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A review of the toxicity of glyphosate

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Dear Dr. Manderville,

For my second co-op work term, I was employed at Frontenac Provincial Park as an Invasive Species Technician where I spent my time working with the invasive species program at the park, as well as with the park rangers. In this role, I was responsible for the environmental monitoring and management of Dog Strangling Vine (DSV), a highly invasive terrestrial plant found within the park. My academic background in toxicology assisted me in both the monitoring and management phases of this position and I continued expanding my abilities to keep record of scientific data in a database, conduct field work and mitigate the potentially harmful effects that pesticides can have on the environment. I was very excited to get hands on experience working through both risk assessment and risk management aspects of a project. I was also able to gain experience using Global Positioning and Geographical Information Systems in the field to mark and locate plots. A secondary responsibility of this position involved providing outreach education on invasive species to the public at local community events. When the hand pulling of the DSV was not a viable option (either due to growing conditions or size of the plot), pesticide spraying was used to manage the plot. Over the course of the summer, I received pesticide application training from the Assistant Zone Ecologist of the Southeast Zone for Ontario Parks. For the management of DSV at Frontenac Provincial Park, we treat the plots with the herbicide glyphosate. The spraying of any pesticide can be cause for controversy, and because glyphosate is the active ingredient in the most widely used herbicide Roundup®, there is a large and global debate over the use of this chemical. As a toxicology student, I was interested in learning about the mechanisms of this herbicide, the past and current studies testing possible adverse effects, and understanding how regulatory limits were being established. Glyphosate is used in the greatest amount for agricultural practices, and is therefore found in trace amounts in much of our foods. It is also used in forestry management and for commercial and residential purposes. Glyphosate can be found in much of our environment and therefore, it is critically important to understand how this chemical effects aspects of the environment such as aquatic organisms, terrestrial organisms, other plants and microbial soil communities as well as us, humans. In my report, I review previously collected data on each of these topics from studies done to help draw a conclusion on the toxicity of glyphosate.

Sincerely,
Katryna Seabrook

A review of the toxicity of glyphosate

Written by: Katryna Seabrook

III. SUMMARY

As the most successful herbicide in history, glyphosate and glyphosate based herbicides are widely used in a variety of sectors for agricultural, forestry, industrial and commercial purposes (Pollegioni *et al.* 2011). Glyphosate is a very effective herbicide as it inhibits an enzyme in the Shikimate pathway in plants, preventing the production of vital amino acids without which the plant dies (Schonbrunn *et al.* 2001). Surfactants have been added to the herbicide to increase its effectiveness, however, these surfactants also increase the herbicide's toxicity. With the increasing use of glyphosate based herbicides, scientific controversy and societal concern over the safety levels of exposure to the chemical for non-target organisms have risen. In this report, the toxicity of glyphosate and glyphosate based formulations are reviewed through the exploration of previous studies evaluating exposure to the herbicide and the effects on humans, vertebrates, soil microbial communities and aquatic organisms.

TABLE OF CONTENTS

Table of Figures

IV. INTRODUCTION

Glyphosate is the most successful herbicide in history (Pollegioni *et al.* 2011). The chemical was originally discovered in 1950 by a Swiss chemist working for a pharmaceutical company, however the product had no pharmaceutical purpose (Gomes *et al.* 2014). Later, a scientist working for Monsanto, John Franz, discovered glyphosate to be a very potent herbicide and the molecule was patented as the active ingredient in Monsanto's herbicide Roundup® in 1974 (Gomes *et al.* 2014). Glyphosate, and the herbicide Roundup®, was registered for use in Canada in 1976 and since then has become the most widely sold and applied herbicide in the country (Gomes *et al.* 2014). The success of glyphosate as an effective herbicide is mainly due to its high specificity towards the plant enzyme enolpyruvylshikimate-3-phosphate synthase (EPSP) in the Shikimate Pathway (Pollegioni *et al.* 2011). Since this enzyme is only present in plants, and some fungi and bacteria, glyphosate-based formulations are believed to exhibit broad-spectrum herbicidal activity with minimal human and environmental toxicity. Because of this, glyphosate is used in a variety of sectors including agriculture, forestry, commercial, residential and aquatic environments (Pollegioni *et al.* 2011). Since 1996, the use of glyphosate in agriculture greatly increased upon the introduction of transgenic glyphosate resistant crops (Tarazona *et al.* 2017). Despite these successes, the widespread use of glyphosate has not only lead to the issue of glyphosate resistant weeds, but has also provoked scientific controversy and societal concern over the toxicity and amount of exposure of the chemical to non-target organisms (including humans, terrestrial and aquatic organisms, and the surrounding environment) (Tarazona *et al.* 2017).

V. BACKGROUND INFORMATION

Mechanism of Action

Glyphosate (N-phosphonomethyl-glycine) is a derivative of glycine in which one of the amino hydrogen atoms of glycine is replaced with a phosphonomethyl group (Gomes *et al.* 2014).

