

Low Cost Monitoring of Glyphosate in Surface Waters Using the ELISA Method: An Evaluation

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Concerns have been raised in the scientific community regarding the environmental implications of a dramatic increase in corn-based ethanol production and associated increases in pesticide use. The use of glyphosate, a broad-spectrum herbicide, for corn production has increased considerably in recent years in Canada and the United States. The cost of measuring concentrations of organic contaminants in the environment using traditional wet chemistry methods can be prohibitive; especially when large numbers of samples are required to quantify the spatial and temporal variability in contaminant concentrations. The goal of our study was to evaluate a cost-effective method to measure glyphosate concentrations in surface waters. The reliability of enzyme-linked immunosorbent assay (ELISA) results was evaluated against liquid chromatography-tandem mass spectrometry, and linear regression results for 30 water samples from urban watersheds revealed a strong relationship ($R^2 = 0.88$). These results suggest that ELISA methods, used in conjunction with traditional methods, represent a cost-effective approach to enhance the spatial and temporal resolution of a water quality monitoring study. Additionally, we measured a total of 739 surface water samples from over 150 sampling locations throughout Ontario using ELISA from April to October 2007. Concentrations exceeded the method detection limit of 0.1 $\mu\text{g/L}$ in 33% of the samples, with a maximum concentration of 12.0 $\mu\text{g/L}$. Glyphosate showed a bimodal temporal distribution with peak concentrations occurring in late spring/early summer and fall, and did not exceed the Canadian Council of Ministers of the Environment (CCME) guideline for the protection of aquatic life (65 $\mu\text{g/L}$) in any of the samples.

Introduction

Glyphosate [*N*-(phosphonomethyl)glycine], the main component in the commercial product RoundUp, was introduced in 1971 as a broad-spectrum, nonselective herbicide to effectively control weeds and vegetation (1–3). Glyphosate is used in urban landscapes on lawns, gardens, and along sidewalks. Since 1996, genetically engineered (“RoundUp

Ready”) products have been developed and widely applied to crops such as soybeans and corn causing glyphosate usage to increase drastically (4, 5). In 1998 and 2003, surveys of agricultural pesticide use in Ontario reported that 647 000 (6) and 1 171 000 kg (7) of glyphosate were applied, respectively. Total glyphosate usage is likely higher than these statistics suggest, as the estimates do not include other applications such as urban uses and forestry.

Glyphosate is fairly persistent in soil, having an average soil half-life of 47 days (1). It is extremely soluble in water (12,000 mg/L), but sorbs strongly to sediment with an adsorption coefficient of 24 000, reducing the amount lost due to runoff (<2%) during precipitation events (1, 8). Glyphosate may be carried into surface waters bound to sediment, and is moderately persistent with a reported water half-life of 49–70 days (1). Glyphosate has been reported to be nontoxic to fish and only slightly toxic to invertebrate species (2). However, a recent study suggests that glyphosate may pose a direct toxic threat to amphibian species (9–11), killing 96–100% of larval and 68–86% of juvenile amphibians when applied at a concentration of 3.8 mg of glyphosate per litre (9). In humans, studies have suggested an increase in incidences of neurologic developmental effects among children born to farmers who used glyphosate (12), and that RoundUp has been found to inhibit the production of steroid hormones in men possibly resulting in a loss of fertility (13). It has also been reported that glyphosate can be detected in the urine of farmers whom have applied it, (14) although a review by Williams et al. (15) implied that RoundUp does not pose a health risk to humans. However, these combined factors demonstrate that water quality monitoring for glyphosate is prudent especially when considering the current increasing trend in its agricultural use.

