# EXPANSION ON
Glyphosate fate and toxicity to salmon and steelhead populations in the lower Skeena River watershed with special reference to environmental and biological parameters that may modify fate and effects

Prepared for:

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**Report Priorities:**

T. Buck Suzuki Environmental Foundation (TBSEF) requested an expansion on a previous report (BioWest Environmental Research Consultants 2017) 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.

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)— and the possibility of adverse effects on salmon, at the organism or at the population level. The previous report highlighted several aspects which may alter glyphosate availability and toxicity to fish in this area compared to other areas of application and included: 1) high soil acidity, 2) high rainfall, 3) temperate climate, 4) proximity of rail line to Skeena and crossing of myriad of small watercourses, streams and rivers, and 5) lack of soil in application areas. In addition, other parameters including high water table, seasonally saturated soils, coarse textured mineral soils/substrates low in organic matter, and closeness to aquatic ecosystems was also to be discussed.

This extension was to include a focus and elaboration on the above aspects of the environment and salmon biology that may predispose these populations to a higher potential for effects compared to other jurisdictions and populations of fish species. This report is written to address these concerns for the benefit of local residents who have a limited scientific vocabulary.

## Executive Summary

The Skeena Watershed is the second largest watershed in BC and supports five salmon species; sockeye (*Oncorhynchus nerka*), pink (*O. gorbuscha*), chum (*O. keta*), Chinook (*O. tshawytscha*), and coho (*O. kitsch*) as well as steelhead/rainbow trout, cutthroat trout, Dolly Varden, bull trout, and lake trout. The lower Skeena River and its tributary streams have high fish values including escapements of over two million salmon and productive resident fish populations. The basin provides a quality habitat which is sensitive to ecosystem disturbance.

Glyphosate (*N*–[phosphonomethyl]glycine) is the active ingredient in Roundup® and other 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.

The potential fate of spraying glyphosate in the area of the location of the CN railway, which closely follows the lower Skeena River, and the possibility of adverse effects on salmon were previously reported on (BioWest Environmental Research Consultants 2017). This report highlighted aspects of glyphosate fate and toxicity that may be modified by jurisdiction- or site-specific attributes that could lead to an increased potential for adverse effects on salmonid populations following glyphosate application to railway ballast sections, and rights of way in close proximity to the CN line in the Lower Skeena River south of Prince Rupert, and upstream to Terrace, BC. Factors that were identified and elaborated on in this report as potential modifiers of exposure and effects include: high soil acidity, high amounts of rainfall, temperate climate, proximity of rail line to Skeena and crossing of myriad of small watercourses, streams and rivers, lack of soil (i.e. long stretches of vegetation over rocky ground), high water table, seasonally saturated soils, and coarse textured mineral soils/substrates low in organic matter.

Glyphosate's tendency to volatilize into the atmosphere is low and it tends to partition to water more so than air; the colder temperatures found in the lower Skeena are not likely to impact volatilization (loss) to any significant

degree. The atmosphere can be a source of glyphosate to the aquatic environment of the Skeena and nearby tributaries through particulate (e.g. mists, droplets etc.) deposition of the herbicide. Deposition by drift can be increased by wind and precipitation events that commonly occur in this area.

Glyphosate is highly soluble in water, and therefore readily dissolves and disperses in aquatic environments. Due to its high water solubility at all typical Skeena river water temperatures, no significant decrease in the dissolved fraction of glyphosate in the water is likely. 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 mainly through sediment adsorption (which will be seasonally- and geographically-dependent) and microbial degradation; it is unclear if colder temperatures would reduce the microbial degradation of glyphosate in water.

Glyphosate possesses unique sorption characteristics in soil by binding tightly to most soil types, although in some soils glyphosate can be highly mobile. Glyphosate sorption can be positively and negatively, correlated with soil organic matter content. Glyphosate sorption to soils also depends on pH; sorption decreases with increasing pH. The highly acidic nature of typical Skeena soils will tend to enhance binding. This sorption rate, however, is expected to decrease at the lower temperatures typically seen in the area. The soil in the lower Skeena area is categorized as a ferro-humic podzol. Glyphosate sorption to soil particles of this type may be reduced in the top soil layers due to the eluviation of sesquioxides from upper layers and the formation of a cementing layer that may prevent water containing glyphosate penetration to lower soil levels where binding would be higher.

The two main loss pathways of glyphosate from soil are leaching and microbial degradation. Glyphosate is moderately persistent in soil with half-lives ranging from 3 to 130 days in the soil environment. Although glyphosate is relatively resistant to leaching and a low tendency to runoff, the soil type in this area coupled with its high water solubility may enhance leaching, particularly with frequent surface runoff events. Microbial degradation in cool temperate areas will be slower than areas of warmer climate. Seasonal fluctuations in temperature will coincide with fluctuations in soil microbial activity and will reduce glyphosate degradation. These factors suggest that the persistence of glyphosate in the environment may be prolonged in the

Skeena area.

The contamination of surface water by glyphosate is primarily through drift during application or as surface and subsurface runoff following application. The transport of glyphosate into surface waters can be highly variable and mainly depends on the level of soil particle adsorption. The leaching of glyphosate through soil is generally low, however, under specific circumstances significant leaching can occur and increasingly, there is evidence for off-site movement of glyphosate into aquatic ecosystems. Glyphosate leaching is expected in soils with low sorption capacity and slow degradation rates, and in areas of high precipitation. This is particularly true 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. In areas such as the lower Skeena with a shallow groundwater table and potentially low soil sorbing capacity (under rail lines) glyphosate may lead to groundwater pollution. In areas where railway ballast is underlain by less pervious soils, the potential exists for precipitation to transport the chemicals downward and along the interface of the ballast and soil into adjacent surface waters.

Dissolved and suspended glyphosate can be transported from terrestrial areas to surface waters by overland runoff as well and transport rates are determined by factors such as rainfall intensity, soil composition, slope characteristics and vegetation.

The toxicity of glyphosate-based herbicides can be attributed to both the active ingredient and to the surfactant portion of the formulation. 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. The 96-h LC50 of formulated glyphosate (Roundup®) for various fish species ranges from 4.3 to >100 mg/L. The surfactant 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 which has relevance to the lower Skeena watershed.

There is currently no consensus on a single mechanism of glyphosate-based herbicide toxicity and it is likely that multiple mechanisms exist leading to a variety of sublethal effects that will occur at much lower concentrations than those for acute mortality listed above. Effects can include general stress, oxidative stress, acetylcholinesterase inhibition, genotoxicity, histopathological alterations, energy metabolism changes, and behavioural effects.

Migratory salmon live a transformational lifestyle and have complex life histories that may preclude them to being more at risk to chemical toxicity. Anadromous salmon may also be more sensitive biologically to toxic injury than other fish species and exhibit a high level of physiological adherence to specific water quality parameters. Pacific salmon at all life stages are inherently prone to stress which may preclude them to being less tolerant of chemical exposure.

