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The Persistence of Glyphosate in Vegetation One Year after Application

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Abstract: Glyphosate-based herbicides are the most widely used herbicides in the world, including in Canadian forestry. In general, glyphosate-based herbicides are considered relatively non-toxic to wildlife species due, in part, to rapid breakdown of the chemical in the environment. However, recent work has shown that glyphosate can persist for at least one year after application at low concentrations leading to concern over the persistence of trace levels in the environment. Using two independent studies we characterize the short- (18 days) and long-term (1 year) persistence of glyphosate in vegetation which are commonly, but differentially, browsed by WhiteiTailed Deer (*Odocoileus virginianus*), Moose (*Alces alces*), and Black Bear (*Ursus americanus*), or used as traditional medicines by Indigenous people and compare the residues to exposure thresholds. In the short-term study, glyphosate concentrations within the application block exceeded the general and maximum residue level (MRL) for fresh fruit set by Health Canada (0.1 ppm) for up to 18 days after application. In the long-term study, glyphosate concentrations were above the MRL one week after application and below the MRL one month and one year after application. Under the assumptions that all vegetation contained glyphosate at the highest observed concentration, animals only consume vegetation from herbicide treated areas, and animals consume the physiological maximum level of vegetation daily, Moose, White-Tailed Deer, and Black Bears could exceed the Acceptable Daily Intake for glyphosate (0.3 mg/kg/day) for up to 18 days after application. Subsequently, given dissipation of residues in vegetative matrices as observed in this study, we consider it highly unlikely that the species considered herein could consume enough vegetation throughout their lives to pose a risk to their health. Overall, our two independent studies demonstrate that trace levels of glyphosate persist in vegetation for up to one year after application, however, observed concentrations are unlikely to pose risk to wildlife. We caution that operational practices as typically imposed in Canadian forestry are very important and effective in minimizing risk.

Keywords: glyphosate; persistence; herbicide; traditional use vegetation

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1. Introduction

Integrated vegetation management aims to manage the course and rate of forest vegetation succession to achieve forest management objectives [1,2]. In young coniferous forests, vegetation management provides desired coniferous species a competitive advantage over undesirable deciduous species early in growth with the goal of accelerating successional development. Vegetation management in conifer stands can be achieved through a variety of techniques, with herbicide application being the most commonly used [3–5]. Alternatives to herbicide use have been developed, in part due to increased public concern about the use of herbicides in forest management, resulting in a shift towards minimizing herbicide use in modern silviculture [2]. Alternatives, such as mechanical control or planting large seedlings can be effective in some conditions [6]. However, herbicides, and in particular glyphosate-based herbicides remain the most cost-effective method to control competitive vegetation in Canadian forestry.

Potential effects on humans and wildlife related to possible short- and/or long-term exposure to glyphosate use in forest management remains a concern [7]. In forest use scenarios, terrestrial fauna could be directly exposed to the herbicide through spray, spray drift, or if rainfall washes residue off leaves [5]. Direct, acute, exposure in a forestry setting has been well studied and the general consensus from environmental risk assessments is that no adverse environmental effects are expected at application rates typically used in Canadian forestry [5,8–11]. Risk to humans is significantly mitigated by limiting human access to spray blocks for a period immediately after application.

Wildlife, and humans, could potentially be exposed to low levels of glyphosate residues remaining on, or in, vegetation over a longer term. Vegetation from within harvest blocks and consumed during the time required for glyphosate or its primary metabolite to degrade or dissipate from plant matrices would pose a conceptually higher risk. Glyphosate persistence in most environmental compartments of forested ecosystems is considered low to moderate, with estimated time to 50% dissipation times (DT50) between 9.7 and 18.9 days on the forest floor [12], between 45 and 60 days in soil [13,14], less than 5 days in static water [11,15–17], and <2 days in flowing streams and rivers (13). Within vegetation, DT50 estimates are as short as 2 days [18], with 98% degradation by 63 days in upper foliage and 99% by 122 days herbaceous vegetation [19]. Within harvest blocks that received an herbicide application, glyphosate has been detected in vegetation, including new leaves, berries, and stems one year after application [20]. Such detections of glyphosate in vegetation one year after application are not surprising because the curvilinear degradation curve predicts that some herbicide will remain in the environment one year after application.

In cases where detectable levels of glyphosate do persist in vegetation, both the magnitude and duration of exposure need to be quantified and compared to threshold values set for the protection of human, wildlife, and aquatic health [21] such as the Maximum Residue Limit (MRL) or Acceptable Daily Intake (ADI). The potential exposure pathways for humans might include consuming berries or through traditional use of vegetation by indigenous peoples. Wildlife exposures might occur through consumption of berries or foliage and/or by pollinators as they forage for nectar in flowers.