Laboratory analysis of water samples for glyphosate can be expensive (>\$300 per sample) using traditional wet chemistry methods. Analytical costs can impose major constraints of the design on monitoring studies that seek to quantify the spatial and temporal variability of glyphosate concentrations in the environment (5). ELISA has been reported as a cost-effective method (\$10 per sample) capable of detecting pesticides in water at concentrations below certain established water quality guidelines (16–19), and has been shown to compare quantitatively and qualitatively with wet chemistry analysis (17, 19, 20). However, ELISA techniques can suffer from false positives based on cross reactivity with other organic contaminants (18, 21–23). In this study, we analyzed more than 700 samples across Ontario. To address the issue of false positives, we evaluated the Abraxis Glyphosate ELISA test kit with traditional wet chemistry techniques using liquid chromatography tandem mass spectrometry (LC/MS/MS) on a subset of samples collected. The data collected were used to illustrate the occurrence of glyphosate in Ontario, establishing a high resolution of spatial and temporal information for surface water concentrations.

Experimental Section

Sample Collection. Freshwater surface grab samples were collected in 20 mL clear glass vials (Fisher Scientific; Mississauga, ON) a few centimeters below the water surface, filling the container approximately half-full and freezing it on its side between -16 and -22 °C. Adsorption to glass did not appear to be an issue. Humphries et al. (24) suggests no preference between collection in either glass or plastic for glyphosate, which is consistent with our own internal study. Samples were stored frozen in the dark in order to inhibit the degradation mechanism (25). A total of 533 samples from

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more than 100 sites in Ontario were collected monthly from May to October 2007 through the Ontario Ministry of the Environment's Provincial Water Quality Monitoring Network. An additional 206 samples from more than 50 sites across Ontario were collected periodically, approximately monthly, from April to October 2007 by Environment Canada (EC). Surface water samples included 210 and 529 from primarily urban and rural watersheds, respectively, marked using global positioning. Twenty percent of samples were analyzed in duplicate.

As a part of the 206 EC samples, 30 surface water samples from three urban creeks in southern Ontario were analyzed via LC/MS/MS and ELISA for comparative purposes. The comparative study samples were collected by grab sampling in 1 L Fisherbrand polypropylene bottles (Fisher Scientific Company; Edmonton, AB) supplied by Alys Analytical Services Ltd. (Sidney, BC, Canada). Subsamples (10 mL) were collected in 20 mL clear glass vials and stored as the other ELISA samples until analysis. The remainder of the samples was submitted to Alys for glyphosate analysis. All ELISA samples were transported to EC at the Canada Center for Inland Waters in Burlington, ON, where they remained frozen until analysis. The samples were stored frozen for less than 21 days after collection until analysis, and were filtered using nylon, nonsterile 0.2 μm syringe filters and Norm-jet 20 mL syringes (Fisher Scientific; Mississauga, ON) to remove suspended materials.

ELISA Method. Glyphosate ELISA test kits were purchased from Abraxis LLC (Warminster, PA). Vortexing was accomplished using a Vortex Genie 2 (VWR International; Edmonton, AB), magnetic separation consisted of a test tube holder fitted over a magnetic separator rack, and analysis was performed with a SDI RPA-II photometric analyzer (Osprey Scientific Inc.; Edmonton, AB). The analysis procedure was performed in accordance with the operating manual accompanying the glyphosate kit (26).

Alys Glyphosate Method. The Glyphosate Method MLA-054 was performed at Alys Analytical Services Ltd. (Sidney, BC, Canada). The method used for surface water samples analyzes for glyphosate, glufosinate and aminomethylphosphonic acid (AMPA). The surface water samples were filtered, derivatized with 9-fluorenylmethylchloroformate (FMOCl) in borate buffer, and extracted with HLB solid-phase extraction cartridges. The extracts were analyzed using liquid chromatography coupled to electrospray ionization tandem MS (LC-ESI-MS/MS). Chromatographic separation of the analytes was carried out using a Waters 2795 Alliance HPLC on a Sunfire C18 (3.5 μm , 4.6 mm \times 30 mm) analytical column. The guard column used was a C18 1 mm opti-guard column. Analysis was performed by using a benchtop Micromass Quattro Ultima tandem quadrupole mass spectrometer (MS). The MS was equipped with Z-spray electrospray (ESI) source and was operated in positive ion mode. The LC/MS/MS was operated running manufacturer's MassLynx version 4.0 software. The MS is run with unit mass resolution in the multiple reaction monitoring (MRM) mode (27). The MDL was reported to be 20 ng/L.