- factors unique to the lower Skeena river system may increase the transport of glyphosate-based herbicides into salmon habitat, result in more glyphosate being bioavailable, and be more toxic to salmonids than in other areas
- this information supports previous conclusions that glyphosate use as proposed may cause adverse effects to Pacific salmonid populations and alternative vegetation control options should be considered

## The toxic action paradigm: understanding the potential for adverse effects

The chemical concentration at the target site within an organism is the final determinant of the magnitude of a toxic response and is dependent on the chemical's fate in both the environment and organism. The risk of adverse effects can be modified by environmental factors through alterations in the chemical's partitioning behaviour in several environmental compartments (e.g. water, air, soil), and its movement into and within an organism. In order to understand how these modifiers might alter risk, information on how they affect a chemical's environmental fate, bioavailability, toxicokinetics, and toxicodynamics is necessary. Together, these 4 'phases' can be described as the toxic action paradigm in Figure 1.

The environmental fate of a chemical is determined by the chemical's physico-chemical properties and the properties of the receiving environment; these determine the partitioning behavior, exposure, and bioavailability. The absorption of a chemical cannot occur unless the organism and chemical come into contact; it is mainly determined by the bioavailability (a measure of the fraction of the chemical in environmental media that is accessible to an organism for absorption). Toxicity is dependent on the actual chemical concentration at a target organ or more specifically, at a target site or receptor (biological entity affected). The concentration at the site of action is dependent on a chemical's disposition (i.e., its absorption, distribution, biotransformation, and excretion) within an organism. Collectively, these processes are known as toxicokinetics. The extent of exposure and the magnitude of effects (toxicodynamics) in an organism form a correlative relationship that is at the foundation of aquatic toxicology and is called the concentration-response (CR) relationship. The CR relationship assumes that exposure to the chemical is causing the responses seen and also assumes that the exposure concentration is related to the concentration at the target site, and as the toxicant exposure is increased, effects will also increase.

The physicochemical properties of a chemical (e.g. water solubility, vapour pressure), the properties of the environment (e.g. water body, soil characteristics, microbial populations), and the biology of the organism (e.g. physiology, life history) can all be modified. In order to assess the risk for adverse effects to occur, an understanding of important site-specific (or jurisdictional) features on processes important to determining environmental fate (e.g. persistence), bioavailability (e.g. binding to soil particles), toxicokinetics (e.g. bioaccumulation), and toxicodynamics (effects) is necessary. In the sections below, various aspects regarding glyphosate fate and effects in the Skeena River watershed in a previous report (Review on glyphosate fate and toxicity to fish with special relevance to salmon and steelhead populations in the Skeena River watershed. BioWest Environmental Research Consultants, 2017) that may be unique this area are highlighted and expanded upon, using the toxic action paradigm (Figure 1) as a guiding framework to assessing the probability for modifying potential adverse effects.

In this report, jurisdiction- or site-specific attributes that may lead to an increased potential for adverse effects on salmonid populations following glyphosate application to railway ballast sections, and rights of way that are in close proximity to the CN line in the Lower Skeena River south of Prince Rupert, and upstream to Terrace, BC were examined. Factors which have been identified as potential modifiers of exposure and effects to be discussed (if information exists) are: high soil acidity, high amounts of rainfall, temperate climate, proximity of rail line to Skeena and crossing of myriad of small watercourses, streams and rivers, lack of soil (i.e. long stretches of vegetation over rocky ground), high water table, seasonally saturated soils, and coarse textured mineral soils/substrates low in organic matter.

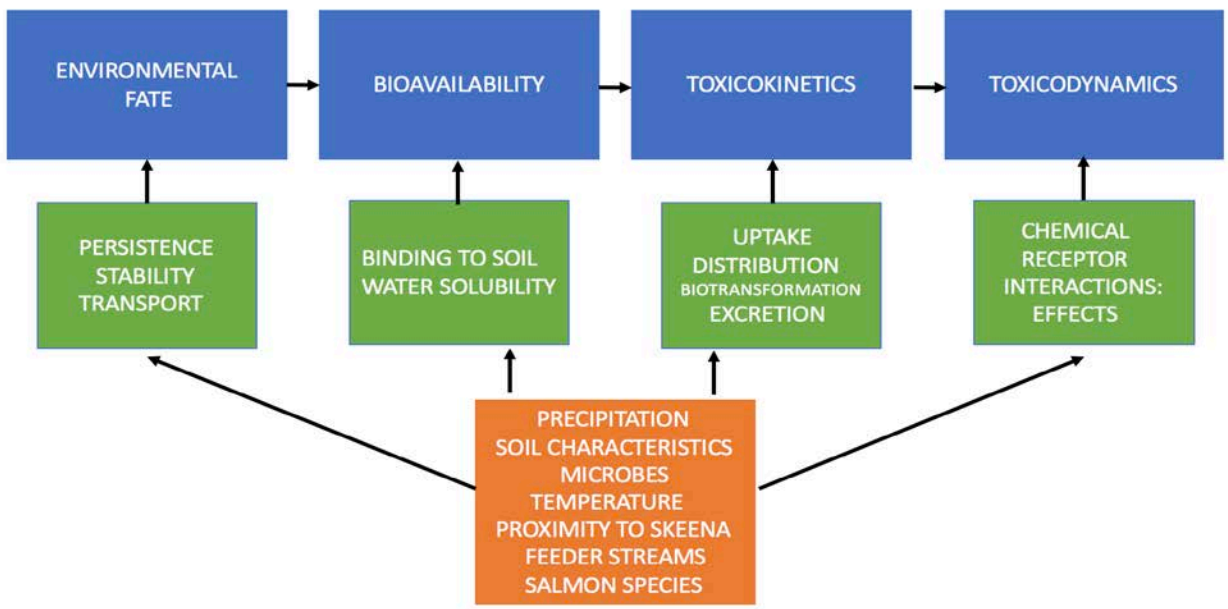


Figure 1. The toxic action paradigm, illustrating the relationships between environmental fate, bioavailability, toxicokinetics (biological movement), and toxicodynamics (effects) and the various parameters that can alter the ultimate responses of fish to environmental contaminants. The orange box illustrates potential environmental parameters (both biotic and abiotic) that can alter factors underlying each phase of the paradigm. For example, in areas of high precipitation water flows over contaminated areas are high, increasing transport into waterbodies (green box) thus altering environmental fate (blue box). In areas of low precipitation, transport of this type may be limited, reducing waterbody concentrations and ultimate bioavailability, uptake, and

effects in fish.

- the toxic action paradigm is a means to logically illustrate the important parameters that lead to adverse effects in organisms
- the paradigm highlights environmental fate, bioavailability, toxicokinetics and toxicodynamics as the most important factors in assessing risk
- a number of abiotic and biotic parameters can alter any of these four ‘phases’ in the paradigm, altering (increasing or decreasing) an organisms risk for adverse effects

## Skeena river watershed and salmon

The Skeena Watershed is located in the northwestern portion of BC with its mouth at 54°N, just south of the Alaska panhandle and is the second largest watershed in British Columbia (54,432 $km^2$). The Skeena River at its eastern extremity drains part of the Nechako Plateau and extends through to the Coast Ranges. Due to this large area, different sections of the watershed exhibit different climatic, hydrological, geological, and biological conditions.

