

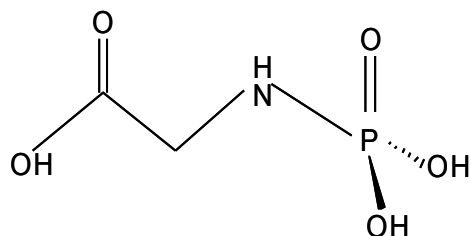
GLYPHOSATE LEVELS IN SOIL, WATER AND AIR BEFORE AND AFTER APPLICATION ON AGRICULTURAL FARMS

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Introduction

Glyphosate [(N-phosphonomethyl)glycine] is one of the most commonly used broad spectrum, nonselective, post emergent herbicide in agricultural, forestry, industrial and domestic areas. It is the active ingredient in the formulation of Roundup and is known to have less adverse effect on mammals than other herbicides such as atrazine¹⁻³. However, recent studies have reported that glyphosate binds to soil, persists in the environment for several months, and elicits negative impacts on the environment and biota²⁻⁶. The major degradation product of



glyphosate is amino methyl phosphonic acid (AMPA)³. Glyphosate has also been detected in runoff water after application⁴. In the recent Agricultural Health Study of Hoppin et al., glyphosate use was shown to be significantly associated with atopic asthma⁵. Similarly, glyphosate use was significantly associated with rhinitis symptoms among Iowa farmers⁶. In addition, glyphosate exposures have been associated with reproductive dysfunction in animals and neurologic developmental effects in humans⁷⁻⁹.

Fig.1. Glyphosate [(N-phosphonomethyl)glycine],
(CAS # 1071-83-6, Molar mass: 169.01g mol⁻¹, MP: 184.5°C)

Further, Kim et al. reported that glyphosate exposure caused skin and eye irritation, gastro-intestinal disorders, reduced blood pressure, swelling of the lungs, hemorrhages, red blood cell destruction, and diarrhea⁹. Long term exposure may also lead to kidney damage, hormonal disturbances and reproductive disorders⁹⁻¹¹. Although glyphosate is responsible for negative health effects, very few studies have documented their occurrence, distribution and persistence in the environment. The objective of the present study was to determine levels of glyphosate contamination in soil, water and air and to elucidate distribution and temporal variation of glyphosate after application on agricultural farms.

Materials and Methods

Surface soils (0-5 cm) were collected from the Murray State University (MSU) agricultural farms (Farm C and Farm D) before and after applications of glyphosate. Details of sampling dates and samples are given in Table 1. Core soils (0 - 25cm) were collected to discern the vertical distribution of glyphosate in the soil. Agricultural runoff water samples (after precipitation) were also collected to study the transport of glyphosate from farms to adjacent creeks. The soil samples were collected using pre-cleaned stainless steel scoops and transferred to pre-cleaned I-Chem bottles and transported to the laboratory on ice. Soil samples were air dried and 5g of soil sample was extracted using KH₂PO₄ buffer /1M NaOH in an automatic shaker followed by freeze drying. The freeze dried samples were dissolved with deionized water and filtered through 0.45 μM Millipore filter. Water samples were extracted using 1ml of KH₂PO₄ buffer in 100 ml of water sample and rotary-evaporated to dryness. Then 10 mL deionized water was added to dissolve the residues and filtered. Atmospheric evaluation was performed using air samples collected before and after application of glyphosate in farms in the Cincinnati area. Researchers at the University of Cincinnati designed and conducted the sampling events. Air samples were collected immediately before or after the glyphosate spray from two farms using three different sampling

methods: a) *NIOSH-developed two-stage cyclone sampler*. At an air flow rate of 3.5 L/min, the 50% cut-off diameters of the first and second stages (tubes) are 1.8 μm and 1.0 μm , respectively. Thus, the sampler collects aerosolized biocontaminants into three particle size ranges: <1.0, 1.0 – 1.8, and >1.8 μm . b) *The Button Inhalable Aerosol Sampler*. This sampler efficiently collects total inhalable particles of up to 100 μm . c) Preloaded 37 mm glass fiber filter cassette, which is a standard OSHA approved method for airborne glyphosate residue sampling. The air samples were extracted similar to soil samples. Soil, water and air sample extracts were analyzed for glyphosate using Abraxis ELISA Kit. Derivatization procedure for the analysis of glyphosate includes addition of 250 μl of the extracts, standards and control to the microtiter plates using micro pipet. One milliliter of the assay buffer was added and vortexed using Bronwill vortexer. One hundred microliter of the diluted derivatization reagent provided in the glyphosate ELISA Assay kit was added and vortexing was done immediately after addition to prevent swirling lines and then incubated at room temperature for about 10 minutes. Absorbance was measured at $\lambda 450\text{ nm}$ using Abraxis Model 6+ Spectrophotometer. Glyphosate quantitation was done using mean slope (response factor) of eight-point calibration ($r^2=0.98$). Some minor modifications were adopted in sample preparation steps for the water samples.

