutes or co-transported bonded to soil colloids (colloid-facilitated or particle-bonded transport). Both dissolved and particle-bonded forms can be moved by leaching through the soil (subsurface runoff) and by overland flow (surface runoff). Subsurface leachates end up in drainage and groundwater, while the direct recipients of surface-runoff-transported materials are open waters such as streams and lakes. In uniform, non-structured (apedal) soils, e.g. many sandy soils, water movement through the soil can be described as piston flow (matrix flow), while in structured (pedal) soils, e.g. many clayey soils, preferential flow bypassing more or less the soil matrix is common.

- the largest proportion of glyphosate contamination, arises from non-point or diffuse sources
- contamination of surface water by glyphosate is primarily through drift during application or as surface and subsurface runoff following application
- transport of glyphosate into surface waters is highly variable and mainly depends on the level of soil particle adsorption
- glyphosate can be transported as a solute or co-transported bonded to soil colloids

Glyphosate in uniform (non-structured) soils

The abilities to sorb and degrade glyphosate seem to be general soil properties but are very soil-dependent. Some soils have high glyphosate sorption capacities, while modest amounts are sorbed by other soils. Similarly, glyphosate degradation is rather fast in certain soils, but slow degradation rates are also commonly seen. Sub-surface glyphosate leaching might therefore be expected in soils with low sorption capacity and slow degradation rate, e.g. on sandy, oxide-poor soils with high hydraulic conductivity that receive high precipitation rates, in particular when glyphosate is sprayed immediately before heavy rainfall.

Glyphosate leaching has been demonstrated in uniform but very coarse-textured soil materials, such as under railway embankments, where high rates of glyphosate have been used for weed control; glyphosate concentrations above the European threshold (0.1 μg/L) (European Community Council Directive 1998) were reported in groundwater samples in such situations (Torstensson et al. 2005). Glyphosate leaching can also be severe on gravelly materials, since glyphosate concentrations up to 1300 μg/L were found in leachates from short columns packed with gravel of different particle sizes (Strange-Hansen et al. 2004).

Accordingly, glyphosate leaching is limited in uniform, non-structured soils without macropores, e.g. many sandy soils, and the risk of surface and groundwater pollution by glyphosate is considered to be low. However, long-term use of glyphosate to control weeds on coarse-textured soil materials such as gravel may lead to glyphosate pollution of groundwater, which indicates that oxide-poor sandy soils with a shallow groundwater table may also be vulnerable.

- glyphosate leaching is expected in soils with low sorption capacity and slow degradation rate, in areas of high precipitation rates, in particular when glyphosate is sprayed immediately before heavy rainfall
- glyphosate leaching has been demonstrated in uniform but very coarse-textured soil materials, such as under railway embankments, where high rates of glyphosate have been used for weed control

Glyphosate in structured soils

Only a few studies have shown or indicated sub-surface leaching of glyphosate in structured soils and soil materials with macropores and bypass flow (Kjær et al. 2005; Landry et al. 2005; Veiga et al. 2001). Kjær et al. (2005) monitored the concentrations of glyphosate over 2 years in drainage water samples from tile drains 1 m below the soil surface at three locations and found that fast transport of glyphosate occurred presumably through macropores and were colloid-facilitated.

- glyphosate sub-surface leaching occurs through macropores and is colloid-facilitated

Factors affecting glyphosate leaching

In addition to soil composition and climate, other factors such as timing of application and the presence of vegetative cover can also affect glyphosate leaching. The importance of timing is clearly demonstrated by substantial leaching in relation to rainstorms shortly after glyphosate application on structured soils where rain events closer to application enhanced leaching (Kjær et al. 2005; Veiga et al. 2001). The presence of vegetation can lead to considerably less leaching of glyphosate compared to bare-soil conditions (Landry et al. 2005).

In short, on structured soils with preferential flow pathways through macropores, which are mainly found in clay soils, glyphosate leaching is limited. Heavy rainfall shortly after glyphosate application seems to be important, while other factors such as vegetation, seem to have some effect on glyphosate leaching. Glyphosate may not leach to the groundwater except in soils with a very shallow groundwater table due to fast and often strong sorption to minerals in sediment layers between the soil and the groundwater table that will remove glyphosate from percolation water. Furthermore, glyphosate can also be degraded in deep layer sediments (Sorensen et al. 2006). Accordingly, glyphosate is rarely found in groundwater (Vereecken 2005). However, in lowland areas with a shallow groundwater table and low soil capacity to sorb and degrade glyphosate, use of glyphosate is expected to lead to groundwater pollution.

- in addition to soil composition and climate, factors such as timing of application and the presence of vegetative cover can affect glyphosate leaching
- in areas with a shallow groundwater table and low soil sorbing capacity glyphosate may lead to groundwater pollution

Overland-transported glyphosate

In addition to the above-mentioned delivery with subsurface water, dissolved and suspended glyphosate can also be transported from terrestrial areas to surface waters by overland runoff. The transport of glyphosate will be determined by factors such as rainfall intensity, soil composition, slope characteristics and vegetation, factors that determine water erosion (Hart et al 2004; Brady and Weil 1999).

Glyphosate running into surface waters because of subsurface leaching or by overland flow may remain in the aqueous phase or be trapped by sorption in bottom sediments. The extent of surface v. subsurface glyphosate transport is unknown, as is the total glyphosate transfer from land to surface waters, because of lack of research. Unfortunately, very few studies on runoff losses of glyphosate have been carried out. In a study carried out in a USA watershed, maximum water losses of glyphosate losses ranged from 0.2 to 1.8% of the applied amount (Edwards et al. 1980). In a three-year study in northern Italy, average annual water runoff losses of glyphosate were 0.003% of the applied amount (Screpanti et al. 2005). The highest glyphosate water losses were observed shortly after herbicide application, coinciding with high intensity runoff phenomena; however, the magnitude of sediment losses of glyphosate is not well known, because of lack of research. Further, glyphosate in open waters can also come from other sources such as direct spraying on open waters to control weeds, or windborne spray drift from neighbouring sprayed areas. According to recent reports, concentrations of glyphosate in glyphosate-polluted surface waters can range from sub-µg/L to mg/L levels (Kolpin et al 2006).

- glyphosate can enter receiving waters from direct spraying on open waters, or windborne spray drift
- the highest glyphosate water run off concentrations are observed shortly after herbicide application
- dissolved and suspended glyphosate can be transported from terrestrial areas to surface waters by overland runoff
- the transport of glyphosate will be determined by factors such as rainfall intensity, soil composition, slope characteristics and vegetation

Conditions in BC related to glyphosate fate

The climatic conditions of the coastal areas of British Columbia frequently include autumn and winter rainstorms: annual rainfall often exceeds 2000 mm. Winter temperatures are cool, with snowpack occurring in the upper reaches. Under such conditions, watersheds with areas of high water table, seasonally saturated soils, and frequent surface runoff events following major storms are common. Soil profiles in the flood plain of coastal forest watersheds are often highly stratified into organic rich (30% or greater organic matter content) upper horizons, underlain by coarse-textured mineral soils, low in organic matter. Climatic, hydrological, and physical characteristics of coastal forest ecosystems are unique, and because glyphosate residue mobility and persistence may be affected by such factors, it is important to note that little reliable information exists on the behavior of glyphosate in a coastal rainforest ecosystems.

The colder climate, consistent rainfall, distinct soil types and biota that characterize temperate locations imply the behavior, fate, effects, and management of herbicides such as glyphosate in these locations may prove quite different from those for warmer or tropical locations. For example, soil half-lives reflect the degradation of glyphosate, which include biodegradation under ambient soil and environmental conditions. When measured in the field, dissipation is also affected by processes including plant uptake, and runoff/leaching as a result of prevailing soil and environmental conditions. Available data suggest that the field dissipation of most pesticides in soils is generally faster in environments that are characterized by warmer climates, which in turn fosters degradation through enhanced microbial activity. Warmer locations are also characterized by relatively uniform soil temperatures throughout the year compared to temperate regions, which experience much greater seasonal variability; these uniform conditions enhance soil microbial activity within soils (Racke et al. 1997). Studies that have examined this influence consistently show faster degradation with higher temperatures including, as examples, the insecticide chlorpyrifos (Getzin 1981) and the herbicide atrazine (Korpraditskul et al. 1992). Much of the recent research on pesticides has endeavored to understand the variations in sorption affinity of pesticides to soils, which can be highly variable across different locations and extrapolation of sorption data from cooler to warmer soils is fraught with difficulties, owing to major differences in the nature of soil types (highly weathered, variable charge) as well as carbon chemistry

(Ahmad and Kiikana 2007; Regitano et al. 2000).

- features unique to the Skeena river area that may affect glyphosate input into salmon habitat include frequent rainstorms and high annual rainfall, cool winter temperatures, areas of high water table, seasonally saturated soils, and frequent surface runoff events
- differences between cool temperate areas and warmer environments suggest that the persistence of glyphosate in the environment may be prolonged

Glyphosate uptake and bioaccumulation potential

The major pathway for the uptake of glyphosate in plants is through the foliage and depending upon soil type and conditions, some root uptake may also occur. Gottrup et al. (1976) demonstrated that the presence of humidity and surfactants increases the absorption of glyphosate by plant foliage. Surfactants increased the diffusion rate across the plasma membrane, but not the cuticle (Wyrill and Burnside 1976). Once absorbed, glyphosate is translocated throughout all plant parts where it prevents regrowth.

Currently, there exists a significant knowledge gap regarding uptake of glyphosate or additives from the environment by aquatic organisms and the resulting tissue concentrations of glyphosate, surfactants, or glyphosate metabolites from exposures.

Glyphosate's low octanol/water coefficient (log K_{ow} value −4.59 to −1.70 [Wang et al. 1994]) and low lipid solubility indicate that it will have a low tendency to bioaccumulate. Limited data available from the early 1980s indicated that glyphosate has little to no potential to bioaccumulate when used in forest systems (Ghassemi et al. 1981). Metabolism studies have found that glyphosate residues have minimal tissue retention and are rapidly eliminated from various animal species including mammals, birds, and fish (Franz et al. 1997). Therefore, large-scale food web contamination caused by biomagnification of glyphosate-based herbicides is unlikely.