The Skeena Watershed is composed of a series of northwest trending mountain ranges: the coastal Kitimat Ranges (composed mostly of granite and granitoid rocks), the Hazelton Mountains in the central part of the watershed (composed of low-grade metamorphic rocks) and the Skeena Mountains and Babine Range (composed primarily of Mesozoic sedimentary and volcanic rocks). Coastal drainages produce mostly sand as rock breakdown products, while interior drainages produce abundant clay and silt as well as sand due to differences in bedrock composition.

The Lower Skeena River extends from the estuary at 54°N, south of Prince Rupert, and upstream 116 km to Terrace, BC. This section of river is bounded to the north and the south by the Kitimat Range, to the east by the Hazelton Range, and to the west by Chatham Sound. (Gottensfeld and Rabnett 2007).

The Skeena Watershed supports five salmon species that are comprised of sockeye (*Oncorhynchus nerka*), pink (*O. gorbuscha*), chum (*O. keta*), Chinook (*O. tshawytscha*), and coho (*O. kitsch*). Trout and char presence includes steelhead/rainbow trout and cutthroat trout, Dolly Varden, bull trout, and lake trout. In general salmon populations are healthy, with relatively few threatened populations and no known recently extinct populations (Morrell 2000; Gottensfeld and Rabnett 2007).

The lower Skeena River and its tributary streams have high fish values; annual escapements of over two million fish from the Skeena River make it the second most important salmon producer in BC. The. The Skeena River main stem and adjacent channels from Kasiks River upstream to Shames (Skeena River West) is extremely important to pink and chum salmon, which spawn in large numbers. One hundred and twenty-two fish species have been identified in the Skeena River system and estuary, each has its own niche and function, contributing to the fish community (Hoos 1976). This community contributes to the ecology, nutrient regime, and structural diversity of the lower Skeena drainage basin. The fish community also provides strong cultural, economic, and symbolic linkages, as well as supporting aboriginal, recreational, and commercial fisheries. All species of salmon, as well as steelhead and cutthroat trout, and Dolly Varden char, are present in this section of river for some period of their life histories. Some species, such as sockeye, mostly migrate through the area. Tributaries support many life stages for anadromous and freshwater species. Juvenile fish migrating downstream to saltwater feed and rear along the extensive network of log jams, side channels, wetlands, and estuary. The basin is productive due to the outstanding spawning and rearing habitat that yields anadromous and resident fish in abundance; however, the fish populations and quality habitat are sensitive to ecosystem disturbance (Gottesfeld and Rabnett 2007).

- the Skeena Watershed is the second largest watershed in BC and due to its large area, different sections of the watershed exhibit different climatic, hydrological, geological, and biological conditions
- the watershed supports five salmon species; sockeye (*Oncorhynchus nerka*), pink (*O. gorbuscha*), chum (*O. keta*), Chinook (*O. tshawytscha*), and coho (*O. kitsch*) as well as steelhead/rainbow trout, cutthroat trout, Dolly Varden, bull trout, and lake trout
- the lower Skeena River and its tributary streams have high fish values including escapements of over two million salmon and

productive resident fish populations
- the basin provides a quality habitat which is sensitive to ecosystem disturbance

**Glyphosate**

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. Commercially available glyphosate-based herbicide formulations are complex mixtures in which glyphosate acts as the active ingredient. 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). Each formula contains a particular concentration of glyphosate with a particular adjuvant profile. With large toxicity differences between individual formulations, it is critical that the complete name and description of the product being investigated is known. The most commonly applied form of glyphosate is in the form of its isopropylamine salt (IPA salt) and 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.

The chemical and physical characteristics for glyphosate that may be altered by several environmental parameters (e.g. temperature) unique to parts of the Skeena watershed are listed in Table 1.

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

| | |
|---|---|
| Water solubility | 11,600 ppm (at 25°C) |
| Vapor pressure | $7.5 \times 10^{-8}$ mm Hg |
| 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. POEA), and water

**Glyphosate use for vegetation control along railways**

According to the CN Pest Management Plan for Integrated Vegetation Control (CN Pest Management Plan for Integrative Vegetation Control, PMP Confirmation #:CN-0128-12/17), the main purpose for controlling problem vegetation along the railway (in the context of this report the CN rail line along the lower Skeena River from Terrace to Prince Rupert) is to maintain the safe functioning of train operations and to protect the public, employees and the environment from potential hazards that are associated with railway operations. Problem vegetation in this regard includes: vegetation that is interfering with railway operations and/or causing safety issues, ballast section vegetation, noxious weeds and invasive plants, and vegetation that interferes with sightline requirements.

Vegetation management can be required on track ballast and right-of-way areas; the railway right-of-way can typically be divided into three vegetation management zones: the ballast section, inner right-of-way and outer right-of-way. Ballast is comprised of the ballast and sub-ballast areas with a typical width of approximately 6.1 m. On a railway, the ballast is the layer of crushed rock that supports the track and ties. The inner right-of-way is the area within the right-of-way where brush control is typically undertaken. This area extends from the ballast shoulder to the maximum reach of a typical mechanical brush cutter, approximately 7 m from centerline of the track. The outer right-of-way is the portion of the right-of-way typically outside of where brush-cutting activity normally occurs, extending to the property line. The outer right-of-way is approximately 6 m in width for the typical right-of-way (CN Pest Management Plan for Integrative Vegetation Control PMP Confirmation #: CN-0128-12/17).

Ballast section treatment includes all tracks within the pest management area. Factors such as track type, site details (e.g. the type of vegetation present and the presence of environmentally sensitive areas adjacent to proposed treatment sites), and past management results determine the priority, frequency, and type of vegetation management treatment selected. The track type is a major factor in determining the prioritization of ballast vegetation management each year. For example, mainline tracks have the highest priority for vegetation management (CN Pest Management Plan for Integrative Vegetation Control, PMP Confirmation #: CN-0128-12/17).

Areas within rights-of-way that are vegetated with a suitable and stable cover of low growing plant species that do not pose a fire or safety risk to the public, CN or its personnel, are not be managed. However, in instances where noxious weeds or invasive plants are present, or where tall growing vegetation is impeding sight line requirements or compromising access to buildings, signals, communication and electrical infrastructure, treatment with one of several herbicide active ingredients including glyphosate are made (CN Pest Management Plan for Integrative Vegetation Control PMP Confirmation #: CN-18/23-BCW). Between July and October 2017, there was an apparent spray application of a herbicide containing the chemical glyphosate along the 100-km stretch of CN track that follows the Skeena river from Terrace to Prince Rupert.