The MRL is not a safety standard per se. No government agency would set an MRL that may pose a health risk, however the MRL is a regulatory standard that reflects use practices and application rates. The MRL is also often taken as the surrogate for exposure. In Canada, the Pest Management Regulatory Agency (PMRA) of Health Canada regulates under the Pest Control Products Act and establishes MRLs for pesticide residues in food intended for human consumption. The MRL is defined as the maximum amount of residue that may be expected to remain in or on raw agricultural commodities or processed food products that contain the commodity and is set in the 95th percentile region of observed residue distributions for specific pesticide product combinations. For fruit, such as blueberry and raspberry, fresh or processed fruits have an established MRL of 0.1 ppm. The general MRL (0.1 ppm) would be applied to foliage as no specific MRL has been developed for that specific commodity.

The ADI is a standard threshold value calculated to characterize toxicological risk from long-term (chronic) exposures to a pesticide. In essence, the ADI is the maximum quantity of a substance that a human could ingest daily throughout their entire lifespan without an appreciable risk to their health. The ADI value is typically set based on the No-Observed-Adverse-Effect Level (NOAEL) as determined through standardized tests on laboratory animals and divided by a value of 100 to account for both intra- and inter-species variation and uncertainty. In Canada, the ADI determined for glyphosate is 0.3 mg glyphosate/kg body weight/day and is based on the NOAEL (32 mg/kg bw/day) from a 26-month chronic toxicity and carcinogenicity study on rats. Since the ADI is established explicitly upon laboratory testing designed to quantify risks to animals, it is considered the more appropriate threshold value for assessing toxicological risks to humans and wildlife.

The goal of this present paper is to characterize residues of glyphosate and its major degradation product AMPA in vegetation used by humans and wildlife immediately after application and for 1 year after application. The measured residues are compared to the MRL and ADI, values that are broadly considered as protective of human health. Comparisons to threshold values characterize the magnitude and duration of risk.

2. Materials and Methods

We conducted two independent projects to quantify the concentration of glyphosate and AMPA in vegetation following aerial applications of a glyphosate-based herbicide typical of Canadian forestry. The goal of study 1 was to measure concentrations at a fine resolution for three weeks after herbicide application, and the goal of study 2 was to measure the concentrations up to one year after herbicide application. Study 2 was conducted in collaboration with Wahnapitae First Nation who have raised concern over the use of glyphosate-based herbicides in forestry operations and its potential to remain in the environment.

2.1. Study 1: Short-Term, Fine Resolution

The first study occurred in six harvest blocks in east central New Brunswick, Canada (Table 1). Each block received an aerial application of the glyphosate-based herbicide Forza (FMC of Canada, Can, 340 g a.i./L) at a target application rate of 1.8 kg a.i./ha between 14 and 20 August 2015.

Table 1. Description of spray blocks used in the short- and long-term studies.

Study	Block	Latitude Longitude	Size (ha)	Application Date	Kg a.e./ha
Study 1	603A	46.105048 −66.117766	16.01	16 August 2015	1.8
	203A	46.11802 −66.108164	43.46	16 August 2015	1.8
	3074171B-I	45.754206 −66.108164	20.49	20 August 2018	1.8
	3074171B-II	45.754206 −65.331638	55.55	20 August 2015	1.8
	569A	45.453345 −66.021355	45.73	14 August 2015	1.8
	569C	45.455908 −66.006574	58.23	14 August 2015	1.8
Study 2	Control	46.802408 −80.873115	6.18	Control	Control
	Herbicide 1	46.799936 −80.873593	14.12	18 August 2018	1.35
	Herbicide 2	46.803052 −80.877352	6.63	18 August 2018	1.35

In each herbicide block, vegetation was sampled along 100 m transects at three locations; within the central portion of the application area, 25 m downwind of the application boundary, and 50 m downwind of the application boundary. Every 10 m along the transects a 10 g sample of vegetation from each of three different vegetation categories (Figure 1), Moose (*Alces alces*) browse, White-Tailed Deer (*Odocoileus virginianus*) browse, and blueberry (*Vaccinium spp.*) fruit was collected. Vegetation from each category was pooled for each transect resulting in one 100 g sample. Moose browse consisted of deciduous woody plants between 1.5 and 2 m above ground such as maples (*Acer spp.*), birches (*Betula spp.*), willow (*Salix spp.*), dogwood (*Cornus spp*), elder (*Sambucus spp.*), cherries (*Prunus spp.*), ash (*Sorbus spp.*), trembling aspen (*Populus tremuloides*), and ground hemlock (*Taxus canadensis*). Deer browse consisted of new shoots, buds, and leaves between 0.5 and 1 m above the ground of maples, birches, ash, cherries, dogwood, honeysuckle (*Lonicera spp.*), raspberry (*Rubus idaeus)*, grasses, sumac (*Rhus spp.*), goldenrods (*Solidago spp.*), sweet fern (*Comptonia peregrine*), wood fern (*Dryopteris spp.*), hobblebush (*Viburnum lantanoides*), wild grape (*Vitis riparia*), wild raisin (*Viburnum nudum*), and blueberry (*Vaccinium spp.*). Blueberry samples consisted of ripe berries, with no stems or leaves, taken from areas with the least amount of overtopping vegetation.