The possibility of dietary exposure and small scale, short-term food chain effects have been considered given the prevalence of glyphosate in the aquatic environment. For example, *Daphnia pulex* exposed to pure glyphosate either through contaminated water or a contaminated diet had variable rates of glyphosate uptake, with higher body burdens resulting from water column exposure (Bengtsson et al. 2004). There is some evidence that the bioaccumulation of glyphosate may be greater than predicted from the log K_{ow} value alone and the bioconcentration factor (BCF) is increased for glyphosate in the presence of POEA in the aquatic environment (Contardo-Jara et al. 2009). It has been suggested that the same function POEA provides by enhancing glyphosate transport into plant cells, also facilitates increased permeability in animal cells (Hedberg and Wallin 2010). The potential for bioaccumulation of glyphosate has also been observed in terrestrial snails (*Helix aspersa*) fed a diet contaminated with glyphosate (Druart et al. 2011), water hyacinth (*Eichhornia crassipes*) exposed to pure glyphosate, and also in carp (*Cyprinus carpio*) and tilapia (*Oreochromis mossambicus*) that were exposed to environmentally relevant concentrations (Wang et al. 1994). Taken together, these results support the slight possibility of food chain contamination.

- a significant knowledge gap exists regarding the uptake and accumulation of glyphosate from the environment (particularly that bound to particles) by aquatic organisms
- large-scale food web contamination caused by the biomagnification of glyphosate-based herbicides is unlikely, however there is some evidence that the bioaccumulation of glyphosate may be greater than predicted, particularly in the presence of POEA

Glyphosate-Based Herbicide Toxicity

Glyphosate is a highly selective inhibitor of 5-enolpyruvylshikimic acid-3-phosphate synthase (EPSPS), the enzyme responsible for the production of chorismate, a molecule necessary for the biosynthesis of the amino acids phenylalanine, tyrosine and tryptophan in plants (this enzyme is not used in animals) (Malik et al., 1989; Mousdale and Coggins, 1984; Rubin et al., 1984), therefore, it was assumed to be completely safe for animals and humans. The USEPA classifies herbicides in four categories: I, II, III and IV (in decreasing order of toxicity). Studies conducted for the USEPA have resulted in glyphosate being ranked as slightly toxic in Class III (a mild irritant). Similarly, glyphosate has been classified in the lowest toxicological risk category (Class

IV; unlikely to present hazard in normal use) according to the criteria of the World Health Organization (WHO) and the Food and Agriculture Organization (FAO).

However, recent toxicological studies have suggested that glyphosate may have been incorrectly described as "toxicologically benign" for both human health and the environment (CONICET 2009; López et al. 2012). Generally, the indiscriminate use of glyphosate-based herbicides such as Roundup® associated with careless handling, accidental spillage or discharge of untreated effluents into natural waterways has been shown to cause harmful effects in aquatic life and may promote long-term biological effects yet to be discovered (Gallardo et al., 2016; Moustafa et al., 2016).

Glyphosate-based herbicides are currently available in a wide variety of formulations, each based on the same active ingredient but with a unique combination of surfactants, adjuvants, and other chemicals whose identity is often proprietary. Across the spectrum of organisms likely to be exposed to glyphosate in the aquatic environment, it has been shown that sensitivity to glyphosate and the constituents of commercial formulas is highly species-specific. Often, there is a greater difference between the sensitivity of two related species than between species with vast taxonomical separation (Lipok et al. 2010; Moore et al. 2012). Across multiple phyla, studies have shown that the primary source of the toxicity of glyphosate-based herbicides can be attributed primarily to the surfactant portion of the formulation. Thus, toxicity can be associated with the type and concentration of the formulation adjuvants. Roundup®, for example, contains the non-ionic surfactant polyoxyethyleneamine (POEA). Several reports had described adverse glyphosate impacts on biota, but mainly due to the formulations (CONICET 2009). The differences in toxicity of glyphosate, Roundup®, and POEA were first identified by Folmar et al. (1979) who compared the toxicity of technical grade glyphosate, the isopropylamine salt of glyphosate, the surfactant POEA and the commercially available glyphosate herbicide, Roundup®. The study provided information on the sensitivities of several species of aquatic organisms, ranging from aquatic invertebrates to teleost fish; the surfactant in the Roundup® was suggested to be a key factor in toxicity.

- recent toxicological studies have suggested that glyphosate may have been incorrectly described as "toxicologically benign" for both human health and the environment

- it has been shown that the sensitivity to glyphosate and the constituents of commercial formulas is highly species-specific and that there can be a greater difference between the sensitivity of two related species compared to that between different species with vast taxonomical separation
- the primary source of the toxicity of glyphosate-based herbicides can be attributed primarily to the surfactant portion of the formulation

Acute toxicity to fish

Previous studies have characterized the effects of individual glyphosate-based herbicide formulations in a wide variety of aquatic organisms including fish using standard toxicity bioassays (Folmar et al. 1979; Glusczak et al. 2011; Hued et al. 2012; Menezes et al. 2011; Modesto and Martinez 2010a). Toxicity bioassays are tools that allow the determination under specific and controlled conditions, the physical and/or chemical effect on a test organism. A classic study to characterize the potential toxicity of a substance is the acute lethal toxicity test, in which the 96-h LC50 value is determined; this is the concentration of a substance that provokes the death of 50% population of organisms in 96 h.

Fish appear to be less sensitive than amphibians (the most sensitive aquatic vertebrate) to direct exposure to glyphosate-based herbicides (Giesy et al. 2000). In general, adult fish are more tolerant to glyphosate herbicide exposure than younger life stages (Jiraungkoorskul et al. 2002). The acute lethal toxicity values are usually well in excess of expected environmental exposure concentration under common use procedures, thus the threat of mortality *via* acute exposure to these herbicide formulations in the environment has been considered minimal under typical environmental conditions.

There are numerous studies regarding the acute toxic effects of glyphosate-based commercial formulations on aquatic animals (Giesy et al. 2000), but few related to the acute toxicity of solutions of glyphosate alone. Results (96-h LC50 values) for several teleosts from several studies range from 7 to 4000 mg/L. Specific values are as follows: *Oncorhynchus mykiss*, a species of high sensitivity, 140 mg/L (Folmar et al. 1979); *Odontesthes bonariensis*, 163 mg/L

(Carriquiriborde 2011); *Poecilia reticulata*, > 400 mg/L (Alvarez et al. 2012) and *Cyprinus carpio*, a highly tolerant species, 620 mg/L (Neskovic et al. 1996). Several authors have noted that glyphosate formulations containing the active principle together with coadjuvants such as POEA present a significant increase in toxicity to aquatic organisms including fish (Pérez et al. 2011). In the review by Giesy et al. (2000), the acute toxicity data for 12 fish species from a variety of studies prior to 2000, reported that the 96-h LC50 values in fish ranged from 4.2 to 52 mg/L for Roundup®. Specific 96-h LC50 values for formulated glyphosate (Roundup®) in various fish species are also available from other studies: *Rhamdia quelen*, 7.3 mg/L (Kreutz et al. 2008); *Oncorhynchus mykiss*, 8.2-27 mg/L (Giesy et al. 2000; Wan et al. 1991); *Cyprinus carpio*, 10 mg/L (Giesy et al. 2000); *Prochilodus lineatus*, 13.7 mg/L (Langiano and Martinez 2008); *Gambusia yucatana*, 17.8 mg/L (Osten et al. 2005); *Leporinus obtusidens*, >100 mg/L (Glusczak et al. 2006). Again, much of the toxicity of the commercial formulation is attributed to the surfactant portion, particularly POEA that has a range of 96-h LC50 values between 0.65 and 7.4 mg/L (Folmar et al. 1979). In a study by Uchida et al. (2011) it was found that the acute lethal toxicity (96-h LC50) of glyphosate was 160 mg/L, but when the surfactant was added to the exposure, the toxicity was increased with a 96-h LC50 value of 8.5 mg/L. The LC50 of the fully formulated mixture was 76.8 mg/L.

In a comprehensive study (Wan et al. 1989) comparing glyphosate, and 3 formulations of the herbicide to 5 species of salmonids (coho, pink, chum, Chinook and rainbow trout) showed that not all formulations are more toxic than glyphosate. In addition most of these species are similar in their sensitivities. This work also shows that the toxicity of glyphosate or formulations was higher in soft water *v.* hard water. The 96-h LC50 value appears to vary considerably for the same fish species tested under similar conditions. For example the 96-h LC50 for rainbow trout of Roundup® in reconstituted water (pH 7.8, hardness 40 mg/L $CaCO_3$) of this test is 18 mg/L. Other studies using the same fish species under comparable rearing techniques and test conditions of fish size, pH, hardness, and water temperature in reconstituted water (APHA 1985) reported values varying from 1.6 mg/L (Folmar et al. 1979) to 22 mg/L (Hildebrand et al. 1982; Mitchell et al. 1987; Servizi et a. 1987). Different strains of rainbow trout tested under similar conditions may have varying 96-h LC50 values, but these values would be unlikely to vary by an order of magnitude.

The toxicity of other formulations of glyphosate have been reported as well; 96-h LC50 values for R-11 was 6 mg/L, for LI 700 it was 17 mg/L, HASTEN 74 mg/L, and for AGRI-DEX the value was 271 mg/L (Smith et al. 2004), indicating that these formulations can be very toxic to juvenile rainbow trout, but that there is a wide range of toxicity which is formulation dependent.

- fish appear to be less sensitive than amphibians to direct exposure to glyphosate-based herbicides
- adult fish are more tolerant to glyphosate herbicide exposure than younger life stages
- for glyphosate, 96-h LC50 values range from 7 to 4000 mg/L for teleosts
- for rainbow trout, *Oncorhynchus mykiss,* the toxicity of glyphosate ranged from 1.3 to 824 mg/L
- the 96-h LC50 of formulated glyphosate (Roundup®) for various fish species ranges from 4.3 to >100 mg/L (for rainbow trout, *Oncorhynchus mykiss*, values ranged from 8.2-27 mg/L)
- polyethoxylated tallow amine (POEA) has a range of LC50 96-h values between 0.65 and 7.4 mg/L to fish
- the toxicity of glyphosate or formulations is typically higher in soft water *v*. hard water

Sublethal effects in fish

Several modes of action have been investigated regarding the source of glyphosate-based herbicide toxicity in non-target organisms, including general stress, the induction of oxidative stress and reactive oxygen species, acetylcholinesterase inhibition, genotoxicity, histopathological alterations, energy metabolism changes, and behavioural effects including those on olfaction. As there is currently no consensus on a single mechanism of glyphosate-based herbicide toxicity, it is likely that multiple mechanisms exist, depending on the particular combination of formula and species. There remains a need to identify the mode of action of glyphosate-based herbicide toxicity across a range of organisms, given that the probability of aquatic exposure will increase as the use of this herbicide continues to expand.

The hazard assessment process for glyphosate-based herbicides has primarily relied on relatively insensitive, mortality endpoints for calculation. Though these endpoints suggest population level effects are possible, they are often only found at concentrations well above those that are responsible for behavioural, cellular and metabolic alterations that may have environmental relevance to exposed organisms.