- the main purpose of the CN Pest Management Plan for integrated vegetation control is to control problem vegetation along the railway in order to maintain the safe functioning of train operations and to protect humans and the environment from potential hazards that are associated with railway operations

- vegetation management can be required on track ballast and right-of-way areas
- treatment with one of several herbicide active ingredients including glyphosate are available for control
- between July and October 2017, there was an apparent spray application of a herbicide containing the chemical glyphosate along the 100-km stretch of CN track that follows the Skeena river from Terrace to Prince Rupert

**Climate and water quality of the Skeena watershed (identification of important modifying parameters)**

The climate of The Skeena watershed varies greatly between its different areas. The coast exhibits abundant precipitation, cool summers, and mild winters with average temperatures near 0°. Precipitation generally reaches maximums in October and November with intense cyclonic storms from the North Pacific frequently moving across the coast (Environment Canada 1993). Rainfall amounts of 50 to 100 mm in a day can occur. The interior exhibits a boreal climate with relatively low precipitation, warm summers, and prolonged cold winters with average temperatures < -10°C for up to 2 to 3 months. Precipitation is relatively uniform throughout the year; summer convective storms can be common but rarely deliver more than 20 mm of rainfall per day (Gottensfeld and Rabnett 2007).

The coastal drainages of the Skeena Watershed receive at least 2500 mm of precipitation per year, with higher amounts in the mountains. The Kitimat-Kitsumkalum trough (Terrace and vicinity) receives 50% or less of this amount. East of the coastal mountains (Smithers area) receives about 600 mm, while further east on the Nechako plateau, annual precipitation is < 500 mm. With these variations in precipitation and winter climate, the interior hydrological pattern differs greatly from that of the coast. Coastal drainages have one or more brief large fall or winter floods most years and are caused by intense rainfall, or rain-on-snow events. Interior drainages (e.g. Bulkley river or the Babine river), usually have a single dominant flood occurring annually at the peak of snow melt in May or June and lasting for several weeks. In coastal-interior transitional areas such as the Kispiox, Kitwanga, and Zymoetz Rivers, a mix of fall rain floods and spring snowmelt floods occur.

Throughout the watershed, prolonged freezing conditions yield low flows in the late winter. In the interior, higher summer temperatures and abundant evapotranspiration by forests contribute to low flows during the late summer and early fall. (Gottensfeld and Rabnett 2007).

The Lower Skeena River has a modified maritime climate that is controlled by the large-scale mid-latitude weather frontal pattern and the topography (Coast Mountain). There is considerable variation of temperature and precipitation from the Skeena River mouth eastward to Terrace. On the coast there is abundant precipitation with cool summers and mild winters with average temperatures near 0° are found on the coast with precipitation reaching a maximum in October and November (Environment Canada 1993; Gottensfeld and Rabnett 2007)

## Environmental fate of glyphosate

The fate and distribution of chemicals in the environment are determined by a complex interplay between the physico-chemical properties of the compound and a multifarious environment. Here, the distribution of glyphosate between several environmental matrices, as well as environmental persistence in soil and water are discussed. Factors specific to the lower Skeena watershed that may affect partitioning or persistence are elaborated on.

*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.

The temperate climate of this area will have little impact on the amount of glyphosate that may partition into the atmosphere. Quantities of mist, drip, drift or splash of glyphosate that may be produced during application (application areas very close to the Skeena) may not have the opportunity to be transported away from the area and may move over and into the waterbody during common precipitation events in this area.

Factors that increase drift are aerial application techniques, high wind speeds (over 10 kph), spray nozzles that produce a high proportion of fine droplets, and calm conditions (without enough turbulence to drive the glyphosate droplets onto plant foliage). Ground application techniques have shown that between 14 and 78% of glyphosate applied can move off-site (Riley et al. 1991).

- glyphosate's tendency to volatilize into the atmosphere is low and it tends to partition to water more so than air; colder temperatures will not impact volatilization to any significant degree
- the atmosphere can be a source of glyphosate to the aquatic environment through particulate (e.g. mists, droplets) deposition of the herbicide to a water body and can be increased by wind and precipitation events

Water: Glyphosate is highly soluble in water (11,600 mg/L at 25°C [Kollman and 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). Temperature is known to affect the solubility of glyphosate in water; with reductions in solubility with lower temperatures (e.g. 10,100 mg/L at 20°C) (https://pubchem.ncbi.nlm.nih.gov/compound/glyphosate), however, the high water-solubility at temperatures found in the lower Skeena river indicate that water temperature will not reduce the availability of dissolved glyphosate to any significant degree.

Temperature for the Babine river before confluence with the Skeena (Babine watershed, the primary source of Sockeye production [80-93%] in the Skeena system in northern BC) have been collected and show typical river temperatures. Based on the limited observed data, mean daily temperatures were 14.5± 2.6°C, surpassing 20°C on occasion. Water temperatures were above average in 2004, 2009, 2013 and 2014, though not significantly. Maximum observed water temperatures peaked over 20°C (but did not exceed 22°C) for extended periods in 2004 (July 15-25 and August 13-20), and brief periods in 2009 (July 31–August 2) and 2014 (July 14-15) (Stiff et al. 2015).

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. Due to the chemical stability of glyphosate in water, colder temperatures are unlikely to significantly affect chemical hydrolysis. Generally speaking, pesticides that are hydrolyzed rapidly in water degrade faster at higher temperatures.

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. 1990a,b; 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. Microbial degradation in water is believed to be temperature dependent, and that lower water temperatures may support a higher carrying capacity of microbes and potential degradation of glyphosate by this route, however, recent studies suggest that this may not be the case, and that temperature, nutrient flows, and bacterial energetics and phylogenies play will all determine microbial activities and that temperature trends cannot easily be predicted (Huete-Stauffer et al. 2015). However, nutrient inputs will predominate on carrying capacity of microbial populations, and certainly low nutrient seasons will reduce the microbial breakdown of glyphosate in water.

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. Seasonal fluctuations of sediment load in the lower Skeena may alter the half-life of glyphosate in this water body; during time of high turbidity (and depending on sediment type), some protection may be offered with respect to reduced $t_{1/2}$ and bioavailability.

Water quality is defined as the natural physical, chemical, and biological characteristics of water. Turbidity is generally low in late summer and through winter in streams of the Skeena Basin. Fine sediment content rises episodically during spring and fall floods in the smaller streams. The pattern in the Skeena River is simpler as it integrates the contributions of many upriver source areas. Turbidity is moderate to high during the spring snowmelt floods and following fall rainstorms. In addition glacial melt and redistribution of silts from alpine areas keeps the turbidity levels moderate for the summer season. The Zymoetz River is a major fine sediment source. The reddish clays derived from weathering of the Telkwa and Nilkitkwa Formations give a brown color to the Skeena following floods. The Telkwa River upstream of Smithers in the Bulkley Valley drains bedrock of similar nature and has a similar effect on fine sediment loading. Following floods the difference in suspended sediment load is conspicuous at the confluences of these rivers with the Skeena. In summary, water quality inputs on the main stem of the Skeena River are in large part controlled by inflowing tributaries fed by turbid glacial meltwater, shallow flow through forest soils, wetlands, and groundwater aquifers replenished by snowmelt. Based on these hydrologic flow path regimes, each basin carries its own chemical signature, and the geology of the basin determines which constituents are available for mobilization.