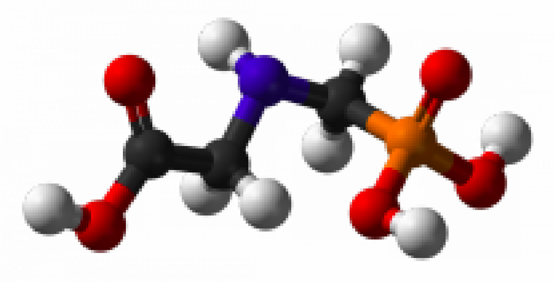

FIGURE 1 - Molecular Structure of Glyphosate where phosphorus is represented by orange, hydrogen by white, oxygen by red and nitrogen by blue (Minol, K. 2013).

The chemical is an odourless, colourless, crystalline solid and is usually formulated with the isopropylamine salt of glyphosate in herbicides which acts as a surfactant to increase its ability to penetrate plants (Schonbrunn *et al.* 2001). It is also a strong chelating agent that creates complexes through links that immobilize the mineral micronutrients in the soil making them unavailable to the plants (Toretta *et al.* 2018). Once glyphosate has penetrated the plant tissue, it will be translocated through vascular tissue reaching active metabolic sites such as the root and shoot meristems (Gomes *et al.* 2014). The shikimate pathway in plants produces aromatic products such as lignin, alkaloids, flavonoids, benzoic acids, plant hormones and amino acids needed for protein synthesis which are all crucial to the plant's survival (Schonbrunn *et al.* 2001). Glyphosate inhibits 5-enolpyruvylshikimate-3 phosphate synthase (EPSPS) which is the sixth enzyme in the shikimate pathway (Schonbrunn *et al.* 2001). The inhibition of EPSPS directly prevents the plant from being able to produce the aromatic amino acids of phenylalanine, tyrosine and tryptophan (Schonbrunn *et al.* 2001).

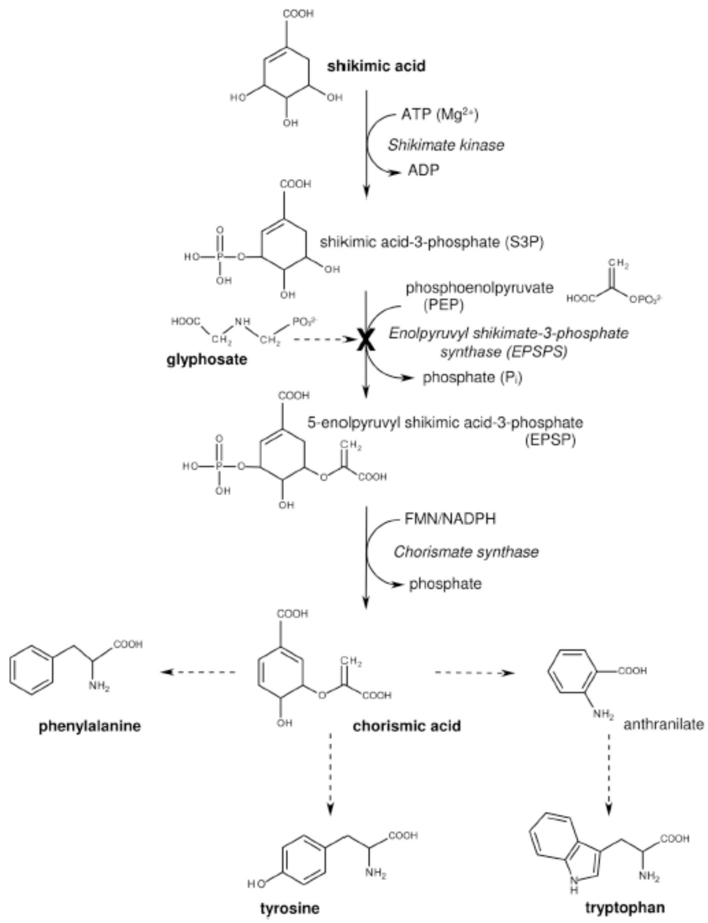

Figure 2 - The biosynthesis of aromatic amino acids and the mode of action of glyphosate in the Shikimate Pathways (Pollegioni *et al.* 2011).

EPSP catalyzes the transfer of an enolpyruvyl moiety from phosphenol pyruvate (PEP) to shikimate-3-phosphate (S3P) forming the products of 5-enolpyruvyl-shikimate-3-phosphate (ESP) and inorganic phosphate (Schonbrunn *et al.* 2001). Glyphosate and PEP have very similar structures and because of this, glyphosate is able to act as a competitive inhibitor of PEP, binding more tightly to the EPSP synthase-S3P complex than PEP. This reaction is reversible, but only very slowly since the dissociation rate for glyphosate is much slower than PEP and therefore rendering the enzyme inactive (Schonbrunn *et al.* 2001). The accumulation of S3P and the prevention of ESP from being formed stops the production of hormones, vitamins, aromatic amino acids and other essential plant metabolites which eventually kills the plant (Gomes *et al.* 2014).