Results and Discussion

Tables 1 shows glyphosate concentration in surface soils from Murray State University farms (Farm C and Farm D) before and after application of glyphosate. Glyphosate concentrations in Farm C and Farm D before glyphosate application were 2.92 ng/g dry wt. and 6.26 ng/g dry wt. respectively. Soil samples were collected 1 day, 3 mos, 6 mos, and 7 mos after glyphosate application. (Table 1). The highest concentrations of 170 ng/g dry wt., and 208 ng/g dry wt. were found in soils from Farm C and Farm D respectively, 24-h after application. Glyphosate concentrations decreased steadily with time. Soil samples collected after seven months revealed glyphosate concentrations of 26.0 ng/g dry wt., and 36.0 ng/g dry wt. in Farm C and Farm D samples respectively. Presences of glyphosate in surface soils seven months after application indicate persistence of glyphosate in agricultural soils.

Table 1. Details of surface soils collected and glyphosate concentrations (ng/g dry wt.) in Murray State University agricultural farms (Farm C and Farm D). Date of glyphosate application: April 8, 2011.

Date of Sampling	Farm C	Farm D
	Glyphosate (ng/g dry wt.)	Glyphosate (ng/g dry wt.)
February, 6, 2011	2.92	6.26
April 9, 2011	170.0	208.0
July 7, 2011	43.4	43.3
October 11, 2011	35.3	48.1
November 1, 2011	26.0	36.0

Vertical distribution of glyphosate in agricultural soils was examined in the core soils and the data is presented below in Table 2..

Table 2. Glyphosate concentrations (ng/g) in core soil samples collected at Murray State University agricultural farms. (Farm C and Farm D)

Core soil Depth (cm)	Farm C [glyphosate ng/g]	Farm D [glyphosate ng/g]
5	3.50	4.53
10	3.14	4.90
15	4.63	6.11
20	6.18	6.36
25	6.82	5.61

Results revealed that glyphosate concentrations increased as depth increased (Table 2). This may be due to permeation of glyphosate from surface soil to deeper layers of soil. Since glyphosate is soluble in water, it can be surmised that water might aid in the permeation process. Although soil microbial degradation is possible, there may be limited photo-degradation of glyphosate due to absence of sunlight¹².

Agricultural runoff water samples were collected after one rainfall event to determine transportation of glyphosate from agricultural farm to adjacent creeks. Water samples from streams representing MSU Farm C and D, other farms and all farms (confluent location of all streams) were analyzed (Table 3). Glyphosate was not detected in any of the water samples collected on February 6th 2011 indicating that very little glyphosate was transported from agricultural farms during this period. However, detectable concentrations of glyphosate were found in farm runoff water samples collected on April 11th 2011 (Table 3). The results showed that glyphosate can be transported from agricultural farms to adjacent water bodies during rainfall events after glyphosate application. Further studies are needed to determine the mass loading of glyphosate to the adjacent creeks (and ultimately into the Clarks River) via agricultural runoff.

Table 3. Glyphosate concentrations ($\mu\text{g/L}$) in runoff of water samples (rainfall events on February 6th and April 11th 2011) collected at Murray State University agricultural farms. Glyphosate application date: April 8, 2011

Locations	Glyphosate ($\mu\text{g/L}$)	
	Water samples collected on February 6, 2011	Water samples collected on April 11, 2011
Creek from Farm C & D	BDL	2.21
Creek from other farms (bridge)	BDL	3.09
Confluence of all creeks (all farms)	BDL	0.91

Table 4 and Table 5 showed details of sampling location, dates and types of sampling devices used for collecting air samples from the Cincinnati farms. Air samples collected using glass fiber filters (GFF) and polycarbonate filters showed higher concentrations of glyphosate in the samples collected after the glyphosate application than the samples collected on the day before glyphosate application (Table 5). Airborne glyphosate residues were collected by multiple size-selective methods for respiratory deposition modeling of the data and also for calculating respiratory doses of inhalable glyphosate in an animal exposure experiment.

Table 4. Details of the air samples collected at University of Cincinnati (UC) agricultural farms

Farm Number	Date of collection before application	Date of collection after application
UC-2	May 12, 2011	May 13, 2011
UC-4	June 24, 2011	June 25, 2011

Table 5. Glyphosate concentration in air samples collected before and after glyphosate application

Farm ID	Sampler	Particle Size Fraction [µm]	Glyphosate [ng/m ³]	
			Before Application	After Application
UC-2	NIOSH Cyclone	>1.8	1.83	4.37
		1.0 - 1.8	0.19	0.30
		<1.0	Not detectable	0.47 [†]
	Button	Total inhalable	Not available	27.1
	Cassette	All sizes	Not available	2.1 [†]
UC-4	NIOSH Cyclone	>1.8	0.113	19.3
		1.0 - 1.8	0.065	17.7
		<1.0	0.089 [†]	146
	Button	Total inhalable	0.314	22.6
	Cassette	All sizes	Not detectable	138.0

[†]Below assay detection limit.

The present study provides evidence that glyphosate application in agricultural farms contribute to contamination of air, water and soil. Glyphosate persist in agricultural farm soils over seven months. Glyphosate concentrations in core soils revealed that glyphosate permeate into deeper layers of soils and may persist for even longer periods. Further studies are warranted to make clear the contamination levels, persistence in the environment, exposure levels and effects on wildlife and human health.

Acknowledgements

The authors are thankful to Mr. Brahmaiah Patibandla, Mrs. Reshmi Indugula, Dr. Michael Yermakov, Dr. Umesh Singh, Mr. Lev Lazinskiy, and Mrs. Sonam Gupta for their help during the sampling, processing samples in the laboratory, and analysis. This research was supported by the NIH/NIEHS Grant Number: R21ES017316.

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