- glyphosate is highly soluble in water, therefore glyphosate readily dissolves and disperses in aquatic environments; due to its high water solubility at all temperatures

- typical Skeena river temperatures are not believed to reduce the dissolved fraction of glyphosate to any significant degree
- glyphosate is stable in water at various pH and temperature values, and is not readily photo-degraded; colder river water temperatures are unlikely to affect this
- glyphosate's eventual removal/loss from water is mainly through sediment adsorption and microbial degradation; it is unclear if colder temperatures would reduce the microbial degradation of glyphosate
- sediment adsorption of glyphosate in river water will be seasonally- and geographically-dependent

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.

From its log Kow-value of -3.4 (Tomlin, 1997), soil organic matter (SOM) would not be expected to sorb glyphosate, and indeed it is often stated, that SOM is not important for the sorption of glyphosate. Madhun et al. (1986) showed, however, that glyphosate could be sorbed by a purified soil humic acid (HA), and one group (Piccolo and co-workers) have published several studies showing a large potential of both pure humic acids (HAs) and HA–iron complexes for sorbing glyphosate (Miano et al.,1992; Piccolo and Celano, 1994; Piccolo et al., 1995, 1996). Sorption studies with six whole soils and with SOM removed showed that several soil parameters including SOM are responsible for the strong sorption of glyphosate in soils. After an 80-d fate experiment, 40% of the added glyphosate was associated with the HA and fulvic acid (FA) fractions in sandy soils, while this was the case for only 10% of the added glyphosate in clay soils. Glyphosate sorbed to humic substances in natural soils seemed to be easier desorbed than glyphosate sorbed to amorphous Fe/Al-oxides (Albers et al. 2009). In a study by Piccolo et al. (1994), glyphosate bound readily to the four soils studied, however, desorption, also occurred readily. In one soil, 80% of added glyphosate desorbed in a 2 h period. The study concluded that glyphosate binding to soils is not permanent can be extensively mobile in the soil environment. 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.

In addition to particle 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). 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). 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). Colder temperatures found in the lower Skeena will likely reduce the sorption rate.

The Skeena soils, covering 7,300 acres, occupy well-drained terraces along the Skeena river. The material in these terraces consists of medium- to coarse-textured deposits upon which a thin or feebly expressed Podzol (an infertile acidic soil having an ash-like subsurface layer [from which minerals have been leached] and a lower dark stratum, occurring typically under temperate coniferous woodland) has developed. The surface texture varies from fine sandy loam to sandy loam, with the latter being the dominant texture. Skeena soils may be distinguished by a 2- to 4-inch mat of forest litter, a thin ashy grey sandy loam, and a brown to yellowish-brown upper subsoil which grades to the pale yellowish-brown and grey brown stratified sandy parent material. Shallower soils than the above occur and are characterized by a gravelly substratum a few inches below the surface. Soil profiles can be underlain by stratified clays which serve as the parent material for these soils (Farstad and Laird 1972).

The soil in the Skeena area is categorized as a Ferro-Humic Podzol (Valentine et al. 1978) of loamy texture which can contain high organic matter and sesquioxide (an oxide in which oxygen is present in the ratio of three atoms to two of another element) concentrations. Sesquioxides can include $Al^{3+}$ and $Fe^{3+}$ oxides that can bind effectively to glyphosate (Farstad and Laird 1972). However, the sandy glacial sediments of the Skeena are derived from igneous rocks that typically have an acidic pH due to the mineralogical composition of the sediments. The acidity of the upper soil is further increased by the organic decomposition products from coniferous leaf litter. This creates an intense chemical weathering zone in the upper part of the soil where primary minerals containing aluminum, iron, and other metal ions are weathered and the ions released into the soil solution. These metal ions form complexes with the organic decomposition products (also called chelates), and the complexes move with vertically draining water into the B horizon (a lower soil level: a B horizon is a mineral horizon meaning it contains ≤ 17% organic C by weight and is characterized by an enrichment in organic matter, sesquioxides, or clay) where they are deposited (the process of podsolization). The deposition of the weathering products from the Ae horizon (a top soil layer: an A horizon is characterized by the eluviation [the transport of soil material from upper layers of soil to lower levels by downward precipitation of water across soil horizons, and accumulation of this material in lower levels] of clay, Fe, Al, or organic matter alone or in combination. Podsolization commonly leads to different degrees of cementation of layers within the soil profile. These cemented layers can form barriers to the vertical penetration of tree roots and of water, leading to water saturation of the layer above the cemented layer. It is unclear, due to these cementing layers, whether glyphosate in water from upper soil layers lacking sesquioxides will penetrate and be bound before being translocated with water at these boundaries.

- glyphosate possesses unique sorption characteristics in soil by binding tightly to most soil types, although in some soil types glyphosate can be highly mobile
- soil organic matter (SOM) plays a controversial role in the soil sorption of glyphosate. Glyphosate sorption can be positively and negatively, correlated with SOM content
- competition between glyphosate and phosphate for surface sites indicates that in phosphate-rich soils reduced glyphosate sorption will occur
- glyphosate sorption also depends on pH; sorption decreases with increasing pH; the highly acidic nature of typical Skeena soils will tend to enhance binding
- the sorption rate is expected to decrease at lower temperatures which are typically found in the area and may increase transport to waterbodies and bioavailability

- the soil in the Skeena area is categorized as a ferro-humic podzol
- glyphosate sorption to soil particles typically found in the lower Skeena watershed may be reduced in top soil layers due to the eluviation of sesquioxides from upper layers and the formation of a cementing layer that may prevent water containing glyphosate penetration to lower soil levels where binding would be higher

*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 the two main loss 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 non-leachable under most circumstances, 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.

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), 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); in experiments, 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). 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).

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. Rates of decomposition depend on soil and micro-floral 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).

The climatic variables in the Skeena area that are deemed important in glyphosate breakdown are moisture and temperature. Degradation rates generally increase as temperature and soil moisture increase, because of increases in both chemical and microbial degradation. 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).

Biodegradation by plants (and perhaps other organisms that degrade glyphosate) tends to increase with higher temperatures. For example, the effect of temperature on the activity and metabolism of glyphosate, as its IPA salt, in single-node rhizome fragments of *Elymus repens* was investigated. Post-treatment temperatures of 26/16° (day/night) reduced the activity of the herbicide compared with that at 10/6°, respectively. Under both temperature regimes and using [$^{14}$C]-glyphosate IPA. At the higher temperature, the rate of glyphosate metabolism was greater, and more $^{14}$C was lost as [$^{14}$C]$CO_2$ (Coupland 1984).