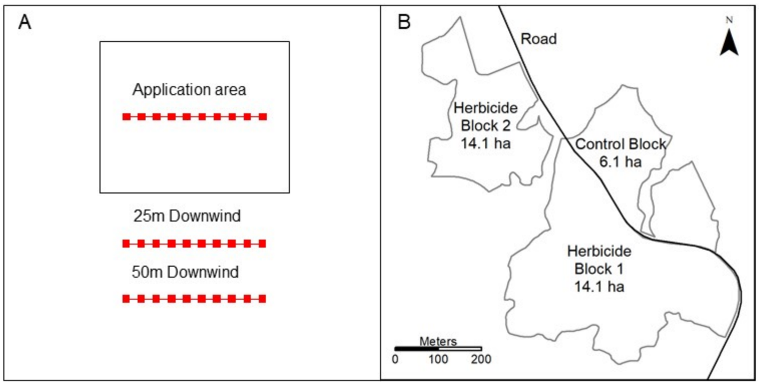

Figure 1. (**A**) Schematic of sampling design for the long-term study, there were six replicate areas. (**B**) A map of the blocks used in the short-term study.

Vegetation samples were collected on days 0, 4/5, 11, and 18 after herbicide application. To avoid cross contamination, for each spray block, the 50 m downwind transects were sampled first, followed by the 25 m downwind transects, and finally sampling along the transect within the application area. During collection, colour coded nitrile gloves were worn for each vegetation category and the gloves were thoroughly washed with water and wiped dry between transects. Samples were collected in clean Ziplock bags, placed in a cooler on ice, and frozen at the end of each field day. Some tissues were not collected during all sampling periods because of the seasonal nature of some of the vegetation tissues. For example, blueberries were ripe when the herbicide was applied, and became over-ripe and dropped to the ground during the post-application sampling periods.

2.2. Study 2: Long-Term, Coarse Resolution

The long-term persistence study occurred in three adjacent harvest blocks in Ontario, Canada, (Table 1). Blocks were spruce-fir stands harvested in 2014 under a clear-cut system. The blocks were assigned to one of two treatments, an herbicide application (n = 2) or as a control (n = 1). The herbicide application blocks received a single aerial application of the glyphosate-based herbicide VisionMax (Monsanto, Winnipeg MB, 540 g a.i./L) at an application rate of 1.35 kg a.i./ha on 18 August 2018. No herbicide was applied to the control block (Figure 1).

The species and tissues collected were chosen in consultation with Wahnapitae First Nation. Community members with knowledge of plants and their traditional uses provided a list of 9 species and 14 tissues types to collect that were grouped into four categories, White-Tailed Deer and Moose browse, Black Bear browse, medicinal, and forestry (Table 2). Vegetation samples were collected one week before (August 2018), one week after (September 2018), one month after (October 2018), and one year (September 2019) after herbicide application. During sampling 10 g of each vegetation tissue was collected separately by haphazardly walking throughout the block and collecting a composite sample from various locations. To avoid cross contamination each tissue was collected separately while wearing nitrile gloves, and the gloves were changed between tissue collections.

Table 2. List of vegetation species and tissue collected and the matrix they were assigned in the long-term study.

Matrix	Species	Tissue
Wildlife browse	Red maple (*Acer rubrum*)	Leaf
	Trembling aspen (*Populus tremuloides*)	Leaf
	White birch (*Betula papyrifera*)	Leaf
Black Bear	Blueberry (*Vaccinium spp.*)	Fruit
	Blueberry (*Vaccinium spp.*)	Leaf
	Raspberry (*Rubus idaeus*)	Fruit
Medicinal	Goldenrod (*Solidago spp.*)	Flower
	Goldenrod (*Solidago spp.*)	Root
	Pearly everlasting (*Anaphalis margaritacea*)	Flower
	Pearly everlasting (*Anaphalis margaritacea*)	Leaf
	Raspberry (*Rubus idaeus*)	Leaf
	Raspberry (*Rubus idaeus*)	Root
	Sweet fern (*Comptonia peregrine*)	Leaf
Forestry	Jack pine (*Pinus banksiana*)	Leaf

During each sampling event, the control block was sampled first and then the treated blocks were sampled. Samples were collected in clean Ziplock bags, placed in a cooler on ice, and frozen at the end of each field day. Not all tissues were collected during all sampling periods because of the seasonal nature of some of the vegetation tissues (e.g., berries).