Glyphosate toxicity to humans

The mechanism through which glyphosate directly exerts its herbicidal effects is present in plants, fungi and bacteria but not in humans or animals, and therefore the pure chemical is low in toxicity to humans (Gomes *et al.* 2014). However, products contain other ingredients that help increase the effectiveness of glyphosate penetrating the plant, creating glyphosate-based herbicides (GlyBH) and formulations (Mesnage *et al.* 2015). These include surfactants that often have greater toxicity than glyphosate, such as polyethoxylated tallowamine (POEA) used in Roundup® (Boggaard *et al.* 2008). Because these adjuvants are not considered directly responsible for the herbicide activity, they are declared as inert diluents and are classified as confidential for regulatory purposes despite the fact that several studies have demonstrated toxic effects of GlyBH formulations and surfactants while these effects were not observed with glyphosate alone (Mesnage *et al.* 2015). This produces a challenge because it can be unclear if toxicity studies used to regulate GlyBH formulations and exposure limits refer to the 'active' ingredient glyphosate or if the adjuvants have been taken into consideration (Mesnage *et al.* 2015). GlyBH formulations and surfactants can contaminate surrounding environments (including surface and groundwater, organisms and soil microbial communities) and therefore pose a risk to human health (Gasnier *et al.* 2009). Humans can be exposed to glyphosate and its formulations through inhalation, ingestion and dermal routes. Most human exposure arises from agricultural practices and the use of the herbicide for commercial and residential purposes. Due to the widespread use of GlyBH and transgenic glyphosate resistant (GR) crops that are designed to tolerate high levels of these compounds, residue levels in food and water are escalating (Gasnier *et al.* 2009). GlyBH-resistant plants do not metabolize or excrete glyphosate and will accumulate it during growth, increasing chemical residue in foods and feeds (Mesnage *et al.*

2015). Therefore, it is critical that future studies aim to identify potential toxicity of all ingredients in GlyBH to identify regulatory limits that accurately reflect environmentally relevant levels of exposure from all possible sources and include possible neurodevelopmental, reproductive and transgenerational toxicological effects (Mesnage *et al.* 2015).

Glyphosate toxicity to terrestrial organisms

Although glyphosate is most well-known for is use in agriculture and GR crops, it is also used for vegetation management in forestry practices such as weed control and invasive species control (Sullivan *et al.* 2003). Ecological sustainability of forestry practices is very important to the conservation of biodiversity, and while the use of GlyBH for the control of weeds and invasive species promotes ecological integrity, the introduction of any chemical into the environment raises concern of possible negative effects. In the case of using GlyBH in forested areas, it is important to study glyphosate's role as a disturbance agent and its impact on species diversity of terrestrial animals that live in this environment (Sullivan *et al.* 2003). Terrestrial animals could be effected directly through exposure to the chemical from aerial sprayings, eating and contact with plants sprayed with the chemical or indirectly through the alteration of their habitat (Sullivan *et al.* 2003). Regulatory limits have been put in place based on oral LD50's and LC50's published in literature, and when applied at recommended rates, glyphosate is virtually non-toxic to mammals, birds, fish, insects and most bacteria (McComb *et al.* 2008). It has also been found that glyphosate does not bioaccumulate in the tissues of animals (Sullivan *et al.* 2003). Studies have used changes in species richness and diversity of terrestrial animals to serve as a measure of the impact of glyphosate on biodiversity, and have demonstrated that the effects from herbicide treatment were within the mean values of natural fluctuations (Sullivan *et al.* 2003).

Glyphosate toxicity to soil

Glyphosate is a polar compound and strongly adsorbs to minerals and clay found in soil (Busse *et al.* 2001). The affinity glyphosate has for trivalent cations found in soil like Al^{3+} and Fe^{+} is due to the fact that glyphosate is a polyprotic acid and within the pH range found in most soils, it will form mono and divalent anions (Borggaard *et al.* 2008). Once in soil, glyphosate may be adsorbed onto soil particles, degraded by microbes, or transferred deeper into the soil (Gomes *et al.* 2014). The mobility and leachability of a compound in soil depends on its adsorption characteristics. For example, if a compound strongly adsorbs to the soil it will be immobilized, but if it weakly adsorbs to the soil, it will be readily leached (Borggaard *et al.* 2008). Glyphosate becomes inactivated in most soil types due to strong adsorption and fast degradation (Borggaard *et al.* 2008). Glyphosate has a relatively short half-life in soil (an average half-life of 47 days, although this can vary from 2-197 days depending on conditions) and therefore quickly degrades to its major metabolite aminomethylphosphonic acid AMPA (Busse *et al.* 2001). Degradation of glyphosate in soils occurs through microbiological processes through one of two pathways. The AMPA pathways involves the cleavage of glyphosate's C-N bond by the enzyme glyphosate oxidoreductase, producing AMPA and glyoxylate. AMPA is then cleaved to produce inorganic phosphorus and methylamine which is mineralized to CO_2 and NH_3 (Borggaard *et al.* 2008). The second pathway begins with the cleavage of the C-P bond to produce sarcosine and phosphate. Sarcosine is cleaved by sarcosine oxidase to produce glycine and formaldehyde, also eventually producing CO_2 and NH_3 (Borggaard *et al.* 2008).