- there is currently no consensus on a single mechanism of glyphosate-based herbicide toxicity and it is likely that multiple mechanisms exist
- glyphosate-based herbicide sublethal toxicity includes general stress, oxidative stress, acetylcholinesterase inhibition, genotoxicity, histopathological alterations, energy metabolism changes, and behavioural effects

Oxidative stress

There is evidence that oxidative stress may be one of the mechanisms of glyphosate toxicity in animals. Reactive oxygen species (ROS) are often generated during normal physiological activities; ROS are also called reactive oxygen intermediaries, oxide free radicals, or radicals and are the product of the reduction of molecular oxygen (O_2) to superoxide radicals ($O_{2\cdot}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH_{\cdot}$). The latter intermediate is a strong oxidant capable of reacting with cellular macromolecules causing various effects such as enzymatic inactivation, membrane lipid peroxidation (mitochondria, lysosomes, endoplasmic reticulum), DNA damage and ultimately cellular death (Helmut 1991; Mitchelmore et al. 1998; van der Oost et al. 2003). The generation of ROS, implicated as a mechanism of toxicity for numerous toxicants, can have a variety of detrimental effects on cells (Bagchi et al. 1995).

Oxidative stress is a cellular response to stressors, caused by an imbalance between the production of high oxidative potential molecules (oxygen-derived) and the ability of a particular biological system to quickly detoxify the intermediates generated or repair the resulting damage (Di Giulio and Meyer, 2008; Repetto and Repetto 2009) leading to the cellular effects listed above (Lushchak 2011). Antioxidant defense systems include antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione S-transferase (GST), and glutathione reductase (GRED) (van der Oost et al., 2003). Other nonenzymatic defenses include liposoluble vitamins (N-tocopherol and β-carotene), and low molecular weight molecules (ascorbic acid, catecholamines, glutathione) (Helmut 1991; Winston and Di Giulio 1991; van der Oost et al. 2003). Exposure to oxidative stressors can be assessed by monitoring changes in the magnitude of some these critical biomarkers. These show either the effects of oxidative stress on the macromolecules or the antioxidant response, such as enzymes of the antioxidant system whose synthesis or activity may be modified as a consequence of the imbalanced intracellular redox state.

The effect of glyphosate herbicide exposure on the activity of key antioxidant enzymes, including SOD, GST, and CAT, varies considerably depending on the experimental protocol and species tested. For example, the activity of liver GST decreased while there was no change in the activity of SOD or CAT in silver catfish (*Rhamadia quelen*) exposed to Roundup® at concentrations up to 0.95 mg/L (Menezes et al. 2011). During a subsequent recovery period, SOD and CAT activity decreased, interpreted by the authors as either a compensatory response by the fish against the toxic conditions, or insufficient recovery time to elicit a complete recovery. An exposure of goldfish (*Carassius auratus*) to high concentrations (2.5–20 mg/L) inhibited SOD in multiple tissues and decreased liver GST activity, whereas CAT activity increased significantly at lower concentrations tested (Lushchak et al. 2009). Further evidence for oxidative stress induction in goldfish liver by Roundup® and its constituents, including POEA, was also provided by Fan et al. (2013). Paiva (*Leporinus obtusidens*) exposed to Roundup® also displayed an increase in CAT activity (Glusczak et al. 2011). A decrease in GST activity (Lajmanovich et al. 2011) was observed in tadpoles acutely exposed to a commercial glyphosate formulation while similar concentrations resulted in either an increase or a decrease in GST activity in some fish species (Modesto and Martinez 2010a, 2010b). To further characterize the link between exposure and oxidative stress, reduced glutathione (GSH) content in tissues can be measured, as the non-protein thiol acts as a sink for free radicals (Schuliga et al. 2002). Decreased GSH levels were observed in fish exposed to Roundup Transorb® (1 and 5 mg/L) with a 24-h exposure, followed by an increase in GSH content after a 96-h exposure (Modesto and Martinez 2010a). This result suggests a recovery after 96-h Roundup® exposure, a pattern that has been observed for exposure to other herbicides (Zhang et al. 2004).

Glyphosate-based herbicide toxicity has been linked to lipid peroxidation (LPO), most often identified through elevated thiobarbituric acid reactive substances (TBARS) levels. The primary target tissue tends to be the liver, (Glusczak et al., 2011), although increased TBARS have been observed in the brain of carp (*Cyprinus carpio*) (Cattaneo et al. 2011) and silver catfish (Menezes et al. 2011) exposed to Roundup®. These observed changes in LPO were found to be transient in silver catfish, as LPO levels returned to normal after a 8-d recovery period to 0.45 mg/L Roundup® (Menezes et al. 2011). However, recovery relative to control values was not observed in carp acutely exposed to Roundup® at 2.5 mg/L (Cattaneo et al. 2011), indicating possible species-specific differences in susceptibility to oxidative stress. Further evidence for the potential of glyphosate-based herbicides to induce oxidative stress is an increase in protein carbonyl, an indicator of protein damage caused by ROS (Glusczak et al. 2011; Menezes et al. 2011). Roundup Transorb® induced LPO in fish after short-term exposure (6 h), although this oxidative damage was mitigated in longer-term exposure (96 h) and correlated with an induction of antioxidant enzymes (Modesto and Martinez, 2010a). Overall, the available evidence suggests that glyphosate-based herbicides may cause oxidative damage in aquatic organisms, depending on species and duration of exposure. Induction of antioxidant systems during chronic, low level exposures, may allow cells to combat oxidative stress effectively and reduce the occurrence of oxidative damage (Lushchak et al. 2009; Modesto and Martinez 2010a, 2010b).

- glyphosate can cause oxidative stress by the production of reactive oxygen species (ROS); the generation of ROS can be measured by changes in the antioxidant enzyme defense system
- glyphosate herbicides can affect the activity of key antioxidant enzymes, including SOD, GST, and CAT, but can vary depending on the experimental protocol and species tested
- glyphosate-based herbicide toxicity by ROS includes lipid peroxidation and protein damage

Neurotoxicity and acetylcholinesterase activity

Acetylcholinesterase (AChE), belongs to the family of enzymes called esterases, and is responsible for the hydrolysis of acetylcholine, a neurotransmitter present in the synapses of the nervous system in both vertebrates and invertebrates. It has been adopted as a biomarker of changes occurring in synaptic junctions in the central nervous system, neuromuscular cholinergic and sympathetic systems, and can affect the behaviour, locomotion, and balance of animals. Measurements of AChE activity can be useful in ecotoxicological studies (Bradbury et al. 2008), such that alterations in the activity of AChE can provide information regarding the effects chemical exposure on the nervous system and may aid in explaining abnormal behaviors in fish (Tierney et al. 2007). This enzyme has previously been used as a sensitive biomarker of organophosphate pesticide exposure. These pesticides are inhibitors of AChE activity leading to an accumulation of acetylcholine in the synaptic cleft, thereby causing over-stimulation of the post-synaptic membrane, a process that can lead to death.

A number of previous studies have associated exposure to glyphosate-based herbicides with the inhibition of AChE activity in brain and/or muscle of aquatic organisms (Cattaneo et al., 2011; Glusczak et al. 2006, 2007; Lajmanovich et al. 2011; Menendez-Helman et al. 2012; Modesto and Martinez 2010a; Salbego et al. 2010; Sandrini et al. 2013). Recently, it has been shown that acute exposure to glyphosate formulations can alter the activity of AChE; in *Leporinus obtusidens* exposed for 96 h to Roundup® (concentrations in the range of 3 to 20 mg/L): AChE activity was significantly decreased in the brain (Glusczak et al. 2006). In a chronic exposure study to 5 mg/L Roundup®, *Leporinus obtusidens* showed brain AChE inhibition (Salbego et al. 2010). A similar result was reported for *Rhamdia quelen* after exposure to 0.2 and 0.4 mg/L Roundup® (Glusczak et al. 2007). In *Prochilodus lineatus*, AChE activity in the brain was significantly reduced by acute exposure to 1 and 5 mg/L Roundup Transorb® (Modesto and Martínez 2010a).

The level of AChE inhibition observed in these studies was below the level deemed life threatening although it may lead to muscle hyperactivity, a condition linked with the production of damaging ROS (Yang et al. 1996). It is unclear what behavioural or locomotor effects may occur with these levels of inhibition. Additionally, it has also been shown that AChE is required for normal muscle and neuron development in fish, a result which suggests that alteration of AChE activity may have survival as well as population level effects (Behra et al. 2002).

- studies have associated exposure to glyphosate-based herbicides with the inhibition of AChE activity in aquatic organisms
- it is unclear what behavioural or locomotor effects may occur with these levels of inhibition as studies linking AChE inhibition and behavioural effects have not been performed

Hematology

Hematological alterations, specifically an increase in hematocrit, were detected in *P. lineatus* exposed for 96 h to Roundup Transorb® at 5 mg/L (Modesto and Martinez 2010a). Increases in both erythrocyte and leukocyte numbers suggest that defense mechanisms in response to contaminant exposure were activated in the fish (Cazenave et al. 2005). This result is different from an earlier study by Glusczak et al. (2006) where a decrease in hematocrit was observed in *L. obtusidens* after exposure to Roundup Original®. Differences in surfactant formulation between Roundup Original® and Roundup Transorb® as well as species-specific responses to contaminant exposure may explain some of this discrepancy (Elahee and Bhagwant 2007). It is of interest to note that the surfactants are not solely responsible for the toxicity of the commercial formulations in these non target species. The immune response, quantified as a phagocytic index of coelomic cells and bacteria agglutination in fingerlings of silver catfish (*Rhamadia quelen*), was decreased after 24-h exposure to 0.73 mg/L pure glyphosate (Kreutz et al. 2011). Even though further studies utilizing commercial formulations should be conducted to provide a more realistic proxy to environmental exposures, these results suggest that the active ingredient alone, rather than the surfactant portion caused the observed decrease in natural immune response.