2.3. Glyphosate and AMPA Quantification

All vegetation samples were shipped frozen, on ice, to the Agriculture and Food Laboratory at the University of Guelph. In the analytical laboratory, concentrations of glyphosate and AMPA in each tissue sample were quantified using advanced Liquid Chromatography tandem mass spectrometery (LC_MS/MS) techniques. The Minimum Detection Limit (MDL) was 0.005 ppm and the Minimum Quantification Limit (MQL) was 0.02 ppm for glyphosate and AMPA.

2.4. Data Analyses

In the short-term persistence study, we tested whether the observed mean glyphosate residue levels exceeded the 0.1 ppm MRL using one sample *T*-Tests for each time period. In the long-term persistence study, we compared the mean measured values for each of the vegetation types to the 0.1 ppm MRL directly. No statistics were performed for the long-term persistence study because only two replicate herbicide application blocks were sampled. All means are reported $\pm$ SE unless otherwise stated.

To assess potential cause for concern with respect to wildlife species consuming vegetation from blocks to which the herbicide was applied, we calculated a worst-case daily intake for a White-Tailed Deer, Moose, or Black Bear and compared the intake to the ADI (0.3 mg/kg bw/day) set by Health Canada. We assumed the average body mass of a White-Tailed Deer is 50 kg, Moose is 300 kg, and Black Bear is 150 kg. We defined a worst-case scenario by assuming the animal only consumed vegetation from the herbicide application blocks and all the vegetation contained the maximum observed concentration of glyphosate. In addition, we assumed that an animal consumes the physiological maximum mass of food possible every day. The physiological maximum amount of dry forage a White-Tailed Deer or Moose can consume in one day is 4.8% of body mass [22], therefore a 50 kg White-Tailed Deer could consume 2.4 kg of browse in a day and a 300 kg Moose could consume 14.4 kg of browse in a day. Captive bears allowed to eat berries ad libitum can consume 34% of their body mass as fruit per day if they are not time constrained by foraging [23]; therefore, a 150 kg Black Bear could theoretically consume 51 kg of berries per day. Together these assumptions create a worst case scenario that is highly unlikely to occur in nature. We did not calculate the ADI for traditional medicinal uses because of uncertainty over how the preparation of medicine could increase or decrease the concentration of glyphosate in the medicine and how the mode of consumption could affect risk.

3. Results

3.1. Study 1: Short-Term, Fine Resolution

Glyphosate was detected in all White-Tailed Deer browse samples from within the application area and downwind during all time periods. The mean concentrations of glyphosate (Table 3) in White-Tailed Deer browse within the herbicide application area was above the 0.01 ppm MRL during all time points ($p < 0.05$). However, mean concentrations were below MRL value 25 m and 50 m downwind at all time periods ($p > 0.05$).

Within the Moose browse samples, glyphosate was detected in all samples, from within the application area and downwind, during all time periods. Mean glyphosate concentrations (Table 3) within the application area were above the MRL on day 0 ($p = 0.011$) and 11 ($p = 0.045$) and below the MRL on day 4/5 ($p = 0.081$) and day 18 ($p = 0.088$). Mean glyphosate concentrations in Moose browse were below the MRL 25 m and 50 m downwind of the application area at all times ($p > 0.05$).

Table 3. Mean glyphosate (95% confident intervals) reside detected in three vegetation matrices at four time periods from the short-term study.

		Glyphosate (ppm)			
Vegetation Matrix	**Location**	**Day 0**	**Day 4/5**	**Day 11**	**Day 18**
Blueberries	Application Area	6.18 (3.18, 9.17)	4.93 (1.14, 8.72)	4.37 (3.79, 4.95)	5.1 (3.74, 6.46)
	25 m Downwind	NA	2.5 (−0.64, 5.64)	NA	NA
	50 m Downwind	NA	0.71 (−0.46, 1.88)	NA	NA
White-Tailed Deer Browse	Application Area	397.50 (236.80, 558.20)	97.25 (21.19, 173.31)	14.75 (9.67, 19.83)	10.35 (4.58, 16.12)
	25 m Downwind	18.42 (−10.87, 47.72)	45.29 (−29.81, 120.39)	1.15 (−0.01, 2.31)	1.14 (−0.01, 2.31)
	50 m Downwind	6.46 (−4.99, 17.92)	2.79 (0.39, 5.19)	0.34 (−0.15, 0.84)	0.17 (−0.08, 0.44)
Moose Browse	Application Area	412.5 (225.98, 599.02)	35.73 (−2.18, 73.64)	11.4 (8.26, 14.54)	10.73 (0.58, 20.89)
	25 m Downwind	11.80 (−7.08, 30.67)	92.0 (−53.32, 237.32)	0.87 (−0.034, 1.78)	1.55 (−0.37, 3.47)
	50 m Downwind	10.51 (−6.72, 27.75)	2.87 (0.16, 5.58)	0.29 (0.076, 0.49)	0.16 (−0.03, 0.34)

Glyphosate was detected in all blueberry samples, however, blueberries were not present on the 25 m downwind transects from two blocks on days 0, 11, and 18 and on day 0 and 11 on two 50 m downwind transects. Due to lack of samples for quantification, statistical analyses were only performed on samples collected from within the application area. Mean glyphosate (Table 3) concentration in blueberries exceeded the MRL on days 0, 11, and 18 ($p < 0.05$) within the herbicide application area, but did not exceed the MRL on day 4/5 ($p = 0.065$).