Quality Control Measures. *Calibration and Control Range.* In an attempt to quantify the effectiveness of ELISA for the analysis of surface water samples, it was necessary to determine the inherent day-to-day variability of the technique and instrument, and to establish acceptable operating limits. First, the three-point calibration curve and control was averaged over 30 determinations. The equation of the line $y = -0.8548x + 0.394$ had an $R^2 = 0.997$ with an average control concentration $0.57 \pm 0.22 \mu\text{g/L}$, compared to the manufacture's listed value of $0.50 \pm 0.13 \mu\text{g/L}$ (27). One calibration curve for glyphosate was below a correlation of 0.990, while the rest were greater than 0.990 with a majority greater than 0.995. The operating manual for the test kit

specifies a 20% standard deviation (SD) for the control, which was exceeded 58% of the time. As a result of the observed day-to-day variability, all results recorded on days where the control deviated by greater than 20% were adjusted to the control by multiplying the actual result by the observed factor (f).

$$f = 1 + [(\text{actual} - \text{theoretical}) / \text{theoretical}] \quad (1)$$

Instrument Variability. Instrument variability was monitored carefully as identical tube replicates were observed to have an average percent relative standard deviation of 13% over 200 samples ranging from 0 to 112%. The instrument also reported numbers below the manufacturers suggested method detection limit (MDL), although the numbers contained an associated nonnumeric (i.e., 0.025nd; where 0.025 is the concentration in $\mu\text{g/L}$, and nd = no detect).

ELISA Analysis. The MDL (0.1 $\mu\text{g/L}$) and the limit of quantification LOQ (0.15 $\mu\text{g/L}$) as defined by Abraxis was used in conjunction with a run detection limit (RDL) rather than simply using the guideline suggested by the manufacturer for reporting data; this adjustment was to account for the day-to-day variability and drift of ELISA response. The RDL was determined by inserting a MilliQ or type 1 laboratory water sample blank at the beginning and end of the sample set in each run to monitor for instrument variability and cross contamination/drift, respectively. The higher recorded MilliQ value was then multiplied by three, setting the resultant value as the RDL, which ranged from 0.01 to 0.30 $\mu\text{g/L}$ over the course of the study. Values obtained less than the RDL were reported as bl.

Results and Discussion

Evaluation of ELISA against LC/MS/MS. The comparison of LC/MS/MS and ELISA results for glyphosate at three urban creeks in the cities of Burlington and Toronto are shown in Table 1. The results for May 14–16 and July 16–19 represent the change in concentration due to a precipitation/runoff event; the sites were sampled at baseflow before a rain event, and then during a rain event for comparison. The data show that peak concentrations occur during periods of high surface runoff in response to large rain events, which is in agreement with the findings of Graziano et al. (20), who observed similar results for atrazine. Table 1 also shows that there is a potential for ELISA to yield false positives for glyphosate, as there was one instance where ELISA detected trace levels of glyphosate when LC/MS/MS had a no detect (Mimico Creek, May 14).

The linear regression of LC/MS/MS against ELISA for the analysis of glyphosate in surface water samples is illustrated in Figure 1, excluding Highland Creek on July 19 because the value is an order of magnitude greater than the next closest result. The R^2 value of 0.88 indicates a close relationship between LC/MS/MS and ELISA; however, the equation of the line ($y = 0.76x - 48.07$) shows that ELISA tends to overestimate the concentration of glyphosate present. The apparent overestimate and the issue of false positives might be explained by the presence of matrix interferences such as cross-reactive related analogues, organic matter, salts, and/or phosphates, to name a few (26). The potential for cross-reactivity is generally defined by the least detectable dose (LDD), the concentration at which a compound interferes with the determination of the target analyte. Abraxis lists the LDD for several compounds of interest (26). However, sufficient data were not collected from every location in our study in order to positively identify a specific cross-reactive analogue as the primary source for the apparent overestimation.