- exposure to glyphosate-based herbicides can alter the hematology of teleosts, however, results have been varied in the direction of these responses
- the immune response of fish can be decreased following short term exposure to low concentrations of glyphosate

Histopathology

Glyphosate exposure has been reported to cause histopathological alterations in the liver of fish (Langiano and Martinez 2008). Alterations in liver histopathology suggesting altered metabolic activity, was also observed at low concentrations in fish exposed to Roundup Max® (0.5 mg/L), suggesting that even at environmentally-relevant concentrations, exposure to commercial glyphosate formulations can alter normal liver and gill structure (Hued et al. 2012). The alterations observed in gill included hyperplasia, epithelial lifting, hypertrophy, and protective responses to limit toxicant diffusion through the gill. The gills are the initial target organ for xenobiotic exposure through water and are particularly susceptible, given their large surface area, vital gas exchange function, and their osmoregulatory role. Gill and liver alterations, similar to those reported in *Jenynsia*, were observed in Nile tilapia (*Oreochromis niloticus*) exposed for 3 months to sublethal concentrations of Roundup (2.4 and 7.2 mg a.e./L), along with kidney lesions which were correlated with significant increases in the activity of plasma aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase (Jiraungkoorskul et al. 2003). These biochemical alterations are indicative of increased metabolism of amino acids in response to elevated energy demand during periods of physiological stress and cellular damage (Jyothi and Narayan 1999). Given that the liver is the primary site of xenobiotic detoxification, significant alterations to structure or normal function will likely have deleterious effects on organism health and performance.

- glyphosate exposure has been reported to cause histopathological alterations in the liver of fish
- glyphosate formulations can alter normal liver and gill structure; alterations observed in gill included hyperplasia, epithelial lifting, and hypertrophy

Genotoxicity

The genotoxic potential of Roundup® has been assessed primarily using comet, micronucleus and erythrocytic nuclear abnormality assays, which determine the quantity of double strand breaks in DNA, induced chromosome damage, and abnormalities in the erythrocyte nucleus respectively. Relatively few studies of the genotoxic effects of glyphosate-based herbicides on aquatic organisms have been published. Grisolia and Starling (2001) reported that intra-abdominal injection of Roundup® increased the formation of micronuclei in *Tilapia rendalli*. Likewise, goldfish and the tropical fish *Corydoras paleatus* showed increased micronuclei frequency as well as dose dependent increases in DNA damage in peripheral erythrocytes following exposure to Roundup® (Cavas and Konen 2007; Ghisi and Cestari 2013). In-ovo exposure of Roundup® to broad-snouted caiman (*Caiman latirostris*) resulted in a dose-dependent increase in the production of micronuclei as well as double strand breaks after direct application of 500 µg/L (Poletta et al. 2009) and at more environmentally relevant exposures (Poletta et al. 2011).

Double-stranded DNA breaks were observed in European eel (*Anguilla anguilla*) exposed to 58 and 116 µg/L after only 24 h, and an increased tendency for erythrocytic nuclear abnormalities was observed after 3 d of exposure to glyphosate-based herbicide (Guilherme et al. 2010). Subsequent studies determined that the type of DNA damage varies with exposure period and concentration, with ROS-dependent DNA damage being the primary mechanism of genotoxicity after 3 d (Guilherme et al. 2012). Roundup® exposure affected DNA integrity in the Neotropical fish, *Prochilodus lineatus*, after only 6 h of exposure at the much higher concentration of 10 mg/L (Cavalcante et al. 2008). These effects were shown to be attenuated over time, with the majority of damage occurring soon after exposure, a pattern which can be explained by engagement of DNA repair systems and the activation of detoxification pathways (Banu et al. 2001). Similar results, with a dose-dependent induction of DNA breaks and decreased effects, suggest repair with time, were also reported in the frog *Eleutherodactylus johnstonei* (Meza-Joya et al. 2013).

In a DNA microarray analysis, Uchida et al. (2011) found no significant gene expression changes in the liver of Japanese medaka (*Oryzias latipes*) exposed to glyphosate for 96 h at concentration between 10 and 160 mg/L. However, when exposed to glyphosate and the fatty acid alkanolamide surfactant, 138 genes were significantly induced.

- the genotoxicity of Roundup® in teleosts has been shown with increases in double strand breaks in DNA, induced chromosome damage, and abnormalities in the erythrocyte nucleus

Energy alterations, reproduction, other effects

Mobilization of energy stores, a common stress response in fish, was observed as a decrease in liver and/or muscle glycogen in Piava exposed acutely (96 h, 3–20 mg/L) (Glusczak et al. 2006) or chronically (90 days, 5 mg/L) to Roundup® (Salbego et al. 2010). An increase in plasma glucose was detected in *P. lineatus* acutely (6 – 96 h) exposed to 7.5 and 10 mg/L of Roundup®, a response that was not associated with a subsequent increase in plasma cortisol (Langiano and Martinez 2008). It should be noted that blood samples were obtained 6 h following exposure and plasma cortisol may have been restored to normal levels by this time, given that cortisol levels typically lag 30 min to 1 h after any disturbance (Barton 2002). Aberrant control of cortisol production is indicative of a xenobiotic affecting the hypothalamus–pituitary–gonadal axis (HPI), a highly controlled hormone system vital for normal stress response in fishes (Hontela and Vijayan 2009). Silver catfish fingerlings acutely exposed to sublethal concentrations of Roundup® (1.2 – 3.65 mg/L) had decreased cortisol production relative to controls, suggesting a decreased ability, when in the presence of glyphosate-based herbicide, to effect the physiological adjustments in response to environmental challenges (Soso et al. 2007). This same study also assessed the endocrine disrupting potential of Roundup® by demonstrating reduced 17β-estradiol levels in correlation with decreased viability of resulting swim-up fry after 40 days of exposure. However, chronic exposures of stickleback (*Gasterosteus aculeatus*) larvae to glyphosate did not induce vitellogenin or spiggin, markers of estrogenic and androgenic effects, respectively (Le Mer et al. 2013). It has been recommended that the use of herbicides such as glyphosate to control aquatic plants should be discouraged until later in a growing season due to their possible effects on fertilized fish eggs and fry (Hiltibran 1967), although data regarding effects on reproduction are few and assessments are premature. Folmar et al. (1979) found that exposure of rainbow trout (*Oncorhyncus mykiss*) to up to 2.0 mg/L of glyphosate or Roundup® for 12 h had no effect on fecundity or gonadosomatic index. In addition, Mitchell et al. (1987) found that exposure to 2.78 mg/L Roundup® for 10 d did not affect seawater adaptation or growth in coho salmon (*Oncorhynchus kisutch*).

- evidence of a physiological stress response to glyphosate-based herbicide exposure has been shown in fish
- studies with both stress and reproductive hormones in fish show that exposure to glyphosate-based herbicides affects hormone profiles and potential whole organism effects

Behaviour

Behavioural endpoints offer several advantages over more traditional measures of aquatic toxicity, including higher sensitivity, relevance in the natural environments, and effects at the organism level which can be linked to effects at the population and species level (Hellou 2011). Concentrations of glyphosate in the aquatic environment are typically orders of magnitude lower than those expected to cause acute toxicity, thus highly sensitive, sub-lethal endpoints such as behaviour are ideal for monitoring the effects of glyphosate-based herbicides in the aquatic environment.

Fish behaviour and the effects of glyphosate-based herbicides have been assessed using a variety of sensitive endpoints, including reproductive displays and preference/avoidance responses (olfaction). Adult zebrafish (*Danio rerio*) were tested as either naïve or pre-exposed to herbicide mixtures containing pure glyphosate, at concentrations equivalent to those detected in the natural environment (0.26–309 ng/L), to determine if fish would avoid the herbicide and if exposure would alter their attraction to L-alanine, a proxy for the presence of food (Tierney et al. 2011). Zebrafish were found to spend more time in the regions of herbicide pulse addition and this response was independent of previous experience. Attraction to food cues was increased in exposed fish, suggesting a link between the energetic costs of contaminant exposure and compensatory increase in food requirement. Juvenile rainbow trout (*Oncorhynchus mykiss*) only avoided Roundup® contaminated water at concentrations in excess of 10 mg a.i./L, whereas exposure at 100 µg/L led to altered L-histidine preference behaviour as well as hypoactivity, suggesting that Roundup® inhibited the ability to react normally to chemical stimuli (Tierney et al. 2007). The mechanism of olfactory recognition of food and the effect of glyphosate herbicide exposure on the ability of fish to recognize odorant cues can be assayed using electro-olfactogram (EOG), wherein evocation of the EOG signals the detection of an odorant. Roundup® was shown to evoke EOG's, demonstrating trout have the ability to sense the presence of this formulation in the aquatic environment. To support this, Hildebrand et al. (1982) determine that rainbow trout juveniles will avoid lethal concentrations of Roundup. However, other studies indicate that rainbow trout will not avoid low concentrations of glyphosate (Folmar 1976). Exposure of rainbow trout to glyphosate in the form of Vision® at sublethal concentrations had little effect on rainbow trout juveniles for 2 months (Morgan and Kiceniuk 1992) with no significant effects of exposure for either 1 or 2 months on any of foraging or agonistic activity variables that were measured or growth.

- trout have the ability to sense the presence of glyphosate-based herbicide formulations
- trout will not avoid low concentrations of glyphosate but will avoid high and potential lethal concentrations
- glyphosate exposure resulted in an increased attraction to food cues in fish suggesting a link between the energetic costs of exposure and compensatory increase in food requirements
- Roundup® contaminated water at high concentrations led to altered olfactory behaviour as well as hypoactivity suggesting an inhibition of the ability to react normally to chemical stimuli

CONCLUSIONS

The recent spraying of a glyphosate-based herbicide in the area of the CN railway which closely follows the Skeena River and the potential effects on the salmonid population in the in the area prompted the generation of this report. A summary of important information that aids in answering specific questions regarding the herbicide fate and effects are as follows. Commercially available glyphosate-based herbicide formulations are complex mixtures in which glyphosate acts as the active ingredient and often include a surfactant and water. In most applications, the strong sorption of glyphosate to vegetation/soil results in it being relatively nonleachable and therefore contamination of water bodies is believed to be limited. However, there are several features of this application and the area in question that may not support this conclusion. First, the contamination of the receiving water (Skeena River and associated tributaries, feeder streams etc.) by glyphosate will be highly dependent on the proximity of herbicide application to the water body. Here, it has been reported that spraying of the CN line occurred directly adjacent to the river without a 5 m buffer zone. In circumstances such as this, direct overspray, or drift during herbicide application can result in significant quantities of glyphosate entering adjacent aquatic environments.

Second, the ability to sorb glyphosate is very soil-dependent; some soils have high glyphosate sorption capacities, while modest amounts are sorbed by other soils. Glyphosate leaching can occur in uniform and very coarse-textured soil materials, such as under railway embankments or in areas of gravelly materials; examples where high rates of glyphosate have been used for weed control show higher glyphosate concentrations in groundwater in these areas. In the spray area, in addition to course substrate, smaller soil particulates will exist to which glyphosate will bind. However, glyphosate sorption capacity, and hence leachability, in the area is unknown and depends on several soil characteristics including mineralogical composition, pH, phosphate content, and perhaps SOM. It is possible that soils in the CN railway area have a low binding capacity for glyphosate, and therefore, along with glyphosate's very high water solubility would result in significant amounts entering the river. Regardless of the binding efficiency of soil in the area, both dissolved and particle-bonded forms will be moved by leaching through the soil and by surface runoff. Subsurface leachates end up in drainage and groundwater, while the direct recipients of surface-runoff-transported materials are open waters such as the Skeena River, particularly during precipitation events. As well, the preponderance of small watercourses and streams in the area will enhance the movement of glyphosate into the main river.