3.2. Study 2: Long-Term, Coarse Resolution

Neither glyphosate nor AMPA was detected in any of the tissue types from the control or herbicide application blocks one week before the herbicide application was made. However, in the control block one week after application, glyphosate was detected in all 12 tissue types, ranging between 0.02 and 0.19 ppm. After one month, glyphosate was detected above the detection limit (0.005 ppm) and below the limit of quantification (0.02 ppm) in 3 tissue types from the control block. Glyphosate was not detected in any of the tissue types collected one year after application in the control block. AMPA was detected below the MQL in 5 of 12 tissue types one week after herbicide application and it was not detected above MDL in any of the 10 tissues sampled one month after application.

Within both of the herbicide blocks glyphosate was detected in all tissue types one week and one month after herbicide application (Figure 2). Whereas, one year after application glyphosate was detected in nine of 14 tissue types in herbicide block 1 and eight of 14 tissue types in herbicide block 2. AMPA was detected in every tissue type from both herbicide blocks one week after application, in all but one tissue one month after application, and in eight of 28 tissue types from both herbicide blocks one year after application, with seven of eight detections below the MDL (0.005 ppm). AMPA was only detected at concentrations above the MRL one week after application, and was rarely detected at all thereafter, therefore we limit discussion to glyphosate residues.

All detected concentrations of glyphosate were above the MRL (0.1 ppm) one week after application in herbicide block 1 and in 12 of 14 tissue types in herbicide block 2 (Figure 2). In general, glyphosate concentrations declined from one week to one month after application, but glyphosate concentrations in all tissues from block 1 and in eight of 11 tissues from block 2 remained above the MRL one month after application. In the one year after application sample, glyphosate was detected in nine of 14 tissue types in herbicide block 1 and eight of 14 tissue types in herbicide block 2. Of the 17 tissue types in which glyphosate was detected, concentrations were above the MRL in four tissues, raspberry leaf (two samples) and raspberry root (two samples).

In plant tissues important for White-Tailed Deer and Moose browse, average concentrations in each block were above the MRL value in block 1 (6.93 $\pm$ 2.58 ppm) and block 2 (9.39 $\pm$ 8.81) one week after application. Glyphosate concentrations declined, but remained above the MRL, one month after application in both herbicide blocks (Block 1: 0.32 $\pm$ 0.19 ppm, Block 2: 0.27 $\pm$ 0.23 ppm). Concentrations in White-Tailed Deer and Moose browse fell below the MRL in each block (Block 1: 0.037 $\pm$ 0.019 ppm, Block 2: 0.018 $\pm$ 0.0092 ppm) one year after application.

Plant tissues used by Black Bear could only be collected in the one week and one year after application samples because berries had not developed before application and had fallen off the plants before the one month after application sample. Immediately after application, glyphosate was detected in fruits eaten by Black Bears at 24.70 $\pm$ 15.37 ppm in herbicide block 1 and 12.46 $\pm$ 7.11 ppm in herbicide block 2, values that exceed the MRL. In the one year after application sample, mean glyphosate concentrations in fruits were below the MRL in both herbicide application blocks (Block 1: 0.063 $\pm$ 0.018 ppm, Block 2: 0.021 $\pm$ 0.011 ppm).

Mean glyphosate concentrations in plant tissues used in traditional medicine were above the MRL one week after application in both herbicide blocks (Block 1: 26.96 $\pm$ 9.64 ppm, Block 2: 15.28 $\pm$ 6.78 ppm) and declined, but remained above the MRL, one month after application (Block 1: 3.97 $\pm$ 2.060 ppm, Block 2: 1.020 $\pm$ 0.283 ppm). After one year, the mean concentration in tissues used in traditional medicine in block 1 (0.065 $\pm$ 0.031 ppm) were below the MRL and above the MRL in block 2 (0.18 $\pm$ 0.16 ppm).