Although the LC/MS/MS method is more sensitive, the ELISA method detection limit is far below water quality guidelines for glyphosate. The Canadian Water Quality Guideline for the protection of freshwater aquatic life is 65 $\mu\text{g/L}$ (2) and the United States Environmental Protection

TABLE 1. Comparison of LC/MS/MS and ELISA for Glyphosate in Urban Creeks (ng/L)

| sampled | Highland Creek | | | Mimico Creek | | | Indian Creek | | |
|------------------------|----------------|-------|-------|--------------|------|--------|-----------------|-----------------|-----------------|
| | LC/MS/MS | | ELISA | LC/MS/MS | | ELISA | LC/MS/MS | | ELISA |
| | AMPA | Gly. | Gly. | AMPA | Gly. | Gly. | AMPA | Gly. | Gly. |
| 17-Apr-07 | nd | nd | bl | 61.7 | nd | bl | nd ^a | nd | nd |
| 14-May-07 | nd | nd | nd | 81 | nd | (74)tc | 42.6 | nd | nd |
| 16-May-07 | 115 | 42.7 | 123 | 167 | 42.2 | (47)tc | 219 | 132 | 367 |
| 16-Jul-07 | 59 | 124 | 231 | 591 | 350 | 581 | 351 | 316 | 371 |
| 19-Jul-07 | 972 | 11800 | 12000 | 324 | 862 | 1090 | 102 | 575 | 686 |
| 21-Aug-07 ^b | 85 | nd | bl | 1270 | 63 | 111 | 85.6 | 20.8 | bl |
| | | | | | | | 57.7 | nd | bl |
| | | | | | | | 65.3 | 27.9 | bl |
| | | | | | | | nd ^c | nd ^c | nd ^c |
| 17-Sep-07 | 73 | nd | nd | 2080 | 260 | 670 | 108 | 32.6 | bl |
| | | | | | | | nd ^c | nd ^c | nd ^c |
| | | | | | | | nd ^d | nd ^d | nd ^d |
| | | | | | | | nd ^e | nd ^e | nd ^e |

^a nd = value <20 ng/L for LC/MS/MS, and "no detect" for ELISA; bl = < 3 × MilliQ; tc = trace level, > 3 × MilliQ < MDL.

^b On Aug 21 samples were collected in triplicate from Indian Creek. ^c Represents field blank. ^d Represent travel blank. ^e Represent laboratory blank.

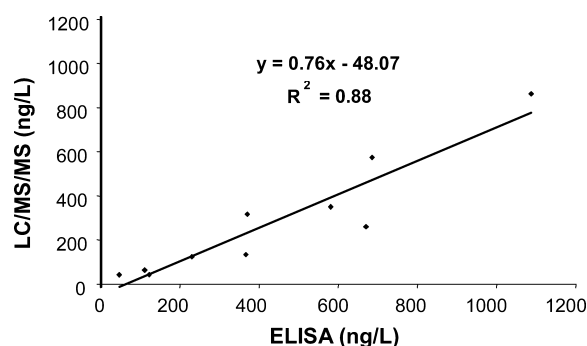


FIGURE 1. Correlation between LC/MS/MS and ELISA for the analysis of glyphosate in surface water samples. Highland Creek on July 19 was excluded from the figure because its concentration is an order of magnitude greater than the next highest concentration value.

Agency drinking water maximum concentration limit (MCL) is 700 µg/L (29) for glyphosate. On the basis of the statistical agreement between LC/MS/MS and ELISA for glyphosate, we monitored the surface water occurrence of glyphosate in Ontario from April to October 2007 using ELISA in order to gain a better understanding of glyphosate concentrations in the environment.