In addition to the above, the coastal areas of British Columbia have climatic conditions that frequently include high precipitation rates in autumn and winter with low temperatures; under such conditions, watersheds with areas of high water table, seasonally saturated or coarse-textured mineral soils, and frequent surface runoff events could lead to appreciable concentrations of chemicals in glyphosate herbicide formulations reaching surface waters following application close to aquatic ecosystems. Glyphosate is highly soluble and stable in water and its loss from water is through sediment adsorption and microbial degradation; its overall loss from aquatic systems ranges from 27 to 146 days. Cool winter temperatures can prolong glyphosate half-life in soils and water through reductions in microbial degradation.

Numerous studies regarding the toxic effects of glyphosate-based commercial formulations on aquatic animals exist, and indicate a clear difference between the toxicity of the active ingredient, other constituents, and formulations. For glyphosate, 96-h LC50 values range from 7 to 4000 mg/L, whereas the values for the formulated herbicide can range from 4.3 to >100 mg/L. Much of the toxicity of the commercial formulation is attributed to the surfactant portion, particularly polyethoxylated tallow amine that has a range of values between 0.65 and 7.4 mg/L. Glyphosate or glyphosate-containing herbicide exposure can also result in the following sublethal effects in various fish species: avoidance behaviour, alterations in olfactory ability, histopathological changes, oxidative stress, genotoxicity, malformations, hematologic changes, alterations of hormone profiles, and decreases in the cell-mediated immune response.

Although the acute lethal toxicity concentrations of glyphosate-based herbicide formulations are considered to be of moderate toxicity and may be unlikely to cause mortality in fish in the Skeena River itself, concentrations in smaller spawning reaches may under some circumstances reach levels that could cause mortality. There exists a knowledge gap regarding many aspects of the application, environmental conditions, and properties of the receiving environments to make firm conclusions in this regard. However, sublethal effects occur at much lower concentrations and include a variety of effects that should be considered significant and may pose a risk to fish in general, and spawning Pacific salmon in areas adjacent to application of the herbicide.

REFERENCES

Aamand J, and OS Jacobsen. 2001. Sorption and degradation of glyphosate and dichlobenil in fractured clay. Proc. Symp. on Pesticide Behaviour in Soils and Water.

Ahmad R, R Kookana. 2007. Geographical extrapolation of pesticide environmental fate data: challenges, risks and opportunities. Am. Chem. Soc. Symposium Ser. 966: 100−119.

Alvarez M, IT Gimenez, H Saitua, RD Enriz, and FA Giannini. 2012. Toxicidad en peces de herbicidas formulados con glifosato. Acta Toxicológica Argentina, 20, 5-13.

Annett R, HR Habibi, and A Hontela. 2014. Impact of glyphosate and glyphosate-based herbicides on the freshwater environment. J. Appl. Toxicol. 34: 458–479.

Bagchi D, M Bagchi, EA Hassoun, and SJ Stohs. 1995. In vitro and in vivo generation of reactive oxygen species, DNA damage and lactate dehydrogenase leakage by selected pesticides. Toxicology 104(1-3): 129–140.

Balthazor TM, and LE Hallas. 1986. Glyphosate-degrading microorganisms from industrial activated sludge. Appl. Environ. Microbiol. 51: 432–434.

Banu BS, K Danadevi, MF Rahman, YR Ahuja, and J Kaiser. 2001. Genotoxic effect of Monocrotophos to sentinel species using comet assay. Food Chem. Toxicol. 39(4): 361–366.

Barja BC, and M Dos Santos Afonso. 2005. Aminomethylphosphonic acid and glyphosate adsorption onto goethite: a comparative study. Environ. Sci. Technol. 39: 585–592.

Barton BA. 2002. Stress in fishes: A diversity of responses with particular reference to changes in circulating corticosteroids. Integr. Comp. Biol. 42(3): 517–525.

Battaglin WA, DW Kolpin, EA Scribner, KM Kuivila, and MW Sandstrom. 2005. Glyphosate, other herbicides, and transformation products in midwestern streams, 2002. J. Am. Water Resour. Assoc. 41(2): 323–332.

Baylis AD. 2000. Why glyphosate is a global herbicide: strengths, weaknesses and prospects. Pest Manag. Sci. 56: 299–308.

Behra M, X Cousin, C Bertrand, JL Vonesch, D Biellmann, A Chatonnet, and U Strahle. 2002. Acetylcholinesterase is required for neuronal and muscular development in the zebrafish embryo. Nat. Neurosci. 5(2): 111–118.

Bengtsson G, LA Hansson, and K Montenegro. 2004. Reduced grazing rates in *Daphnia pulex* caused by contaminants: Implications for trophic cascades. Environ. Toxicol. Chem. 23(11): 2641–2648.

Borggaard OK, and AL Gimsing. 2008. Fate of glyphosate in soil and the possibility of leaching to ground and surface waters: A review. Pest Manag. Sci. 64(4): 441–456.

Borggaard OK, and B Elberling. 2004. Pedological Biogeochemistry. Paritas, Brøndby, Denmark, 483 pp.

Botta F, G Lavison, G Couturier, F Alliot, E Moreau-Guigon, N Fauchon, B Guery, M Chevreuil, and H Blanchoud. 2009. Transfer of glyphosate and its degradate ampa to surface waters through urban sewerage systems. Chemosphere 77(1): 133–139.

Bradbury SP, RW Carlson, TR Henry, S Padilla, and J Cowden. 2008. Toxic responses of the fish nervous system. In: Di Giulio, R.T., Hinton, D.E. (Eds), The Toxicology of Fishes, CRC Press-Taylor and Francis Group, Boca Raton, FL. Pp. 417-455.

Brady, N and R Weil. 1999. The Nature and Properties of Soils, 12th edition. Prentice-Hall, Upper Saddle River, NJ, 881 pp.

Bronstad, JO, and Friestad HO. 1985. The Herbicide Glyphosate. p. 200-205. Butterworth and Co. Ltd. Toronto.

Carlisle SM, and JT Trevors. 1988. Glyphosate in the environment. Water Air Soil Pollut. 39: 409–420.

Carriquiriborde P. 2011. Toxicidad de glifosato en peces autóctonos: Estudios de laboratorio y campo. In: Camino M, Aparicio V (Eds), Aspectos ambientales del uso del glifosato. Ediciones INTA (Argentina), 57-68.

Carter A. 2000. How pesticides get into water - and proposed reduction measures. Pestic. Outlook 11: 149–156.

Cattaneo, R, B Clasen, VL Loro, CC de Menezes, A Pretto, B Baldisserotto, A Santi, and LA de Avila. 2011. Toxicological responses of *Cyprinus carpio* exposed to a commercial formulation containing glyphosate. Bull. Environ. Contam. Toxicol. 87(6): 597–602.

Cavalcante D, CBR Martinez, and SH Sofia. 2008. Genotoxic effects of Roundup® on the fish Prochilodus lineatus. Mut. Res. Gen. Toxicol. Environ. Mutagen. 655(1-2): 41–46.

Cavas T, and S Konen. 2007. Detection of cytogenetic and DNA damage in peripheral erythrocytes of goldfish (*Carassius auratus*) exposed to a glyphosate formulation using the micronucleus test and the comet assay. Mutagenesis 22(4): 263–268.

Cazenave J, DA Wunderlin, AC Hued, and MDA Bistoni. 2005. Haematological parameters in a neotropical fish, *Corydoras paleatus* (Jenyns, 1842) (Pisces, Callichthyidae), captured from pristine and polluted water. Hydrobiologia 537: 25–33.

CONICET. 2009. Comisión Nacional de Investigación sobre Agroquímicos. Consejo Científico Interdisciplinario creado en el ámbito del Consejo Nacional de Investigaciones Científicas y Técnicas. "Informe - evaluación de la información científica vinculada al glifosato en su incidencia sobre la salud humana y el ambiente". Ciudad Autónoma de Buenos Aires.

Contardo-Jara, V, E Klingelmann, and C Wiegand. 2009. Bioaccumulation of glyphosate and its formulation Roundup ultra in Lumbriculus variegatus and its effects on biotransformation and antioxidant enzymes. Environ. Pollut. 157(1): 57–63.

De jonge H, and LW de jonge. 1999. Influence of pH and solution composition on the sorption of glyphosate and prochloraz to a sandy loam soil. Chemosphere 39: 753–763.

De jonge H, LW de Jonge, OH Jacobsen, T Yamaguchi, and P Moldrup. 2001. Glyphosate sorption in soils of different pH and phosphorus content. Soil Sci. 166: 230–238.

Di Giulio RT, and JN Meyer. 2008. Reactive oxygen species and oxidative stress. The Toxicology of Fishes, CRC Press-Taylor and Francis Group, Boca Raton, FL. Pp. 273-324.

Dick RE, and JP Quinn. 1995. Glyphosate-degrading isolates from environmental samples: occurrence and pathways of degradation. Appl. Microbiol. Biotechnol. 43: 545–550.

Dideriksen K, and SL Stipp. 2003. The adsorption of glyphosate and phosphate to goethite: a molecular-scale atomic force microscopy study. Geochim. Cosmochim. Acta 67: 3313–3327.

Dion HM, JB Harsh, and HH Hill. 2001. Competitive sorption between glyphosate and inorganic phosphate on clay minerals and low organic matter soils. J. Radioanal. Nucl. Chem. 249: 385–390.

Domitrovic HA. 1997. Toxicidad y respuesta histopatológica en Aequidens portalegrensis (Pisces, Cichlidae) expuestos a Glifosato en ensayos de toxicidad aguda y en ensayos subletales. Univ Nac Nordeste (Argentina), Actas Reunión Comunicaciones Científicas Tecnológicas. Tomo II: 4-29/4-32.

Druart C, M Millet, R Scheifler, O Delhomme, and A de Vaufleury. 2011. Glyphosate and glufosinate-based herbicides: Fate in soil, transfer to, and effects on land snails. J. Soils Sediments 11(8): 1373–1384.

Duke SO, and SB Powles. 2008. Glyphosate: A once-in-a-century herbicide. Pest Manag. Sci. 64(4): 319–325.