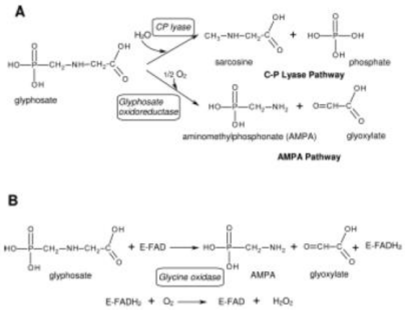

Figure 3 - The different degradation pathways of glyphosate in soil (Pollegioni *et al.* 2011).

Transport of glyphosate and AMPA from terrestrial to aquatic environments can occur in solution or suspension through subsurface or surface runoff into drainage and groundwater, as well as open water (lakes and streams) (Borggaard *et al.* 2008). The leaching of glyphosate into aquatic environments can occur through macropores in structured soils as well as after heavy rainfalls. Long-term use of glyphosate over course-textured soil materials (such as gravel) can lead the pollution of groundwater (Borggaard *et al.* 2008).

Glyphosate toxicity to aquatic organisms

Glyphosate can be used for the control of aquatic weeds and invasive species in water bodies, wetlands and ditches that can lead to an increase in diversity and productivity of organisms (Solomon *et al.* 2008). Glyphosate is highly water soluble, yet it readily ionizes and as an anion it is strongly adsorbed to sediments, much like it is in soil (Solomon *et al.* 2008). Therefore, glyphosate has very limited mobility and is quickly removed from water to sediments and suspended particulate matter. Much like as in soil, glyphosate does not degrade rapidly in sterile water, but will be quickly broken down into AMPA and CO_2 in the presence of microflora in the water (Solomon *et al.* 2008). Glyphosate can bind to sediments or undergo microbiological breakdown, following the same two pathways as in its degradation in soil. None of the metabolic products are believed to be toxic to aquatic organisms at the environmentally relevant concentrations (Solomon *et al.* 2008). In aquatic organisms, the surfactants added to GlyBH are often more toxic as glyphosate itself does not bioaccumulate, biomagnify or persist in the environment (Solomon *et al.* 2008). Surfactants such as POEA are adsorbed readily into soils and have been observed to have a half-life of 2 weeks (which is longer than the half-life of glyphosate) (Solomon *et al.* 2008). Therefore, from a risk assessment point of view, it is acute exposure of the herbicide to aquatic organisms is most likely to cause a toxic effect and have therefore been the most appropriate measures of effect in studies. The aquatic organisms most at risk are shallow freshwater organisms (such as algae, invertebrates, immature fish and amphibians) as the sediments are rich in organic matter (Solomon *et al.* 2008). In this way, other organisms in the ecosystem may be indirectly affected from the exposure to GlyBH.

VI. LITERATURE REVIEW

Glyphosate exposure increasing risk of cancer in humans

A study completed by Koller *et al.* investigated the cytotoxic and genotoxic properties of the chemical glyphosate and the GlyBH of Roundup® which is composed of POEA and glyphosate. To determine if exposure to glyphosate causes DNA damage and cancer, the buccal epithelial cell line TR146 was used to observe the results of four different endpoints. The first endpoint required the extracellular LHDe essay to measure the oxidation of NADH to NAD+. This was used to monitor the release of lactate dehydrogenase, providing information on the damage done to cell membranes after exposure (Koller *et al.* 2015). The second endpoint tested used the XXT assay to assess the changes to mitochondrial functions through measuring the activity of succinate dehydrogenase in viable cells (Koller *et al.* 2015). The third endpoint looked at total protein synthesis, which was monitored as a marker of cell proliferation using the SRB test (Koller *et al.* 2015). The final endpoint used the uptake of dye by healthy cells in neutral red

(NR) assays to observe the changes of the integrity of the membranes and lysosomal activities. In addition to these endpoints, single cell gel electrophoresis (SCGE) assays were used to observe the formation of single and double stranded breaks after exposure to the herbicide, and a cytokinesis-block micronucleus (CBMN) cytome assay was used to measure nuclear anomalies after exposure to the herbicides.

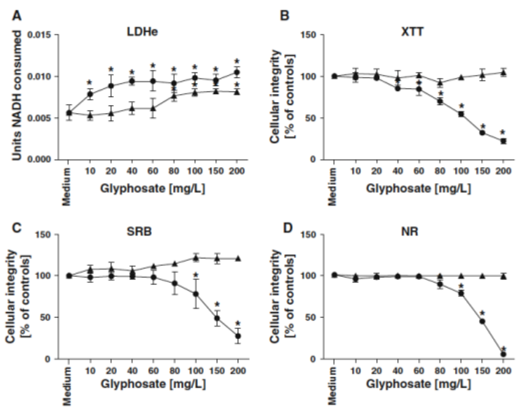

Figure 4 - The impact of glyphosate and Roundup in cell line TR146 after 20 minutes of exposure in each of the four assays. Each point represents the means +/- SD of three measurements and * represent significant differences from control values (Koller *et al.* 2015).