Current Environmental Levels. Grab sample timing was random for the most part; however, a few precipitation events were targeted. As a result, the data represent a wide range of surface water conditions ranging from high-flow to low-flow periods. Figure 2 shows the concentration distribution for samples with detected levels of glyphosate. Most of the data represented as outliers in the box plot correspond to precipitation events. The urban and rural samples had detection frequencies of 33% (70 of 210) and 32% (170 of 529), respectively. One hundred and fifty-eight samples were analyzed in duplicate (about 20%), of which 54 had quantifiable levels. The resulting average relative percent standard deviation was 33%, higher than the recommended 20% by the manufacturer (24), with a range from 0–119%. The majority of the samples with detectable concentrations (204 of 240) was less than 0.5 µg/L, with maximum recorded urban and rural values of 12.0 and 5.38 µg/L, respectively. These data are similar to other studies. Battaglin et al. (8) and Scribner et al. (29) recorded maximum glyphosate concentration in comparable U.S. streams of 8.7 and 9.7 µg/L, respectively, using traditional wet chemistry methods. Kolpin

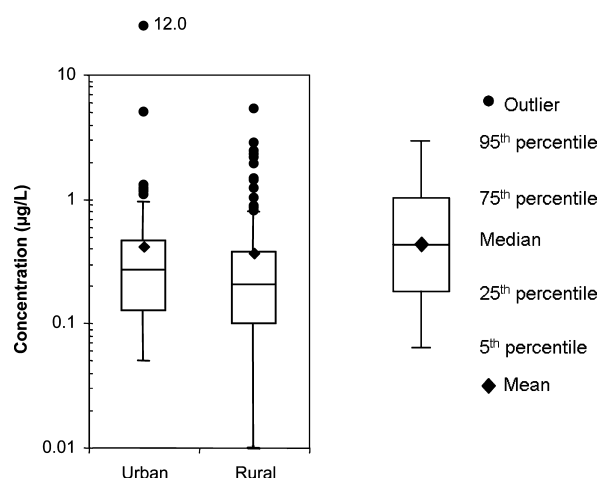


FIGURE 2. Glyphosate concentration in surface waters from various locations across Ontario from April to October 2007. The numbers of urban and rural samples represented are 70 and 170, respectively, representing only the detectable concentration levels.

et al. (4) also reported levels as high as 2.2 µg/L for glyphosate in urban streams upstream and downstream of ten water treatment plants in the U.S.

Spatial and Temporal Trends. Factors that can affect the spatial and temporal variability of surface water concentrations for glyphosate in Ontario include (1) the hydrology of a region; (2) latitude versus length of the growing season; (3) land use, i.e., residential, commercial, or agricultural; (4) population density; (5) the frequency of application; (6) the frequency and intensity of precipitation events; (7) application practices; and (8) soil type.

All of the surface water samples in this study were collected within the Great Lakes Basin, which is part of the Great Lakes–St. Lawrence River system. The precipitation patterns in southern Ontario are strongly influenced by the Great Lakes. Generally, this means that precipitation is greatest in southwestern, central and southeastern Ontario. However, in 2007, the summer months were unseasonably dry, particularly in July and August, which experienced below average precipitation (30). Environment Canada's monthly Ontario Weather Review (31) reports accumulated precipitation for several locations across southern Ontario to be <50 mm, which in some cases is more than 50% below normal.