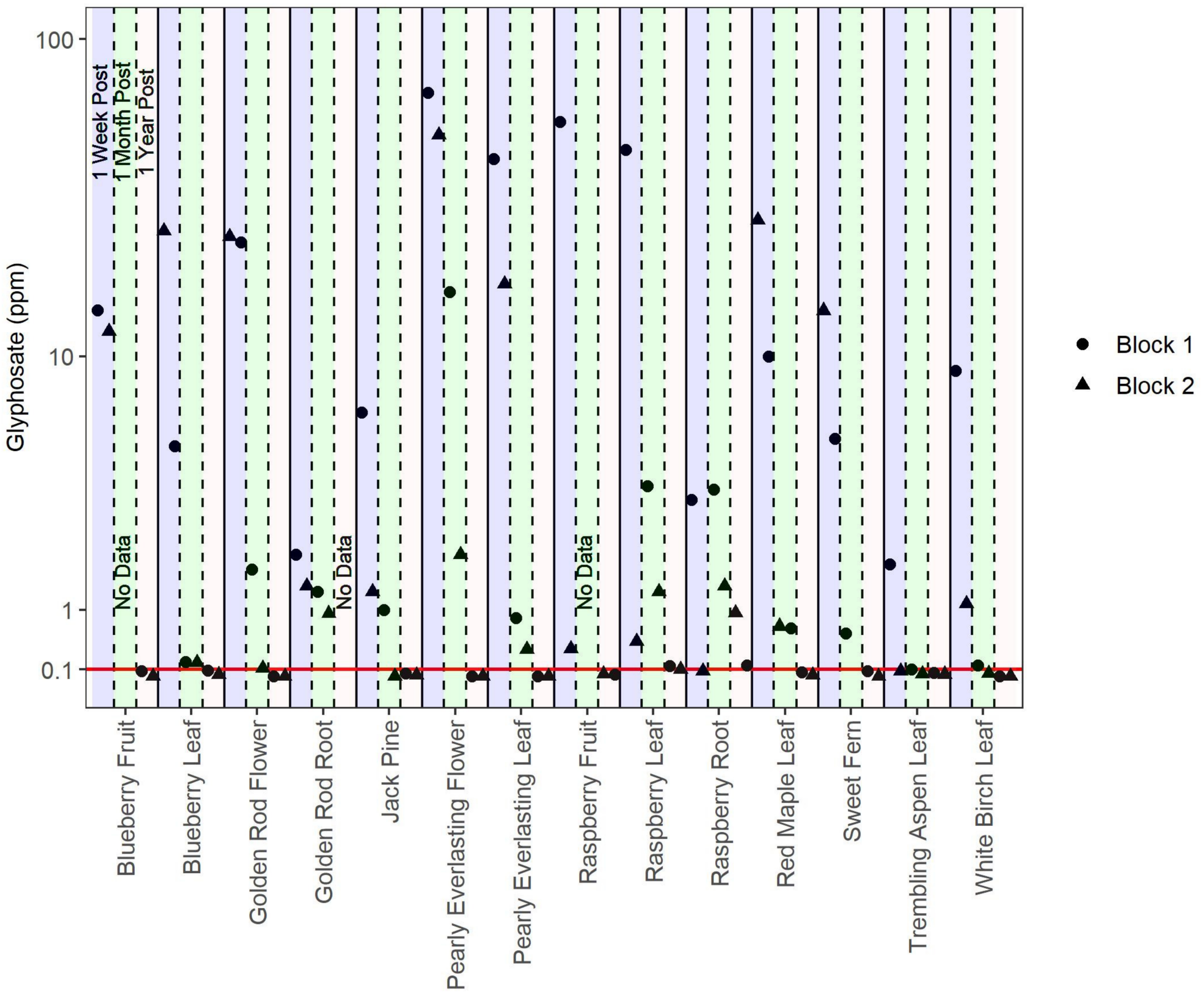

Figure 2. Glyphosate concentrations (ppm) detected in 14 vegetation types one week, one month, and one year after application in two harvest blocks that received an aerial application of a glyphosate-based herbicide. The horizontal line represents the Maximum Residue Limit for fresh fruits and the general MRL for untested food set by Health Canada (0.1 ppm). Note that *Y*-axis is log scale.

3.3. Acceptable Daily Intake

The values from both studies are combined in the ADI analyses to aid interpretation because the same consistent pattern was found in both datasets. As expected, the worst case maximum daily intake of glyphosate by animals declined with time as the residue concentration declined (Figure 3). It is possible for an adult White-Tailed Deer or Moose to exceed the ADI for up to 18 days after application by consuming vegetation from harvest blocks that received an herbicide application. For an adult White-Tailed Deer or Moose to exceed the ADI 18 days after application, they must consume 0.78 kg and 4.28 kg of browse every day, respectively which is lower than the physiological daily limits we calculated; White-Tailed Deer: 2.40 kg, Moose: 14.40 kg. However, one month after application it is unlikely that an adult White-Tailed Deer or Moose could exceed the ADI. Exceeding the ADI one month after application would require a 50 kg adult White-Tailed Deer to consume 20.20 kg of vegetation and a 300 kg adult Moose to consume 121.60 kg of vegetation daily.