Overall, in all of the essays, the Roundup® formulations were more toxic than the straight glyphosate, and all effects were observed to increase as a function of the increasing exposure concentrations (Koller *et al.* 2015). In the CBMN assay, it was concluded that the effects of exposure were dose dependent. In conclusion, Roundup®, but not the active principle glyphosate, caused cytotoxic effects in the TR146 cell line (Koller *et al.* 2015). Genotoxicity tests concluded that both glyphosate and Roundup® induce strand breaks leading to formation of nuclear anomalies in DNA and chromosomal damage (Koller *et al.* 2015). Glyphosate did not induce toxic effects in the NR, SRB or XXT assays up to doses of 200mg/L, but did show toxic effects in the LHDe assay at a dose at 80mg/L (Koller *et al.* 2015). Significant cytotoxic effects were seen with all endpoints using Roundup® and LHDe and XXT assays demonstrated effects at doses less than 10mg/L (Koller *et al.* 2015). This reflects that the formulation can damage cell membranes and interfere with protein synthesis at low concentrations. Because significant acute and genotoxic effects were observed at doses between 10-20mg/L after 20 minutes of exposure to Roundup®, it can be concluded that after inhalation or short contact with this formulation, adverse effects in cells could occur (Koller *et al*. 2015). Overall, the findings of this study support the assumption of a possible linkage between GlyBH exposure and increased cancer risks (Koller *et al.* 2015).

Toxic hazards of glyphosate on terrestrial vertebrates

In a study by McComb *et al.* the effect of glyphosate on terrestrial animals was examined through studying the adequacy of dose responses to glyphosate of model organisms (lab rodents) at predicting that of free ranging animals. Intraperitoneal (IP) dosing of glyphosate (in the form of an isopropylamine salt) was used to compare the LD50's for nine species of terrestrial vertebrates to that of white lab mice. Based on previous literature, the mortality for terrestrial vertebrates from exposure to the maximum allowable application rate of glyphosate and its formulations in forest management is presumed to be very low based on oral LD50's and LC50's.

Species	N	LD50	Lower CI	Upper CI	NML
MAMMALS					
White lab mice (IP)	24	1,100	930	1,280	900
Deer mouse (IP)	49	1,370	1,120	1,680	600
Deer mouse (gavage)	29	>6,000	–	–	6,000
Townsend's chipmunk (IP)	16	1,320	980	1,790	900
Trowbridge's shrew (IP)	14	1,340	820	2,190	<900
Oregon vole (IP)	15	800	610	1,050	450
AMPHIBIANS					
Rough-skinned newt (IP)	30	1,250	1,030	1,430	500
Rough-skinned newt (gavage)	15	>2,600	–	–	>2,600
Ensatina salamander (IP)	10	1,070	700	1,640	<900
Tailed frog (IP)	5	>2,000	–	–	>2,000
Pacific giant salamander (IP)	3	<2,000	–	–	<2,000
Western red-backed salamander (IP)	9	1170	–	–	<1,200
COMPARATIVE DATA [a]					
Mallard duck (oral)		>4,000			
Bobwhite quail (oral)		>4,000			
Norway rat (oral)		4,300			
White lab mouse (oral)		1,500			
Rabbit (oral)		3,000			

[a] U.S. Forest Service 1988

Table 1 - LD50's and No Mortality Levels (mg/kg) for the mammals and amphibians after IP and gavage with comparative lab animal data (McComb *et al.* 2008).

In this study, McComb *et al.* looked at acute lethality and the influence of sublethal doses on the behaviour of the animals. To accomplish this, the acute LD50's of glyphosate in each of the four terrestrial mammals and five amphibian species were compared with that of the white mice. Animals were assessed for toxic responses daily during 96 hours after injection. After the initial dose, the concentration was adjusted to determine LD50's (McComb *et al.* 2008). A maximum dose at which no individual died following exposure was also defined as the No Mortality Level (NML). The sublethal effects of glyphosate on these species were examined using histopathological methods and liver and kidney samples were analyzed for tissue damage. The field survival in two of the species were assessed following the administration of a sublethal dose of glyphosate. This was used to determine if the toxicity of the does influenced the behaviour of the animal enough to make them susceptible to other sources of mortality under normal environmental conditions (McComb *et al.* 2008). The following null hypotheses were tested; first, the animals a receiving sublethal dose of glyphosate that would be considered in extreme forestry applications will experience a mortality rate no greater than animals that receive a control dose, and secondly, movement patterns will not differ between the animals receiving a sublethal dose of glyphosate and a control dose (McComb *et al.* 2008).