Edwards WM, WB Triplett, and RM Kramer. 1980. A watershed study of glyphosate transport in runoff. J. Environ. Qual. 9: 661-665.

Elahee KB, and S Bhagwant. 2007. Hematological and gill histopathological parameters of three tropical fish species from a polluted lagoon on the West coast of Mauritius. Ecotoxicol. Environ. Saf. 68(3): 361–371.

El-Gendy KS, NM Aly, and AH El-Sebae. 1998. Effects of Edifenphos and glyphosate on the immune response and protein biosynthesis of bolti fish (Tilapia nilotica). J. of Environ. Sci. Health B 33: 135-149.

Eriksson, KE. 1975. Roundup. Weeds and Weed Control. 16: J5-6.

European Community Council Directive. 1998. Concerning the quality of water intended for human consumption. Off. J. Eur. Commun. L330: 32–54.

Fan JY, JJ Genga, HQ Rena, XR Wang, and C Han. 2013. Herbicide Roundup® and its main constituents cause oxidative stress and inhibit acetylcholinesterase in liver of *Carassius auratus*. J. Environ. Sci. Health, Part B: Pesticides, Food contaminants, and agricultural wastes 48: 844–850.

Feng JC, and DG Thompson. 1990. Fate of glyphosate in a Canadian forest watershed. 2. Persistence in foliage and soils. J. Agric. Food Chem. 38: 1118–1125.

Feng JC, DG Thompson, and PE Reynolds. 1990. Fate of Glyphosate in a Canadian Forest Watershed. 1. Aquatic Residues and Off Target Deposit Assessment. J. Agric. Food Chem. Vol. 38. pp. 1110-1118.

Folmar, LC. 1976. Overt avoidance reaction of rainbow trout fry to nine herbicides. Bull. Environ. Contam. Toxicol. 15: 509-514.

Folmar LC, HO Sanders, and AM Julin. 1979. Toxicity of the herbicide glyphosate and several of its formulations to fish and aquatic invertebrates. Arch. Environ. Contam. Toxicol. 8(3): 269–278.

Franz JE, MK Mao, and JA Sikorski. 1997. Glyphosate: A Unique Global Herbicide. American Chemical Society. Chap. 4 pp. 65-97.

Gallardo K, J Verbel, J Freeman. 2016. Toxicogenomics to evaluate endocrine disrupting effects of environmental chemicals using the zebrafish model. Current Genomics. 17: 515-527.

Gerritse RG, J Beltran, and F Hernandez. 1996. Adsorption of atrazine, simazine and glyphosate in soils of the Gnangara Mound, Western Australia. Aust. J. Soil Res. 34: 599–607.

Getzin, L. 1981. Dissipation of chlorpyrifos from dry soil surfaces. J. Econ. Entomol. 74: 707−713.

Ghassemi M, L Fargo, P Painter, S Quinlivan, R Scofield, and A Takata. 1981. Environmental fates and impacts of major forest use pesticides. Office of Pesticides and Toxic Substances. Washington D.C. U.S. EPA. p. A-149-168.

Ghisi NC, and MM Cestari. 2013. Genotoxic effects of the herbicide Roundup® in the fish *Corydoras paleatus* (Jenyns 1842) after short-term, environmentally low concentration exposure. Environ. Monit. Assess. 185: 3201–3207.

Gianessi, LP, and N Reigner. 2006. Pesticide use in U.S. Crop protection: 2002. Washington, D.C.

Giesy JP, S Dobson, and KR Solomon. 2000. Ecotoxicological risk assessment for Roundup® herbicide. Rev. Environ. Contam. Toxicol. 167: 35–120.

Gilbert N. 2013. A hard look at GM crops. Nature 497: 24–26.

Gimsing AL, and OK Borggaard. 2001. Effect of KCl and CaCl2 as background electrolytes on the competitive adsorption of glyphosate and phosphate on goethite. Clays Clay Miner 49: 270–275.

Gimsing AL, and OK Borggaard. 2002. Competitive adsorption and desorption of glyphosate and phosphate on clay silicates and oxides. Clay Miner 37: 509–515.

Gimsing AL, and OK Borggaard. 2007. Phosphate and glyphosate adsorption by hematite and ferrihydrite and comparison with other variable-charge minerals. Clays Clay Miner 55: 110–116.

Gimsing AL, C Szilas, and OK Borggaard. 2007. Sorption of glyphosate and phosphate by variable-charge tropical soils from Tanzania. Geoderma 138: 127–132.

Gimsing AL, OK Borggaard, and M Bang. 2004. Influence of soil composition on adsorption of glyphosate and phosphate by contrasting Danish surface soils. Eur. J. Soil Sci. 55: 183–191.

Glozier NE, J Struger, AJ Cessna, M Gledhill, M Rondeau, WR Ernst, MA Sekela, SJ Cagampan, E Sverko, C Murphy, JL Murray, and DB Donald. 2012. Occurrence of glyphosate and acidic herbicides in select urban rivers and streams in Canada, 2007. Environ. Sci. Pollut. Res. Int. 19: 821–834.

Glusczak L, DdS Miron, BS Moraes, RR Simões, MRC Schetinger, VM Morsch, and VL Loro. 2007. Acute effects of glyphosate herbicide on metabolic and enzymatic parameters of silver catfish (*Rhamdia quelen*). Comp. Biochem. Physiol. 146C: 519-524.

Glusczak L, DdS Miron, M Crestani, MB da Fonseca, FDA Pedron, MF Duarte, and VUP Vieira. 2006. Effect of glyphosate herbicide on acetylcholinesterase activity and metabolic and hematological parameters in Piava (*Leporinus obtusidens*). Ecotoxicol. Environ. Saf. 65: 237–241.

Glusczak L, VL Loro, A Pretto, BS Moraes, A Raabe, MF Duarte, MB da Fonseca, CC de Menezes, and DMD Valladao. 2011. Acute exposure to glyphosate herbicide affects oxidative parameters in Piava (Leporinus obtusidens). Arch. Environ. Contam. Toxicol. 61: 624–630.

Goldsborough LG, and AE Beck. 1989. Rapid dissipation of glyphosate in small forest ponds. Arch. Environ. Contam. Toxicol. 18: 537–544.

Goldsbourough LG and DJ Brown. 1993. Dissipation of Glyphosate and Aminomethylphosphonic Acid in Water and Sediments of Boreal Forest Ponds. Envir. Tox. And Chem. Vol. 12. pp. 1139-1147

Gottrup O, PA O'Sullivan, RJ Schraa, and WH Vanden Born. 1976. Uptake, translocation, metabolism, and selectivity of glyphosate in Canada Thistle and Leafy Spurge. Weed Research. 16: 197-201.

Grisolia CK, and F Starling. 2001. Micronuclei monitoring of fishes from lake Paranoa, under influence of sewage treatment plant discharges. Mut. Res. Gen. Toxicol. Environ. Mutagen 491: 39–44.

Grunewald K, W Schmidt, C Unger, and G Hanschmann. 2001. Behavior of glyphosate and aminomethylphosphonic acid (AMPA) in soils and water of reservoir Radeburg II catchment (Saxony/Germany). J. Plant Nutr. Soil Sci. 164: 65–70.

Guilherme S, I Gaivao, MA Santos, and M Pacheco. 2010. European eel (*Anguilla anguilla*) genotoxic and pro-oxidant responses following short-term exposure to Roundup® - a glyphosate-based herbicide. Mutagenesis 25: 523–530.

Guilherme S, I Gaivao, MA Santos, and M Pacheco. 2012. DNA damage in fish (Anguilla anguilla) exposed to a glyphosate-based herbicide - elucidation of organ-specificity and the role of oxidative stress. Mut. Res. Gen. Toxicol. Environ. Mutagen 743: 1–9.

Hance RJ. 1976. Adsorption of glyphosate by soils. Pestic. Sci. 7:363–366.

Hanke I, I Wittmer, S Bischofberger, C Stamm, and H Singer. 2010. Relevance of urban glyphosate use for surface water quality. Chemosphere 81: 422–429.

Hart M, B Quin and M Nguyen. 2004. Phosphorus runoff from agricultural land and direct fertilizer effects: a review. J. Environ. Qual. 33: 1954–1972.

Hedberg D, and M Wallin. 2010. Effects of Roundup and glyphosate formulations on intracellular transport, microtubules and actin filaments in Xenopus laevis melanophores. Toxicol. In Vitro 24: 795–802.

Hellou J. 2011. Behavioural ecotoxicology, an "early warning" signal to assess environmental quality. Environ. Sci. Pollut. Res. 18: 1–11.

Helmut S. 1991. Oxidative Stress, Oxidants and Antioxidants. Academic Press, London.

Hildebrand LD, DS Sullivan, and TP Sullivan. 1982. Experimental studies of rainbow trout populations exposed to field applications of Roundup® herbicide. Arch. Environ. Contam. Toxicol. 11: 93-98.

Hiltibran, RC. 1967. Effects of some herbiceds on fertilized eggs and fry. Amer. Fish Soc. Trans. 96: 414.

Hontela A, and MM Vijayan. 2009. Adrenocortical Toxicology in Fishes, Chapter 10. In Adrenal Toxicology, Target Organ Toxicology Series, Harvey PW, Everett DJ, Springall CJ (eds). Informa Healthcare USA, Inc.: New York; 233–256.

Horner LM. 1990. Dissipation of glyphosate and aminoethylphosphonic acid in forestry sites. Monsanto Company. Report #MSL-9940.

Hued AC, S Oberhofer, and MD Bistoni. 2012. Exposure to a commercial glyphosate formulation (Roundup®) alters normal gill and liver histology and affects male sexual activity of Jenynsia multidentata (*Anablepidae, Cyprinodontiformes*). Arch. Environ. Contam. Toxicol. 62(1): 107–117.

Jacob GS, J Schaefer, EO Stejskal, and RA McKay. 1985. Solidstate NMR determination of glyphosate metabolism in a *Pseudomonas sp.* J. Biol. Chem. 260: 5899–5905.

Jacob GS, JR Garbow, LE Hallas, NM Kimack, GM Kishore, and J Schaefer. 1988. Metabolism of glyphosate in *Pseudomonas sp*. strain LBr. Appl. Environ. Microbiol. 54: 2953–2958.

Jacobsen OS. 2003. Degradation, sorption and persistence of glyphosate and the metabolite AMPA in a fractured clay soil profile. Proc. XII Symp. on Pesticide Chemistry, Piecenza, Italy.

Jaworska J, H van Genderen-Takken, A Hanstveit, E van de Plassehe, and T Feijtel. 2002. Environmental risk assessment of phosphonates, used in domestic laundry and cleaning agents in the Netherlands. Chemosphere 47(6): 655–665.