Similarly, it is possible for a 150 kg Black Bear consuming the physiological maximum daily amount of berries (51 kg) to exceed the ADI for up to 18 days after application, and unlikely the ADI could be exceeded one month after application (Figure 3). A 150 kg adult Black Bear would need to consume 14.28 kg of berries every day to exceed the ADI 18 days after application and 214.30 kg of berries every day one month after application to exceed the ADI.

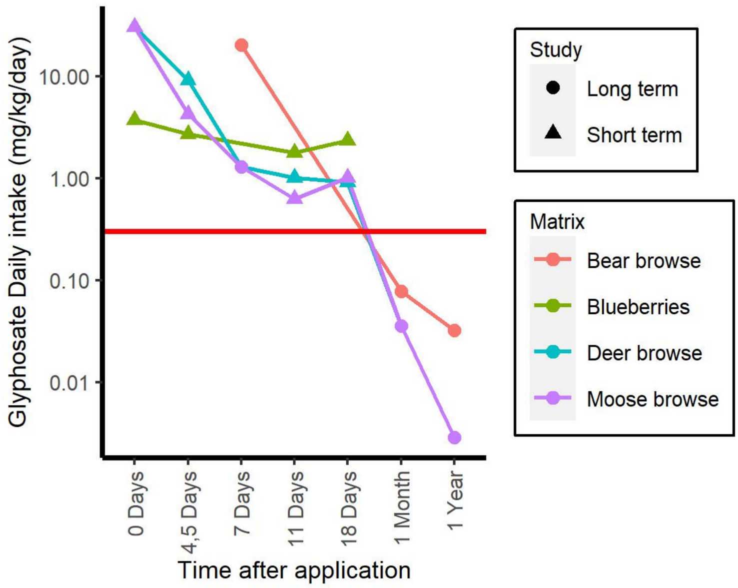

Figure 3. Worst case daily intake of glyphosate for four vegetation matrices (mg glyphosate/kg body weight/day). Calculations assume all vegetation contains the maximum observed concentration, animals only consume glyphosate treated vegetation, and animals consume the physiological maximum of food each day. The horizontal line is the Acceptable Daily Intake set by Health Canada (0.3 mg glyphosate/kg body weight/day). Note that *Y*-axis is log scale.

4. Discussion

Prior scientific and regulatory reviews have concluded that glyphosate-based herbicides, used according to label directions and under conditions typical of Canadian forestry do not pose a substantive risk to humans or wildlife [5,24,25]. The continued use of glyphosate-based herbicides in Canadian forestry depends, in part, on whether new research supports or contradicts prior conclusions. Results of the present study demonstrate that glyphosate can be detected in many vegetation matrices for at least one year after application. Detecting glyphosate is not surprising because it is applied by broadcast spray from a helicopter specifically to control unwanted plants and advantage commercially valuable species. Moreover, it is well known that such applications are imperfect, not all vegetation intercepts equivalent proportions of the depositing spray cloud, not all vegetation is equally susceptible to the herbicide, and some plants survive the exposure [26]. Previous studies established a generalized half-life of glyphosate in plant material of approximately 14 days [13,27]. In contrast to popular misperception, a half-life value does not indicate that 100% of the residues will be dissipated in two half-lives (i.e., at 28 days) but rather that residues will continue to decline by approximately 50% in each subsequent 14 day time window, thus yielding typical curvilinear decline curves which often characterize dissipation of labile chemicals, such as glyphosate in the environment.

Thus, it is not surprising that we observed relatively low-level residues in forest plant matrices over longer periods of time, consistent with several previously published studies [13,19,20,28]. However, as many other researchers have noted, the issue of interest is not the detection of residues at small concentrations, but whether such concentrations pose an unacceptable risk of toxicological effect to either humans or wildlife. Estimating the risk of negative effects to wildlife and humans requires characterizing the exposure (magnitude and time) and comparing the exposure to thresholds, such as MRL and ADI. When estimating risk, ADI is more appropriate because it is a risk-based estimate calculated from detailed toxicological investigations specifically designed to determine the NOAEC. In contrast, the MRL is based on observed residue distributions on various food commodities and is generally set well below concentrations expected to cause effects in humans.

Within application blocks, glyphosate concentrations were above the MRL (0.1 ppm) threshold for humans, and it is possible for animals to exceed the ADI for up to 18 days after application. The greatest risk to human health occurs if fruit is picked and consumed without being washed within 18 days of the herbicide being applied and that such consumption is repeated throughout an individual's life. Such a combination of factors is unlikely, but significant mitigation actions are required by law to minimize such potential human exposures. Mitigations include public notification of areas to be sprayed prior to the season, explicit mapping of proposed spray sites, site reconnaissance before and during spray applications to ensure humans are not unexpectedly in the area at the time of treatment, and signage of treated areas posted 7 days prior and retained after applications are made [9,29]. Such mitigations substantially reduce the probability of human exposure to glyphosate residues in berries or vegetation in treated spray blocks.