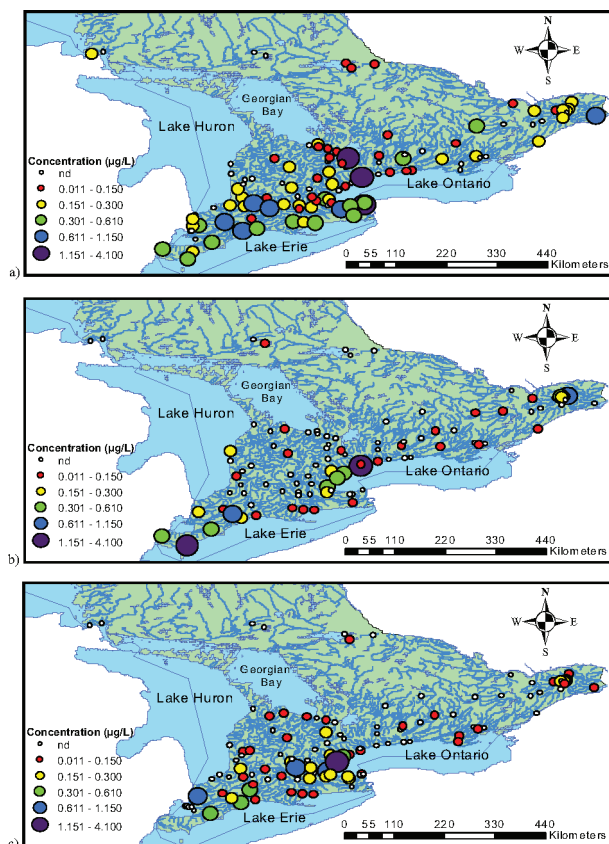


FIGURE 3. Average glyphosate surface water concentrations in the (a) spring, (b) summer, and (c) fall 2007.

Precipitation may play an important role in glyphosate's fate as is shown by instances where samples collected before and during a rain event at the same location, demonstrated a concentration increase of up to 10-fold. This may suggest that surface runoff is a major source pathway to glyphosate water concentrations; however, there may be other contributing factors including leaching (32), macropore transport, and/or atmospheric deposition (33–35).

The length of the growing season in southern Ontario is several weeks longer than in northern Ontario because of the difference in latitude. Also, soils on the Canadian Shield, a vast area of granite bedrock that comprises most of northern Ontario, are relatively thin compared to the nutrient-rich soils of southern Ontario. As a result, the majority of agricultural activities in Ontario occur in the southern regions, amounting to about 95% (9.35 of 9.83 million acres) of the total arable land, which is defined as the total land in crops, seeded pasture and summerfallow land (36). Glyphosate concentration in surface waters reflected this trend, showing higher levels in southern Ontario in relation to the northern region throughout our study (Figures 3). Spatial patterns show measurable amounts of glyphosate in surface waters throughout southern Ontario. Glyphosate is used for a variety of purposes including forestry, agriculture, and residential applications, and correspondingly, we monitored it in a number of different land-use zones during the course of this study. However, usage statistics for forestry and urban inputs are not currently available in Ontario. None of the values exceeded the Canadian Water Quality Guideline. Elevated glyphosate concentrations were recorded in both large urban centers and in well-established agricultural areas.

Glyphosate occurrence in surface water was found to be strongly dependent on application practices in commercial, agricultural, and residential land-use zones. For residential and commercial applications, glyphosate may be applied

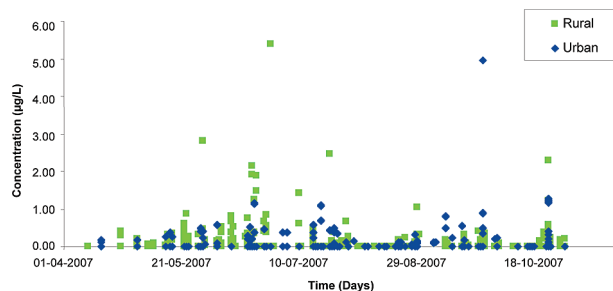


FIGURE 4. Glyphosate occurrence in surface water samples in Ontario from April to October 2007. A sample with concentration 12.0 µg/L collected on July 19 during a precipitation event in an urban creek is not shown.

multiple times throughout the growing season, whereas for agriculture, it is generally applied in the spring in preparation for planting, and in the late summer/fall during harvest for preparation of future crops. The temporal trend showed a bimodal distribution of glyphosate in Ontario exhibiting peak values in the spring and fall (Figure 4). This distribution is likely attributable to the two-season agricultural application practice. By contrast, large urban centers tended to have consistent measurable glyphosate detections throughout the growing season.