Jiraungkoorskul W, ES Upatham, M Kruatrachue, S Sahaphong, and S Vichasri-Grams. 2002. Histopathological effects of Roundup, a glyphosate herbicide, on Nile tilapia (*Oreochromis niloticus*). Science Asia 28: 121–127.

Jiraungkoorskul W, ES Upatham, M Kruatrachue, S Sahaphong, S Vichasri-Grams, and P Pokethitiyook. 2003. Biochemical and histopathological effects of glyphosate herbicide on Nile tilapia (*Oreochromis niloticus*). Environ. Toxicol. 18(4): 260–267.

Jyothi B, and G Narayan. 1999. Certain pesticide-induced carbohydrate metabolic disorders in the serum of freshwater fish *Clarias batrachus* (Linn.). Food Chem. Toxicol. 37: 417–421.

Kier LD and DJ Kirkland. 2013. Review of genotoxicity studies of glyphosate and glyphosate-based formulations. Crit. Rev.Toxicol. 43: 283-315.

Kirkwood RC. 1979. Advance in Pesticide Science. Geissbuhler, H. Ed. Pergamon Press. Oxford. pp 420-429.

Kishore GM, and GS Jacob. 1987. Degradation of glyphosate by Pseudomonas sp. strain PG2982 via a sarcosine intermediate. J. Biol. Chem. 262: 12164–12168.

Kjær J, P Olsen, M Ullum, and R Grant. 2005. Leaching of glyphosate and amino-methylphosphonic acid from Danish agricultural field sites. J. Environ. Qual. 34: 608–620.

Kollman W, and R Segawa. 1995. Interim report of the pesticide chemistry database. Environmental hazards Assessment Program. Department of Pesticide Regulation.

Kolpin DW, EM Thurman, EA Lee, MT Meyer, ET Furlong, and ST Glassmeyer. 2006. Urban contributions of glyphosate and its degradate AMPA to streams in the United States. Sci. Total Environ. 354: 191–197.

Korpraditskul R, V Korpraditskul, S Kuwatsuka 1992. Degradation of the herbicide atrazine in five different Thai soils. J. Pestic. Sci. 17: 287−289.

Kreutz LC, LJG Barcellos, SD Valle, TD Silva, D Anziliero, ED dos Santos, M Pivato, and R Zanatta. 2011. Altered hematological and immunological parameters in silver catfish (Rhamdia quelen) following short term exposure to sublethal concentration of glyphosate. Fish Shellfish Immunol. 30: 51–57.

Kreutz LC, LJG Barcellos, TO Silva, D Anziliero, D Martins, M Lorenson, A Marteninghe, and LB Da Silva. 2008. Acute toxicity test of agricultural pesticides on silver catfish (*Rhamdia quelen*) fingerlings. Ciencia Rural, 38: 1050-1055.

Lajmanovich RC, AM Attademo, PM Peltzer, CM Junges, and MC Cabagna. 2011. Toxicity of four herbicide formulations with glyphosate on Rhinella arenarum (Anura: Bufonidae) tadpoles: B-esterases and glutathione s-transferase inhibitors. Arch. Environ. Contam. Toxicol. 60: 681–689.

Landry D, S Dousset, J-C Fournier and F Andreux. 2005. Leaching of glyphosate and AMPA under two soil management practices in Burgundy vineyards (Vosne-Roman´ee, 21-France). Environ. Pollut. 138: 191–200.

Langiano VC, and CBR Martinez. 2008. Toxicity and effects of a glyphosate based herbicide on the neotropical fish Prochilodus lineatus. Comp. Biochem. Physiol. C: Toxicol. Pharmacol. 147: 222–231.

Le Mer C, RL Roy, J Pellerin, CM Couillard, and D Maltais. 2013. Effects of chronic exposures to the herbicides atrazine and glyphosate to larvae of the three spine stickleback (*Gasterostreus aculeatus*). Ecotoxicol. Environ. Saf. 89: 174–181.

Lerbs W, M Stock, and B Parthier. 1990. Physiological aspects of glyphosate degradation in Alcaligenes spec. strain GL. Arch. Microbiol. 153: 146–150.

Lipok J, H Studnik, and S Gruyaert. 2010. The toxicity of Roundup® 360 SL formulation and its main constituents: Glyphosate and isopropylamine towards non-target water photoautotrophs. Ecotoxicol. Environ. Saf. 73: 1681–1688.

Liu C-M, PA McLean, CC Sookdeo, and FC Cannon. 1991. Degradation of the herbicide glyphosate by members of the family Rhizobiaceae. Appl. Environ. Microbiol. 57: 1799–1804.

López SL, D Alassa, S Benítez-Leite, R Lajmanovich, F Mañas, G Poletta, N Sánchez, MF Simoniello, and AE Carrasco. 2012. Pesticide used in South American GMO-based agriculture: a review of their effects on humans and animal models. Advances Mol. Toxicol. 6: 41-75.

Lushchak OV, OI Kubrak, JM Storey, KB Storey, and VI Lushchak. 2009. Low toxic herbicide Roundup induces mild oxidative stress in goldfish tissues. Chemosphere 76: 932–937.

Mackay D, WY Sieu, and KC Ma. 1997. Illustrated Handbook of Physical-Chemical Properties and Environmental Fate of Organic Chemicals. Lewis: New York.

Malik J, G Barry, and G Kishore. 1989. The herbicide glyphosate. BioFactors 2: 17-25.

Mamy L, E Barriuso, and B Gabrielle. 2005. Environmental fate of herbicides trifluralin, metazachlor, metamitron and sulcotrione compared with that of glyphosate, a substitute broad spectrum herbicide for different glyphosate-resistant crops. Pest Manag. Sci. 61: 905–916.

McConnel JS, and LR Hossner. 1985. pH dependent adsorption isotherms of glyphosate. J Agric. Food Chem. 33: 1075–1078.

McConnel JS, and LR Hossner. 1989. X-ray diffraction and infrared spectroscopic studies of adsorbed glyphosate. J Agric. Food Chem. 37: 555–560.

Menendez-Helman RJ, GV Ferreyroa, MD Afonso, and A Salibian. 2012. Glyphosate as an acetylcholinesterase inhibitor in Cnesterodon decemmaculatus. Bull. Environ. Contam. Toxicol. 88: 6–9.

Menezes CC, MB Fonseca, VL Loro, A Santi, R Cattaneo, B Clasen, A Pretto, and VM Morsch. 2011. Roundup effects on oxidative stress parameters and recovery pattern of Rhamdia quelen. Arch. Environ. Contam. Toxicol. 60: 665–671.

Meza-Joya FL, MP Ramírez-Pinilla, and JL Fuentes-Lorenzo. 2013. Toxic, cytotoxic, and genotoxic effects of a glyphosate fromulation (Roundup®SL-Cosmosflux®411F) in the direct-developing frog Eleutherodactylus johnstonei. Environ. Mol. Mutagen. 54: 362–373.

Mitchell D, P Chapman, and T Long. 1987. Acute toxicity of Roundup and Rodeo herbicides to rainbow trout, chinook, and coho salmon. Bull. Environ. Contam. Toxicol. 39: 1028-1035.

Mitchelmore CL, C Birmelin, JK Chipman, and DR Livingstone. 1998. Evidence for cytochrome P-450 catalysis and free radical involvement in the production of DNA strand breaks by benzo[a]pyrene and nitroaromatics in mussels (Mytilus edulis) digestive gland cells. Aquat. Toxicol. 41: 193-212.

Modesto KA, and CBR Martinez. 2010a. Effects of Roundup Transorb on fish: Hematology, antioxidant defenses and acetylcholinesterase activity. Chemosphere 81: 781–787.

Modesto KA, and CBR Martinez. 2010b. Roundup® causes oxidative stress in liver and inhibits acetylcholinesterase in muscle and brain of the fish Prochilodus lineatus. Chemosphere 78: 294–299.

Moore LJ, L Fuentes, JH Rodgers Jr, WW Bowerman, GK Yarrow, WY Chao, and WC Bridges Jr. 2012. Relative toxicity of the components of the original formulation of Roundup® to five north american anurans. Ecotoxicol. Environ. Saf. 78: 128–133.

Moshier LJ, and D Penner. 1978. Factors influencing microbial degradation of 14C-glyphosate to $^{14}C\text{-}CO_2$ in soil. Weed Sci. 26: 686–691.

Mousdale DM, and JR Coggins. 1984. Purification and properties of 5-enolpyruvylshikimate 3-phosphate synthase from seedlings of Pisum sativum L. Planta, 160: 78-83.

Moustafa G, F Shaaban, A Hadeed, W Elhady. 2016. Immunotoxicological, biochemical, and histopathological studies on Roundup and Stomp herbicides in Nile catfish (*Clarias gariepinus*). Vet. World. 9: 638-647.

Nešković NK, V Poleksić, I Elezović, V Karan, and M Budimir. 1996. Biochemical and histopathological effects of glyphosate on carp, Cyprinus carpio. Bull. Environ. Contam. Toxicol. 56: 295-302.

Newton M, LM Homer, JE Cowell, DE White, and EC Cole. 1994. Dissipation of Glyphosate and Aminomethylphosphonic Acid in North American Forests. J. Agric. Food Chem. Vol. 42. pp. 1795-1802

Oliveira Jr RS, WC Koskinen, and FA Ferreira. 2001. Sorption and leaching potential of herbicides in Brazilian soils. Weed Res. 41: 97–110.

Osten JR v-, A Ortíz-Arana, L Guilhermino, and AMVM Soares. 2005. In vivo evaluation of three biomarkers in the mosquitofish (Gambusia yucatana) exposed to pesticides. Chemosphere 58: 627-636.

Pérez GL, MS Vera, and L Miranda. 2011. Effects of Herbicide Glyphosate and Glyphosate-Based Formulations on Aquatic Ecosystems. In: Kortekamp A (Ed), Herbicides and Environment. In Tech, pp. 343-368.

Peruzzo, PJ, AA Porta, and AE Ronco. 2008. Levels of glyphosate in surface waters, sediments and soils associated with direct sowing soybean cultivation in North Pampasic region of Argentina. Environ. Pollut. 156(1): 61–66.

Piccolo A, G Celano, and P Conte. 1996. Adsorption of glyphosate by humic substances. J Agric. Food Chem. 44: 2442–2446.

Pipke R, and N Amrhein. 1988. Degradation of the phosphonate herbicide glyphosate by Arthrobacter atrocyaneus ATCC13752. Appl. Environ. Microbiol. 54: 1293–1296.