For wildlife, concerns are primarily related to species which forage in treated sites during the late summer early fall application window. Our study results suggest that ADI thresholds could be exceeded for species such as Moose, White-Tailed Deer, and Black Bear for approximately 3 weeks after application. The vegetation we collected and analyzed represents potential exposures in nature because we sampled new, live growth and White-Tailed Deer and Moose preferentially browse on leaves and new vegetation growth [30,31]. We emphasize that our ADI calculations assume wildlife consume the physiological maximum amount of vegetation every day for their entire lifespan only from herbicide treated forestry blocks and all vegetation has glyphosate residue at the maximum detected concentration. The scenario is possible, but highly unlikely to occur.

Glyphosate can penetrate the cuticle and exocarp of fruits and can take greater than 61 days to degrade within the fruit [28]. Berries were only present in the harvest blocks one week and one year after herbicide application in both the short- and long-term studies. Thus, our studies may have missed the highest potential concentrations in fruit. However, our studies are environmentally realistic because they occurred in operational harvest blocks and the timing of herbicide application and the presence of fruit are operationally and ecologically relevant and the concentrations detected one year after application are within the ranges reported in other studies [13,20].

The long-term study incorporated vegetation used for traditional medicine by Indigenous people. A similar trend of high concentration immediately after application and then a steady decline was observed in the vegetation tissues used in traditional medicines. After one year the mean concentration in tissues used in traditional medicine in one of the two blocks was above the MRL. The highest concentrations of glyphosate detected one year after application were in raspberry leaves and roots. Roots can function as storage tissues and presence in root demonstrates that glyphosate can be stored within plant tissues for at least one year after application. Whereas, leaves are tissues that were grown after herbicide application, and detection in this tissue provides evidence that glyphosate is stored and then transported to new rapidly growing tissues in the plant as previously reported [20,32,33]. For example, Wood [20] concluded that glyphosate is stored and then transported to new shoot and leaves of perennial plants. Both storage and transport are not surprising because not all vegetation is killed by glyphosate, and some plants can tolerate low levels of glyphosate within their tissues [26,27].

Vegetation used in Indigenous traditional medicine is often prepared to extract the medicine. To accurately determine the potential exposure and calculate an ADI, tests need to be performed with the prepared medicine and the route of exposure needs to be considered. Further, the presence of glyphosate within the vegetation has the potential to alter the medicinal properties of the vegetation, which could decrease the effectiveness of the medicine. In this regard, it would be irresponsible to offer conclusions or recommendations here as we did not test for residue concentrations in the plant matrices subsequent to preparations which may be typical of their use in traditional medicines. Future, more comprehensive work characterizing concentrations in roots and leaves and the potential risk in traditional medicines should be conducted in collaboration with knowledge holders.

In both the short- and long-term persistence studies glyphosate was detected outside of the herbicide application blocks. Detection outside of the application blocks is likely due to wind-assisted drift of the spray cloud. In the short-term study the downwind sites were relatively close to the herbicide application blocks and in the long-term persistence study the control block was separated from the spray blocks by a forest road (width ~30 m) and the helicopter flight lines ran parallel to the road. Deposition in the long-term persistence study was modelled using spray advisor [34], which predicted low deposition rates throughout the control block that were consistent with the residue values measured in the short-term study. Prior work has shown that herbicide drift from aerial application can occur, with amounts varying according to meteorological conditions, in particular wind speed [35].

5. Conclusions

Results of this study are consistent with prior work showing that low levels (trace concentrations) of glyphosate can persist for at least one year in terrestrial matrices, such as soils [13] and vegetation [20], following typical applications for vegetation control in forestry. To predict risk of effects to wildlife it is important to compare observed residues to toxicological thresholds such as ADI. Under a worst-case scenario, that makes several improbable assumptions, it would be possible for White-Tailed Deer, Moose, and Black Bear to consume enough vegetation to exceed the ADI for up to 18 days after application. However, after one month it is extremely unlikely that the animals considered herein could consume enough vegetation to exceed the ADI. We conclude that there is a relatively short period during which glyphosate residue concentrations are high enough for there to be any significant risk to the wildlife species considered herein. In the long term, no adverse effects from glyphosate consumption are expected in wildlife consuming vegetation from harvest blocks that receive a glyphosate-based herbicide treatment under scenarios typical of use for forest vegetation management in Canada. This conclusion derived from our case-specific field studies in eastern Canadian forests is congruent with results of many different regulatory agencies and independent scientific reviews internationally that reach the same general conclusion that typical uses of glyphosate-based herbicides do not pose a significant risk to wildlife species. Future work characterizing risk from the use of traditional medicines and the potential loss of medicinal properties should be conducted in collaboration with knowledge holders.

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