Increased Demand for Biofuels and its Implications on Glyphosate Usage. Although total agricultural pesticide use has declined in Ontario since the 1970s and 80s (6, 7), the recent increasing trend in the use of glyphosate demonstrates the need for continued monitoring. Glyphosate concentrations in surface waters should be routinely measured, specifically in the spring/early summer and during rain events, in order to effectively monitor for water quality guideline exceedances for the protection of freshwater aquatic life.

The need for ongoing glyphosate monitoring may be further enhanced considering recent developments in government regulations concerning biofuels and ethanol production. In Ontario, new regulations effective January 1, 2007, state that all commercial gasoline formulations must contain five percent ethanol-blend (37). This will similarly be mandated by the Canadian federal government by 2010. It is projected that in Canada, ethanol production will grow from 42 million liters in 2006 to approximately 799 million liters by 2010 (38). In the United States, the call for the production of 35 billion gallons of renewable fuel by 2017 (5) has led to an increase in ethanol production. In 2006 ethanol accounted for about 3.5% of the total U.S. fuel consumption and is expected to reach seven percent by the end of the decade. Currently corn grain, which makes up 98% of all U.S. ethanol production, provides the most immediate and economic way to mass-produce raw materials for ethanol production. Most of the increase in corn production has been accomplished by converting existing croplands from soybeans to corn. Within the Great Lakes states and provinces, corn acreage is projected to increase by 13.1%. Furthermore, to produce more corn, farmers are choosing to forego soybean crop rotation and instead plant corn in consecutive growing seasons. This practice increases the threat from pests, disease, and poor weather. To protect against large-scale crop failure in a monocultural crop system, farmers increasingly rely on pesticide use as well as genetically modified varieties of corn. These two aspects are often linked as in the case of RoundUp ready crops (37).

Implications for Water Monitoring. The ELISA methodology may allow us to conduct broad scale monitoring programs designed to measure whether herbicides such as glyphosate are increasing in surface waters as we strive to meet ethanol targets.

A suggested application for glyphosate monitoring would be a complementary approach between ELISA and traditional wet chemistry methods. All samples analyzed by ELISA include a complementary duplicate subset analyzed by LC/MS/MS to ground truth the values generated using ELISA. Additionally, the traditional wet chemistry analysis should also include AMPA measurements to determine if ELISA cross-reactivity is a factor. For example, if one were to collect 100 samples over the course of the growing season and analyze the samples for glyphosate, traditional analysis would cost about \$30 000, whereas analysis using ELISA would cost approximately \$1000. One approach would be to analyze samples only by LC/MS/MS, which show levels measured by ELISA. On the basis of our results from surface water samples in 2007, about 30% of the samples collected would have to be analyzed using wet chemistry methods. The resulting total cost of the complementary approach would be \$10 000, which equates to a cost savings of 67%. This approach would provide greater return on investment, as samples analyzed by wet chemistry would presumably have higher detected levels of glyphosate and samples with lower levels would have been prescreened by ELISA.

There are numerous potential applications for the ELISA technique because of factors such as little to no sample pretreatment and fast turn around time. This technique used in conjunction with traditional wet chemistry analysis could provide a cost-effective alternative for improving temporal and/or spatial monitoring. This would allow for greater flexibility to monitoring agencies which can develop broad scale projects to determine environmental impacts of the changing global energy demands in the 21st century.