Species	1.1 kg/ha Normal	1.1 kg/ha Extreme	4.4 kg/ha Normal	4.4 kg/ha Extreme	IP LD50
Deer mouse	0.41	0.94	1.60	3.70	1,370
Townsend's chipmunk	1.70	5.60	6.80	22.40	1,320
Oregon vole	0.49	1.20	2.02	4.86	800
Trowbridge's shrew	0.27	0.47	1.07	1.87	1,320
Rough-skinned newt	0.13	0.28	0.52	1.12	1,250
Ensatina salamander	0.05	0.09	0.22	0.33	1,070
Tailed frog	0.07	0.13	0.28	0.52	>2,000
Pacific giant salamander	0.68	2.25	2.73	8.98	<2,000
Western red-backed salamander	0.03	0.04	0.10	0.15	1,170

Table 2 - Predicted normal and extreme field exposure rates (mg/kg) following glyphosate application (computer simulation) (McComb *et al.* 2008).

The results of this experiment indicated that glyphosate has low intraperitoneal toxicity and very low oral toxicity (McComb *et al.* 2008). The levels of intraperitoneal exposure required to reach LD50's were generally consistent across all test animals and therefore, the white lab mice can be thought of as an acceptable model for the species studied.

Although species do very in their responses to glyphosate exposure, overall, the margins of safety determined for small mammals and amphibians by laboratory mice appear to be large under any environmentally relevant exposure in forestry management (McComb *et al.* 2008).

Aquatic toxicity of glyphosate and effects of environmental factors

In a study performed by Tsui and Chu, the acute toxicity of Roundup® (containing POEA as a surfactant) was observed in the aquatic organisms of Microtox® bacterium, microalgae, protozoa and crustaceans. In addition to this, the effects of environmental factors on the acute toxicity of Roundup® to crustaceans such as temperature, water pH, suspended sediment and algal food concentration was also studied (Tsui *et al.* 2003). An interesting aspect to this study involved the separation of the surfactant POEA toxicity from the Roundup® toxicity to see the relative contribution of POEA to the toxic effects of the herbicide.

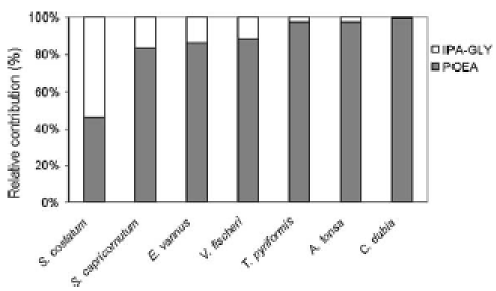

Figure 5 - The relative toxicity to Roundup® of IPA salt of glyphosate (white bar) and POEA (grey bar) to different organisms (Tsui *et al.* 2003).

The results of the study concluded that POEA was the most toxic chemical in the formulation, and was the only chemical that exerted toxic effects on all of the organisms tested. Overall, microalgae and crustaceans were 4-5 times more sensitive to the herbicides than the bacterium and protozoa (Tsui *et al.* 2003). It is likely that the microalgae were most sensitive because it is photosynthetic and therefore has similar metabolic pathways to higher plants which the herbicide is designed to target. It is thought that non-photosynthetic organisms (such as bacteria, protozoa, crustaceans and fish) should be more tolerant to the toxicity of the IPA salt of glyphosate (Tsui *et al.* 2003).

Species	Endpoint[a]	Glyphosate acid (mg AE/l)[b]	IPA salt of glyphosate (mg AE/l)	POEA (mg AE/l)	Roundup® (mg AE/l)
Vibrio fischeri	15 min IC50	17.5 (15.8–19.5)[c]	162 (150–177)	10.2 (9.80–10.7)	24.9 (23.9–26.0)
Selenastrum capricornutum	96 h IC50	24.7 (22.8–26.7)	41.0 (29.4–59.1)	3.92 (1.57–9.58)	5.81 (2.36–8.14)
Skeletonema costatum	96 h IC50	2.27 (0.82–11.1)	5.89 (3.14–10.4)	3.35 (2.02–5.40)	1.85 (0.33–10.49)
Tetrahymena pyriformis	40 h IC50	648 (430–1280)	386 (95.2–2020)	4.96 (2.90–8.98)	29.5 (11.3–66.0)
Euplotes vannus	48 h IC50	10.1 (6.47–14.5)	64.09 (19.0–325)	5.00 (4.62–5.42)	23.5[d]
Ceriodaphnia dubia	48 h LC50	147 (141–153)	415 (339–508)	1.15 (1.04–1.27)	5.39 (4.81–6.05)
Acartia tonsa	48 h LC50	35.3 (30.9–40.3)	49.3 (38.4–63.1)	0.57 (0.50–0.65)	1.77 (1.33–2.34)

[a] IC50 = median growth inhibition concentration; LC50 = median lethal concentration.
[b] AE = acid equivalent.
[c] 95% confidence interval was in parentheses.
[d] 95% confidence interval cannot be calculated.