- the two main loss pathways of glyphosate from soil are leaching and microbial degradation
- 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 non-leachable, and has a low tendency to runoff
- glyphosate has a high water solubility which tends to enhance leaching particularly through frequent surface runoff events as occur in the Skeena watershed
- microbial degradation in cool temperate areas will be slower than areas of warmer climate. Seasonal fluctuations in temperature and concomitant with fluctuations in soil microbial activity will also reduce glyphosate degradation. These factors suggest that the persistence of glyphosate in the environment may be prolonged in the Skeena area

## Glyphosate transport to the aquatic environment

### *Glyphosate transport*

The contamination of surface and groundwater by glyphosate 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). 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 nearby waterbodies by glyphosate; primarily through drift during application, as surface runoff, and as subsurface runoff following application (Borggaard and Gimsing 2008). Subsurface leachates end up in drainage and groundwater (and eventually open waters), while the direct recipients of surface-runoff-transported materials are open waters such as streams and lakes. 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).

Transport of glyphosate into waterbodies is highly variable and mainly depends on the level of soil particle adsorption (which can be highly variable based on soil chemistry and physical characteristics as mentioned above). 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). 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. As mentioned previously, glyphosate has the potential to bind tightly to soil particles depending on pH, soil texture and phosphate levels (Sprankle et al. 1975). Although most data indicate that the leaching of glyphosate is generally low, it seems that under specific circumstances significant leaching can occur (Veiga et al., 2001; Kjær et al., 2005) and that increasingly, there has been evidence for off-site movement of glyphosate into aquatic ecosystems. As well, in some studies based on laboratory experiments show that this compound can be extensively mobile in certain soils (Piccolo et al., 1994; Beltran et al., 1998; Maqueda et al., 1998). Piccolo et al. (1995, 1996) suggested that the mobility of glyphosate in soil might be controlled by the formation of complexes between glyphosate and water-soluble HAs or fulvic acids (FAs). Maqueda et al. (1998) found that glyphosate is highly adsorbed by natural FAs and since the binding mechanism is most likely hydrogen bonding or some other relatively weak and reversible bonding, glyphosate could be later released from the HAs or FAs.

In another study example, Veiga et al (2001) showed that residues of glyphosate and its main metabolite AMPA in a forest soil in north-west Spain, previously treated with 5 and 8 l ha of glyphosate were highly mobile, results that were not in agreement with the literature (Newton et al., 1994; Roy et al., 1989). Both glyphosate and AMPA quickly reached lower soil sub-horizons, and furthermore, the concentrations were similar in both sub-horizons. The statistical analysis revealed no significant differences between glyphosate concentrations in either sub-horizons. This fact was interpreted as a result of the high water solubility of glyphosate, which allows its migration in solution, together with the high porosity and moderate permeability of the treated soil and the rainfall during the studied period. Glyphosate could also transfer bound to suspended material, or be translocated through plants and exuded through roots (Rodrigues et al., 1982) or incorporated into soil after plant decay (Brønstad and Friestad, 1985). Transportation as suspended material seems plausible during some storm events that occurred in the monitoring period (Veiga 2001). In another study, glyphosate was detected in runoff waters 4 months post application following spraying and rainfall (Edwards et al. 1980). Residues of glyphosate, and its main metabolite AMPA, have been found in both food and water sources, indicating the potential risks for frequent, long-term exposure (Kwiatkowska et al. 2013; Landry et al. 2005; Mañas et al. 2009; Mesnage et al. 2015).

- 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
- leaching of glyphosate is generally low, however, under specific circumstances significant leaching can occur and increasingly, there is evidence for off-site movement of glyphosate into aquatic ecosystems
- laboratory experiments show that glyphosate can be extensively mobile in certain soils
- 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 transport 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 (e.g. top surface levels of Skeena soils) 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, all conditions that are likely to occur in the lower Skeena region.

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 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

*Glyphosate transport 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.

- 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 or water saturated soils (as can be found in the Skeena watershed) 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. However, in lowland areas with a shallow groundwater table and low soil capacity to sorb and degrade glyphosate which may be situation in the Skeena watershed, the use of glyphosate may 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 such as the Skeena 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. The lower Skeena watershed is an area of extremely high precipitation compared to many other jurisdictions such as those from the above studies. It is expected that the high water solubility of glyphosate, couple with high rainfall events will increase the likelihood of significant glyphosate surface runoff. The lack of soil in ballast and the close proximity of the rail line to the Skeena increase the potential of glyphosate entering the Skeena.

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 neighboring sprayed areas. The close proximity of the area of glyphosate application on the rail line in this area, ensure the contamination of the adjacent river with glyphosate from spray drift.

- 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

*Area of glyphosate application for vegetation control on railways*

Track ballast forms the track bed upon which railroad ties are laid. It is packed between, below, and around the ties. It is used to bear the load from the railroad ties, to facilitate the drainage of water, and also to keep down vegetation that might interfere with the track structure. This also serves to hold the track in place as the trains move over the tracks. It is typically made of crushed stone or riprap. Riprap is the most commonly used bank protection material in BC. Rocks are used in BC and the density of rock used for riprap typically varies from about 2,400 kg/m$^3$ to 2,800 kg/m$^3$, with a density of about 2,600 kg/m$^3$ common for the granitic or granodioritic rocks that are often quarried in BC.

For vegetation control on rail lines, glyphosate is used as a non-selective, non-residual herbicide used to control a very large number of herbaceous broadleaf and grass species and species of woody vegetation. It is most useful in areas where low soil residual is required because of the close proximity of wells, water bodies and other environmentally sensitive features. The use of these herbicides necessitates more frequent re-treatment, as they typically degrade rapidly and are effective only on plants sprayed directly.

Herbicide mobility is an important consideration when used on the free draining ballast section of railways. In areas where the ballast section is underlain by less pervious soils, the potential exists for precipitation to transport the chemicals downward and along the interface of the ballast and soil. It is believed, although unknown, that this movement seldom occurs as the lower layer of the ballast and the sub-ballast layer, containing contaminants of fine particles and organic matter, provide a zone with intermediate properties that retain the herbicide. The retentive properties of any such zone depend upon a variety of soil characteristics and binding capacities as listed above. For example, if this layer consists of mostly Ae horizon soils typical of this area, then binding will be low as the sesquioxide concentrations will also be low. This reliance on a lower soil level 'barrier or sink' for applied glyphosate that permeates the ballast rocks may be misinformed.

- in areas where railway ballast is underlain by less pervious soils, the potential exists for precipitation to transport the chemicals downward and along the interface of the ballast and soil into adjacent surface waters

**Glyphosate toxicity**

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 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.

- the toxicity of glyphosate-based herbicides can be attributed to both the active ingredient and 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 2010).

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. 1976); *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 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.