Poletta GL, A Larriera, E Kleinsorge, and MD Mudry. 2009. Genotoxicity of the herbicide formulation Roundup® (glyphosate) in broad-snouted caiman (Caiman latirostris) evidenced by the comet assay and the micronucleus test. Mut. Res. Gen. Toxicol. Environ. Mutagen. 672(2): 95–102.

Poletta GL, E Kleinsorge, A Paonessa, MD Mudry, A Larriera, and PA Siroski. 2011. Genetic, enzymatic and developmental alterations observed in Caiman latirostris exposed in ovo to pesticide formulations and mixtures in an experiment simulating environmental exposure. Ecotoxicol. Environ. Saf. 74(4): 852–859.

Racke K, M Skidmore, D Hamilton, J Unsworth, J Miyamoto, S Cohen. 1997. Pesticide fate in tropical soils. Pure Appl. Chem. 69: 1349–1371.

Reichenberger S, M Bach, A Skitschak, and HG Frede. 2007. Mitigation strategies to reduce pesticide inputs into ground and surface water and their effectiveness; a review. Sci. Total Environ. 384(1-3): 1–35.

Regitano J, L Alleoni, P Vidal-Torrado, J Casagrande, V Tornisielo and V Imazaquin. 2000. Sorption in highly weathered tropical soils. J. Environ. Qual. 29: 894–900.

Repetto Jiménez M, and G Repetto Kuhn. 2009. Toxicología Fundamental. Ediciones Díaz de Santos, Madrid.

Roy DN, SK Konar, S Banerjee, DA. Charles, DG Thompson, and R Prasad. 1989. Persistence, movement, and degradation of glyphosate in selected Canadian Boreal forest soils. J. Agric. Food Chem. 37: 437.

Rubin JL, CG Gainess, and RA Jensen. 1984. Glyphosate inhibition of 5-enolpyruvylshikimate–3-phosphate synthase from suspension-cultured cells of Nicotina silvestris. Plant Physiology, 75: 839-845.

Rueppel ML, BB Brightwell, J Schaefer, and J Marvel. 1977. Metabolism and degradation of glyphosate in soil and water. J Agric. Food Chem. 25: 517–528.

Salbego J, A Pretto, CR Gioda, CC de Menezes, R Lazzari, JR Neto, B Baldisserotto, and VL Loro. 2010. Herbicide formulation with glyphosate affects growth, acetylcholinesterase activity, and metabolic and hematological parameters in Piava (Leporinus obtusidens). Arch. Environ. Contam. Toxicol. 58(3): 740–745.

Sandrini JZ, RC Rola, FM Lopes, HF Buffon, MM Freitas, CMG Martins, and CE Rosa. 2013. Effects of glyphosate on cholinesterase activity of the mussel Perna perna and the fish Danio rerio and Jenynsia multidentata: *In vitro* studies. Aquat. Toxicol. 130-131: 171–173.

Schuette J. 1998. Environmental Fate of Glyphosate. Environmental Monitoring and Pest Management Department of Pesticide Regulation 95824-5624.

Schuliga M, S Chouchane, and ET Snow. 2002. Upregulation of glutathione-related genes and enzyme activities in cultured human cells by sublethal concentrations of inorganic arsenic. Toxicol. Sci. 70(2): 183–192.

Schwarzenbach RP, PM Gschwend and DM Imboden. 1993. Environmental Organic Chemistry. John Wiley and Sons, Inc., New York, NY, 681 pp.

Screpanti C, C Accinelli, A Vicari, and P Catizone. 2005. Glyphosate and glufosinate-ammonium runoff from a corn-growing area in Italy. Agron. Sustain. Dev. 25: 407-412.

Servizi J, R Gordon and D Martens. 1987. Acute toxicity of Garlon 4 and Roundup herbicides to salmon, daphnia and trout. Bull. Environ. Contam. Toxicol. 39: 15-22.

Sheals J, S Sjōberg, and P Persson. 2002. Adsorption of glyphosate on goethite: molecular characterization of surface complexes. Environ. Sci. Technol 36: 3090–3095.

Smith B, C Curran, K Brown, J Cabarrus, J Gown, J McIntyre, E Moreland, V Wong, J Grassley and C Grue. 2004. Toxicity of four surfactants to juvenile rainbow trout; implications for use over water. Bull. Environ. Contam. Toxicol. 72: 647-654.

Solomon KR, and DG Thompson. 2003. Ecological risk assessment for aquatic organisms from over-water uses of glyphosate. J. Toxicol. Environ. Health- Part B-Crit. Rev. 6(3): 289–324.

Sørensen SR, A Schultz, OS Jacobsen, and J Aamand. 2006. Sorption, desorption and mineralisation of the herbicides glyphosate and MCPA in samples from two Danish soil and subsurface profiles. Environ. Pollut. 141: 184–194.

Soso AB, LJG Barcellos, MJ Ranzani-Paiva, LC Kreutz, RM Quevedo, D Anziliero, M Lima, LB da Silva, F Ritter, AC Bedin, and others. 2007. Chronic exposure to sub-lethal concentration of a glyphosate-based herbicide alters hormone profiles and affects reproduction of female Jundia (Rhamdia quelen). Environ. Toxicol. Pharmacol. 23(3): 308–313.

Sprankle P, WF Meggitt, and D Penner. 1975. Adsorption, mobility, and microbial degradation of glyphosate in soil. Weed Sci. 23(3): 229–234.

Stehr CM, TL Linbo, DH Baldwin, NL Scholz, and JP Incardona. 2009. Evaluating the effects of forestry herbicides on fish development using rapid phenotypic screens. N. Amer. J. Fish. Manag. 29(4): 975-984.

Strange-Hansen R, PE Holm, OS Jacobsen, and CS Jacobsen. 2004. Sorption, mineralization and mobility of N-(phosphonomethyl) glycine (glyphosate) in five different types of gravel. Pest Manag. Sci. 60: 570–578.

Szarek J, A Siwicki, A Andrzejewska, E Terech-Majewska and T Banaszkiewicz. 2000. Effects of the herbicide Roundup® on the ultrastructural pattern of hepatocytes in carp (*Cyprinus carpio*). Mar. Environ. Res. 50: 263-266.

Thurman EM, and JD Fallon. 1996. The deethylatrazine/atrazine ratio as an indicator of the onset of the spring flush of herbicides into surface water of the Midwestern United States. Int. J. Environ. Anal. Chem. 65(1-4): 203–214.

Tierney KB, CR Singh, PS Ross, and CJ Kennedy. 2007. Relating olfactory neurotoxicity to altered olfactory-mediated behaviors in rainbow trout exposed to three currently-used pesticides. Aquat. Toxicol. 81(1): 55–64.

Tierney KB, MA Sekela, CE Cobbler, B Xhabija, M Gledhill, S Ananvoranich, and BS Zielinski. 2011. Evidence for behavioral preference toward environmental concentrations of urban-use herbicides in a model adult fish. Environ. Toxicol. Chem. 30(9): 2046–2054.

Torstensson L, E Börjesson, and J Stenstrōm. 2005. Efficacy and fate of glyphosate on Swedish railway embankments. Pest Manag. Sci. 61: 881–886.

Uchida M, S Takumi, K Tachikawa, R Yamauchi, Y Goto, H Matsusaki, H Nakamura, Y Kagami, T Kusano and K Arizono. 2011. Toxicity evaluation of glyphosate agrochemical components using Japanese medaka (*Oryzias latipes*) and DNA microarray gene expression analysis. J. Toxicol. Sci. 37: 245-254.

USEPA. 1986. Guidance for the reregistration of pesticide products containing glyphosate. Washington, D.C.: Office of Pesticide Programs.

USEPA. 1990. Pesticide Fact Handbook. Vol. 2. p. 301-312. Noyes Data Corporation. Park Ridge, New Jersey.

USDA Forest Service. 1984. Pesticide background statements. p. G1-G72. In Agriculture Handbook No. 633. Vol. 1. Herbicides. Part 2.

van der Oost R, J Beyer, and NPE Vermeulen. 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environmental Toxicology and Pharmacology, 13: 57-149.

Veiga F, JM Zapata, MLF Marcos, and E Alvarez. 2001. Dynamics of glyphosate and aminomethylphosphonic acid in forest soil in Galicia, north-west Spain. Sci. Total Environ. 271: 135–144.

Vereecken H. 2005. Mobility and leaching of glyphosate: a review. Pest Manag. Sci. 61: 1139–1151.

Wan M, R Watts and D Moul. 1989. Effects of different dilution water types on the acute toxicity to juvenile Pacific salmonids and rainbow trout of glyphosate and its formulated products. Bull. Environ. Contam. Toxicol. 43: 378-385.

Wan M, R Watts and D Moul. 1991. Acute toxicity to juvenile Pacific northwest salmonids fo Basacid Blue NB755 and its mixture with formulated products of 2,4-D, glyphosate and triclopyr. Bull. Environ. Contam. Toxicol. 47: 471-478.

Wang CJ, and ZQ Liu. 2007. Foliar uptake of pesticides - present status and future challenge. Pestic. Biochem. Physiol. 87(1): 1–8.

Wang YJ, DM Zhou, and RJ Sun. 2005. Effects of phosphate on the adsorption of glyphosate on three different types of Chinese soils. J. Environ. Sci. 17: 711–715.

Wang YS, JH Yen, YN Hsieh, and YL Chen. 1994. Dissipation of 2,4-d glyphosate and paraquat in river water. Water Air Soil Pollut. 72(1-4): 1–7.

Wauchope RD, TM Buttler, AG Hornsby, and PWM Augustijnbeckers. 1992. The SCS ARS CES pesticide properties database for environmental decision making. Rev. Environ. Contam. Toxicol. 123: 1–155.

Weed Science Society of America (Ed.). 1989. Glyphosate. Herbicide Handbook of the Weed Science Society of America. 6th ed. p. 146-149. Champaign, Illinois.

Winston GW, and RT Di Giulio. 1991. Prooxidant and antioxidant mechanisms in aquatic organisms. Aquat. Toxicol. 19: 137-161.

Wyrill JB, and OC Burnside. 1976. Adsorption, translocation, and metabolism of 2,4-D and glyphosate in Common Milkweed and Hemp Dogbane. Weed Sci. 23: 557-566.

Yang ZP, J Morrow, AP Wu, LJ Roberts, and WD Dettbarn. 1996. Diisopropylphosphorofluoridate-induced muscle hyperactivity associated with enhanced lipid peroxidation in vivo. Biochem. Pharmacol. 52(2): 357–361.

Zhang JF, H Shen, XR Wang, JC Wu, and YQ Xue. 2004. Effects of chronic exposure of 2,4-dichlorophenol on the antioxidant system in liver of freshwater fish *Carassius auratus*. Chemosphere 55(2): 167–174.