Table 3 - A summary of the results to the toxicity tests (Tsui *et al.* 2003).

Out of the environmental factors tested, increasing the pH and increasing the concentration of suspended sediment significantly increased the toxicity of Roundup® to crustaceans. In terms of pH increase, it is reasoned that the POEA is cationic at acidic and neutral pH, and is therefore non-ionic at alkaline pH. Therefore, in the non-ionic form, POEA exerts greater toxicity to organisms through non-specific membrane disruption and greater toxicity in alkaline mediums (Tsui *et al.* 2003). In literature, the maximum expected environmental concentration of glyphosate in 15cm of water is 2.88mg AE/L, and based on the results of this experiment, this concentration would be toxic to aquatic organisms when considering the contribution of POEA, but not toxic with glyphosate alone. Therefore, in risk assessment of herbicides, the toxicity of all chemicals in the herbicide and the expected environmental exposure factors should be considered (Tsui *et al.* 2003).

The effects of glyphosate toxicity to soil microbial communities

In a study performed by Busse *et al.* the direct and indirect effects of glyphosate on soil microbial communities from ponderosa pine plantations were assessed using culture media and soil bioassays with concentrations of glyphosate up to 100 times larger than expected after a single field application. The indirect effects of microbial biomass, respiration and metabolic diversity were compared after 9-13 years of vegetation control using repeated glyphosate applications in a long term field study (Busse *et al.* 2001). Other purposes of this study included determining whether microbial responses to glyphosate varied with soil type, site quality and time of year, as well as determining the appropriateness of using artificial media studies to predict environmental responses to glyphosate. Interestingly enough, the addition of glyphosate to culture media resulted in a reduction of culturable bacteria and spore forming fungi whereas when added to soil at normal field concentrations, glyphosate had no measured effect on soil respiration (Busse *et al.* 2001).

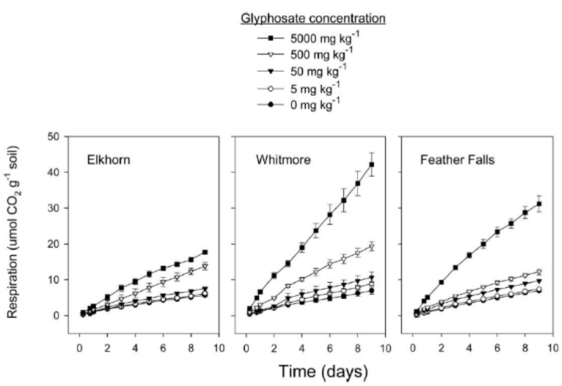

Figure 6 - Soil respiration after glyphosate exposure (Busse *et al.* 2001).

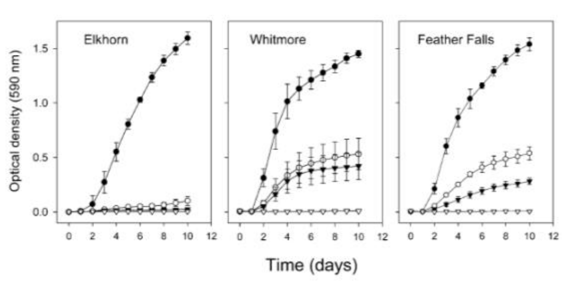

Figure 7 - Bacterial growth rate on Biolog GN culture medium after glyphosate exposure (Busse *et al.* 2001).

The differences in toxicity between artificial media and soil reflect the chemical nature of glyphosate as both an anti-microbial and polar compound that is easily inactivated by the soil through adsorption. Therefore, experiments using artificial, soil-free media do not accurately predict microbial responses to glyphosate in terrestrial systems as they lack ecological relevance (Busse *et al.* 2001).

VII. DISCUSSIONS

Due to the widespread use of glyphosate and GlyBH, the herbicidal effects have been seen throughout many faucets of the environment and society. With applications to major industries such as agriculture and forestry, as well as its use for industrial, commercial and residential purposes, GlyBH can cause direct and indirect toxic effects to workers, farmers, consumers as well as other non-target organisms in the environment. The widespread use of this herbicide has also lead to GlyBH resistant weeds, rendering the herbicide less effective. This, along with the potential health concerns, leads to the question if there are other methods of weed management that would be equally as effective while offering fewer toxic effects. Some examples of alternate methods of weed management include the use of biological controls or eobiotic herbicides (Pesticide Action Network Europe, 2018). Biological control refers to the use of living organisms (such as insects, bacteria/fungi and nematodes) that will naturally reduce the weed population as part of their normal function (Pesticide Action Network Europe, 2018). However, it is important to carefully research which biological control to use in a certain environment to ensure that an invasive species or harmful pest is not introduced. Eobiotic herbicides are substances that are endogenous to the environment already and are therefore biodegradable, leaving no residues (Pesticide Action Network Europe, 2018). However, eobiotic herbicides are non-selective and may also impact non-target organisms. It is important to note that there will be benefits and consequences when using any of these methods, including the use of GlyBH. In 2017, the Pest Management Regulatory Agency (PMRA) of Canada re-evaluated the regulations surrounding glyphosate, as there has been much controversy over the widespread uses and possible toxicity of the herbicide. During this re-evaluation, both active ingredients (glyphosate) and added formulated products (surfactants) were included (Pest Management Regulatory Agency, 2017). In terms of risk to humans, the PMRA concluded that glyphosate does not pose a carcinogenic or genotoxic risk. In addition to this, exposure to the chemical from food and water is not expected to pose any risks of concern to human health. In terms of non-target organisms including terrestrial vertebrates and aquatic invertebrates, spray buffer zones were found to be necessary to mitigate toxic effects, but overall when applied following label directions, GlyBHs are not expected to pose risks to the environment (Pest Management Regulatory Agency, 2017). Overall, the benefits of glyphosate and GlyBHs as a weed control product in both agriculture and non-agricultural management still outweighs any possible toxic effects of the chemical in today's society.