Water quality is important in glyphosate toxicity, particularly water hardness. Water quality data for the Skeena River was collected from 1966−1990 at Usk. Remington (1996) reported waters are soft, with a mean pH of 7.4, which is neutral to slightly alkaline (Remington 1996). Glyphosate in soft water was 20 times more toxic to rainbow trout than was glyphosate in hard water. For Roundup, the reverse is true: it is more toxic in hard water than in soft Wan et al. (1989, 1991). Finally, glyphosate toxicity increases with increased water temperature. In both rainbow trout and bluegills, toxicity about doubled between 7 and 17°C (Folmar 1979).

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.

- 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 and at higher temperatures which has relevance to the lower Skeena water shed

*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, feeding and growth (Percilia et al. 2017; for full review see BioWest Environmental Research Consultants, 2017). 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 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

**Salmon sensitivity**

Pacific salmon habitat consists of freshwater, estuarine, and marine components, necessitating dramatic physiological transformations and a migration through a variety of natural and anthropogenic stressors.

Migratory salmon live a transformational lifestyle and have complex life histories that that may preclude them to being more sensitive to chemical toxicity. As they mature from alevin to adult, they physically change, and these changes may make them more or less sensitive to health challenges, such as contaminant exposures. Changes can include gill re-modelling, lipid storage or usage rates, and hormone profiles. Furthermore, salmon species can differ markedly in their sensitivities to contaminants (Tierney et al. 2007).

While anadromous salmon may be considered as “ecologically sensitive” to the effects of contaminants because of their complex life history and their ever-changing habitat use, they may also be more sensitive to toxic injury than other fish species on a physiological level. Several meta-analyses have found that compared with other fish species, salmonids are often the most vulnerable to toxic injury associated with several contaminants, including metals, some pesticides, and dioxin-like compounds (Teather and Parrott 2006; Hrovat et al. 2009; Vittozzi and De Angelis 1991). Toxicokinetic (uptake, distribution, biotransformation, and excretion) and toxicodynamic factors (receptor–xenobiotic interactions) likely explain differential species sensitivity among fish, as highlighted by the following examples.

The metabolism of some xenobiotics appears to be different in salmonids compared with other teleosts. For example, in most teleosts exposed to the polycyclic aromatic hydrocarbon benzo[a]pyrene (BaP), conjugated metabolites predominate in excretory fluids such as bile (Kennedy et al. 1991). However, when coho salmon are exposed to BaP, the predominant metabolite group in bile consists of unconjugated metabolites (Seubert and Kennedy 1997), suggesting that coho are unable to conjugate these compounds effectively. Similar alterations in other salmonids are also seen with seawater acclimation and BaP exposure; there is a higher production of unconjugated metabolites in saltwater-adapted rainbow trout, as observed in juvenile coho salmon undergoing smoltification (Lance et al. 2001).

Differential sensitivity to about one-third of organophosphate (OP) pesticides exists among fish species, with salmonids being relatively sensitive (Macek and McAllister 1970; Vittozzi and De Angelis 1991). The mechanism of action for OP pesticides is through the inhibition of the acetylcholinesterase (AChE) enzyme, leading to overstimulation in the nervous system. In one study, four fish species were compared using *in vitro* measures of AChE sensitivity, by the pesticide diazinon (Keizer et al. 1995). The most sensitive species was rainbow trout which had a highly sensitive AChE.

The effects of tetrachlorodibenzo-*p*-dioxin and related dioxin- like compounds are mediated through interaction of the ligand with the aryl hydrocarbon receptor (AhR), leading to association with the AhR nuclear translocator (ARNT), translocation from the cytoplasm to the nucleus, and subsequent transcription of genes containing dioxin response elements. Trout and other salmonids have been shown to be the most sensitive to developmental toxicity and early life stage mortality (blue-sac disease) caused by dioxin exposure among 10 different fish species (Elonen et al. 1998). Differences in sensitivities among fish species are thought to be due to differences in AhR sensitivity for ligands, expression levels, tissue distributions, and components of the signalling pathway or AhR cross-talk with other signalling pathways (Powell and Hahn 2000; Hahn 2001; Zhou et al. 2010). In addition, studies have shown that there are multiple forms of the AhR expressed in rainbow trout and Atlantic salmon, which possess different binding affinities for dioxin and have differential tissue distributions, potentially contributing to the high sensitivity of salmonids to dioxins (Abnet et al. 1999; Hansson and Hahn 2008).

Anadromous salmonids also exhibit a physiological sensitivity and are highly sensitive to changes in water temperature and oxygen content and, as active animals, they have high metabolic requirements. For example, with temperatures exceeding 20°C, salmon may increasingly lose the battle with fungal infections, such as *Saprolegnia* sp. (Tierney and Farrell 2004).

Anadromous salmon are inherently sensitive to stress and handling. From the time of capture onward, stress hormone concentrations may remain elevated. Elevated cortisol can affect metabolic rate and have other consequences, such as negatively affecting the immune system (Barton et al. 1987). Anadromous salmon move through fresh- water and marine environments using very long migratory corridors. During their lifetime, they must overcome a number of natural stresses, as well as human-caused changes to their habitat. Natural stresses include such things as hydrological conditions during their freshwater period (temperature, flow, turbidity), ocean productivity (sufficient food at critical windows), predation at all points along their migration, and disease. Human-caused impacts on their habitat include forestry (physical alteration of waterways, siltation, increased UV-B radiation), agriculture (altered flow, damming, nutrients), gravel extraction from river beds, and river crossing construction and maintenance (rail and roadway bridges, pipelines, electrical lines, dams). It is unclear if stress accumulated during particular life history periods will be cumulative with respect to exposure and effects caused by contaminants. Evidence suggests that animals under stressful conditions are more susceptible to toxic effects.

- migratory salmon live a transformational lifestyle and have complex life histories that that may preclude them to being more sensitive to chemical toxicity
- anadromous salmon may also be more sensitive to toxic injury than other fish species
- anadromous salmonids also exhibit a high level of physiological sensitivity to water quality parameters such as temperature and oxygen levels
- anadromous salmon are inherently sensitive to stress

## Modifiers of glyphosate toxicity

According to the paradigm of glyphosate toxic action in Figure 1, abiotic and biotic parameters may alter both the toxicokinetics (absorption, distribution, biotransformation, excretion), and toxicodynamics (effects) of glyphosate leading to altered toxicity. The susceptibility of fish to xenobiotic action can be modulated by a variety of abiotic factors including water pH, dissolved $O_2$ content and temperature. The only water quality parameters in the Skeena river watershed compared to other jurisdictions that may play a role in altering the inherent sensitivity of salmonids to glyphosate are environmental temperature and water hardness.

In toxicology, as in other areas of biology such as ecology, physiology and biochemistry, the influence of temperature at all levels of biological organization is pervasive and often of dominant importance (Hochachka and Somero 1984). The toxicity of many xenobiotics to fish is altered by changes in water temperature, however, its influence on chemical toxicity is complex. This is because temperature alone may be a lethal factor with thermal limits that may be altered by a specific toxicant. Temperature may act as a modulator of toxicity through effects on chemical availability (e.g. solubility in water), toxicokinetics (uptake, distribution, metabolism and excretion), or toxicant-receptor interactions. No consistent pattern for the effects of temperature on toxicity emerges from the literature; a temperature change may increase, decrease or result in no change in toxicity. For example, a general increase in the susceptibility of trout and bluegills to many pesticides including dieldrin, chlordane and malathion was noted as temperature increased (Macek et al. 1969). Contrary to these results, several studies have also shown that decreasing temperature leads to an increase in the toxicity of organic compounds such as phenol (Brown et al. 1967) and DDT (Cairns et al. 1975) as well as several forms of metals (Lemly 1993; Brown 1968). These examples indicate that although temperature can significantly alter the toxicity of specific chemicals, it is difficult to predict the course of temperature's modulating effects.

One study exists, as reported above, that glyphosate toxicity increases with increased water temperature. In both rainbow trout and bluegills, toxicity about doubled between 7 and 17°C (Folmar 1979). This may have important relevance to the timing of glyphosate application for vegetation management.

Water hardness is among the well-known factors that modify the toxicity of trace metals and some organic chemicals in many species of fish (Pascoe et al. 1986). Skeena river waters are soft, (mean pH of 7.4), and as reported above, glyphosate in soft water was 20 times more toxic to rainbow trout than was glyphosate in hard water.

- Water temperature can affect the toxicity of glyphosate to salmonids and should be taken into consideration during glyphosate application proposals
- Water hardness as found in the Skeena river (soft) will lead to higher glyphosate toxicity in salmonids

## Conclusions

Past spraying of a glyphosate-based herbicide in the area of the CN railway which closely follows the lower Skeena River between Terrace and Prince Rupert BC, and concerns regarding the potential effects on the salmonid population in the area prompted the generation of an earlier report (BioWest Environmental Research Consultants 2017). A summary of important information that would aid in answering specific questions regarding the herbicide fate and effects were included. From that report, and due to a proposal to apply glyphosate to the same area, an elaboration on several factors unique to the lower Skeena river that may increase the exposure and effects in salmonids was undertaken. Specifically, the factors could potentially alter the environmental fate, bioavailability, and toxicity to salmonids in this area compared to other areas of application included the following: 1) high soil 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) coarse textured mineral soils/substrates low in organic matter and a general lack of soil in application areas, 6) high water table and seasonally saturated soils, and 7) close proximity to aquatic ecosystems.

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 non-leachable and therefore the 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. Estimates have shown that between 14 and 78% of applied glyphosate can move offsite through ground application techniques. 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 as is found under railway embankments. In the spray area, the soil is categorized as a ferro-humic podzol and glyphosate sorption may be reduced in top soil layers due to the eluviation of sesquioxides from upper layers and the formation of a cementing layer that may prevent water containing glyphosate from penetrating to lower soil levels where binding would occur. In addition, sorption rates decrease at lower temperatures and may increase transport in water. High water solubility, low binding capacity in the rail area, high water table, seasonally-saturated soils, and high precipitation rates all may result in significant amounts entering the river either subsurface or overland.

In addition to the above potential increases in surface water concentrations, the coastal areas of BC have climatic conditions including low temperatures that may increase the $t_{1/2}$ of glyphosate in soil and water. Microbial degradation in cool temperate areas may be slower than areas of warmer climate. Seasonal fluctuations in temperature and concomitant with fluctuations in soil microbial activity will also reduce glyphosate degradation. These factors suggest that the persistence of glyphosate in the environment may be prolonged in the Skeena area under such conditions.

Migratory salmon live a transformational lifestyle and have complex life histories that that may preclude them to being more sensitive to chemical toxicity. As they mature from alevin to adult, they physically change, and these changes may make them more or less sensitive to health challenges, such as contaminant exposures. Salmonids may also be more sensitive to toxic injury than other fish species on a physiological level. Several meta-analyses have found that compared with other fish species, salmonids are often the most vulnerable to toxic injury associated with several contaminants, including metals, some pesticides, and dioxin-like compounds. Anadromous salmonids also exhibit a physiological sensitivity and are highly sensitive to changes in water temperature and oxygen content. Anadromous salmon are inherently sensitive to stress.

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. The only water quality parameters in the Skeena river watershed compared to other jurisdictions that may play a role in altering the toxicity to salmonids are environmental temperature and water hardness. Glyphosate toxicity increases with increased water temperature and therefore, the timing of application in this area should be considered carefully. During warmer seasons and water temperatures, bioavailable glyphosate will be more toxic to fish. Skeena river waters are soft, and glyphosate in soft water is 20 times more toxic to salmonids *v*. hard water. 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.

In the lower Skeena river area, there are a number of climatic, hydrological, geographical, and biological conditions that may alter the risk of salmonid populations to the use of glyphosate on railways near the Skeena river. This information warrants an examination and reevaluation of its use in this regard. This report provides information showing that factors unique to this river system may increase the transport of glyphosate-based herbicides into salmon habitat, result in more glyphosate being bioavailable, and be more toxic to salmonids than in other areas. This information supports previous conclusions that glyphosate use as proposed may cause adverse effects to Pacific salmonid populations.

**Statement of limitations**

This review has been prepared and the work referred to in this review has been undertaken by Biowest Research Consultants for the T. Buck Suzuki Environmental Foundation (TBSEF). It is intended for the sole and exclusive use of the TBSEF and its authorized agents for the purpose(s) set out in this review. Any use of, reliance on or decision made based on this report by any person other than TBSEF for any purpose, or by TBSEF for a purpose other than the purpose(s) set out in this review, is the sole responsibility of such other person or TBSEF. TBSEF and BIOWEST RESEARCH CONSULTANTS make no representation or warranty to any other person with regard to this review and the work referred to in this review and they accept no duty of care to any other person or any liability or responsibility whatsoever for any losses, expenses, damages, fines, penalties or other harm that may be suffered or incurred by any other person as a result of the use of, reliance on, any decision made or any action taken based on this review or the work referred to in this review.

This report has been prepared based on the TBSEF Statement of Work and the literature identified during the review. It is based on the literature specifically identified in the review at the time of the review. BIOWEST RESEARCH CONSULTANTS expresses no warranty with respect to the accuracy of the data reported in the literature.

The evaluation and conclusions reported herein do not preclude the identification of additional literature pertinent to the compounds discussed in this review. If new literature/studies become available, modifications to the findings, conclusions and recommendations in this review may be necessary.

Where information obtained from reference sources is included in the review, no attempt to verify the reference material was made. BIOWEST RESEARCH CONSULTANTS expresses no warranty with respect to the toxicity data presented in various references or the validity of the toxicity studies on which it was based. Scientific models employed in the evaluations were selected based on accepted scientific methodologies and practices in common use at the time and are subject to the uncertainties on which they are based.

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