VIII. CONCLUSIONS

Glyphosate is a non-selective herbicide used for weed control in agriculture, forest management, commercial and residential settings (Gomes *et al.* 2014). It exerts its direct herbicidal effects by inhibiting the enzyme EPSP in the Shikimate Pathway in plants, but can also cause indirect effects mainly caused by the addition of surfactants to the glyphosate herbicide (Mesnage *et al.* 2015). In most cases, it is these indirect effects that are a concern to human health, non-target organisms and aquatic environments. In the four studies reviewed above, it can be concluded that when glyphosate is applied according to regulated values, it poses very low toxicity to non-target organisms. For example, in the study by Busse *et al.* it was concluded that the addition of glyphosate to soil at normal field concentrations resulted in no measurable effect on soil respiration (Busse *et al.* 2001). Furthermore, in the study performed by McComb *et al.* it was concluded that glyphosate poses low toxicity to terrestrial vertebrates and confirmed that the margins of safety determined by laboratory mice for the application rates of the herbicide appear to be large under any relevant condition in forestry management (McComb *et al.* 2008). It can also be concluded that overall, it is the surfactants in the GlyBH that exert the most toxicity. For example, in the study by Koller *et al.* exposure to the surfactant POEA in Roundup® was found to be toxic to humans at doses between 10-20mg/L after 20 minutes of exposure. Overall, Koller *et al.* observed the trend that the Roundup® formulations were more toxic to human buccal epithelial cell line TR146 than glyphosate on its own (Koller *et al.* 2012). However, toxic effects have been observed in environments treated with glyphosate, and in humans, the possibility of carcinogenic effects has been expressed. Due to these uncertainties, many studies have been completed, however it can be difficult to reconcile the data as it is not always clear if the study is testing glyphosate alone or testing the formulation and taking into account to added toxicity of each adjuvant. It is therefore critically important that more comprehensive studies are done, examining the effects of all the chemicals included in GlyBHs and it is also important that this data is reflected on the product's label. As the presence of this chemical in our environment continues to increase, it is necessary that both acute and chronic toxicological studies continue to be completed, analyzing the effects of GlyBHs on humans, non-target organisms and the environment as a whole. This is crucial in providing the most accurate regulatory limits to ensure the safe and responsible use of this herbicide.

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XI. EMPLOYER EVALUATION FORM

Co-op Work Term Report Employer Comments

UNIVERSITY of GUELPH

The Co-op Work Term Report is a critical assignment that integrates academic theory and practical experience for our Co-op students. Supervisors are well positioned to comment on the content and accuracy of student reports.

Please take a moment to provide feedback on the report and ensure there are no proprietary concerns. Note that confidential work reports are not accepted by the university. The Faculty Advisor will assign the final grade and your feedback will be taken into consideration for grade calculation.

Your feedback may reference the following areas:

- Ideas, topic and analytic content
- Organization, presentation and professional appearance
- Data collection, research results and conclusion
- Clarity of expression and understanding of new concepts

Good Summary of the history, Chemical make up, routes of Possible harm, and toxicity of Glyphosate, including the interactions between Glyphosate and other chemicals that make up Round up®. Information is presented in a Clear format that makes following along and understanding Concepts covered easy. There is no concern with this report containing any Ontario Parks proprietary information. An enjoyable read!

Please sign and return this form to the student once you have reviewed the report. The student must submit the Co-op Work Term Report including this form to their Faculty Advisor no later than the fifth class day of the following semester.

Supervisor's Name:	Mike Holm	Student's Name:	Katryna Seabrook
Supervisor's Email:	mike.holm@ontario.ca	Student's ID Number:	0956251
Supervisor's Phone #:	613 376-6950	Work Term Semester:	Summer 2018 (WT2)
Organization:	Ontario Parks - Frontenac PP	Academic Discipline:	Biomedical Toxicology
Supervisor's Signature:	M. H[illegible]	Date:	September 1, 2018

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