Acknowledgments

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Literature Cited

- (1) *Extension Toxicology Network: Pesticide Information Profiles-Glyphosate*; Oregon State University: Corvallis, OR; <http://extoxnet.orst.edu/pips/glyphosa.htm>. (accessed on Nov 13, 2007).
- (2) Canadian Water Quality Guidelines for the Protection of Aquatic Life. In *Canadian Environmental Quality Guidelines*; Canadian Council of Ministers of the Environment: Winnipeg, Manitoba, 1999.
- (3) Ibanez, M.; Pozo, O. J.; Sancho, J. V.; Lopez, F. J.; Hernandez, F. Re-evaluation of glyphosate determination in water by liquid chromatography coupled to electrospray tandem mass spectrometry. *J. Chromatogr., A* **2006**, *1134* (1–2), 51–55.
- (4) Kolpin, D. W.; Thurman, E. M.; Lee, E. A.; Meyer, M. T.; Furlong, E. T.; Glassmeyer, S. T. Urban contributions of glyphosate and its degradate AMPA to streams in the United States. *Sci. Total Environ.* **2006**, *354* (2–3), 191–197.
- (5) Engelhaupt, E. Biofueling Water Problems. *Environ. Sci. Technol.* **2007**, *41*, 7593–7595.
- (6) Hunter, C.; McGee, B. *Survey of Pesticide Use in Ontario, 1998*; Ontario Ministry of Agriculture, Food and Rural Affairs, Policy Analysis Branch: Guelph, Ontario, 1999.
- (7) McGee, B.; Berges, H.; Callow, K. *Survey of Pesticide Use in Ontario, 2003*; Ontario Ministry of Agriculture, Food and Rural Affairs, Policy Analysis Branch: Guelph, Ontario, 2004.
- (8) Battaglin, W. A.; Koplin, D. W.; Scribner, E. A.; Kuivila, K. M.; Sandstrom, M. W. Glyphosate, other herbicides, and transformation products in Midwestern streams, 2002. *J. Am. Water Resour. Assoc.* **2005**, *41*, 323–332.
- (9) Relyea, R. A. The lethal impact of Roundup on aquatic and terrestrial amphibians. *Ecol. Appl.* **2005**, *15*, 1118–1124.
- (10) Relyea, R. A. The lethal impacts of Roundup and predatory stress on six species of North American tadpoles. *Arch. Environ. Contam. Toxicol.* **2005**, *48*, 351–357.
- (11) Relyea, R. A. The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities. *Ecol. Appl.* **2005**, *15*, 618–627.
- (12) Garry, V. F.; Harkins, M. E.; Erickson, L. L.; Long-Simpson, L. K.; Holland, S. E.; Burroughs, B. L. Birth defects, season of conception, and sex of children born to pesticide applicators living in the Red River valley of Minnesota, USA. *Environ. Health Perspect.* **2002**, *110* (3), 441–449.
- (13) Walsh, L. P.; McCormick, C.; Martin, C.; Stocco, D. M. Roundup inhibits steroidogenesis by disrupting steroidogenic acute regulatory (StAR) protein expression. *Environ. Health Perspect.* **2000**, *108* (8), 769–776.
- (14) Acquavella, J. F.; Alexanders, B. H.; Mandel, J. S.; Gustin, C.; Baker, B.; Chapman, P.; Bleeke, M. Glyphosate biomonitoring for farmers and their families: results from the farm family exposure study. *Environ. Health Perspect.* **2004**, *112* (3), 321–326.
- (15) Williams, G. M.; Kroes, R.; Munro, I. C. Safety evaluation and risk assessment of the herbicide Roundup and its active ingredient, glyphosate, for humans. *Regul. Toxicol. Pharmacol.* **2000**, *31*, 117–165.
- (16) Rubio, F. M.; Itak, J. A.; Scutellaro, A. M.; Selisker, M. Y.; Herzog, D. P. Performance characteristics of a novel magnetic-particle-based enzyme-linked immunosorbent assay for the quantitative analysis of atrazine and related triazines in water samples. *Food Agric. Immunol.* **1991**, *3*, 13–125.
- (17) Hall, J. C.; Van, Deynze, J.; Struger, J.; Chan, C. H. Enzyme immunoassay based survey of precipitation and surface water for the presence of atrazine, metolachlor and 2,4-D. *J. Environ. Sci. Health, Part B* **1993**, *28*, 577–597.
- (18) Lawruk, T. S.; Hottenstein, C. S.; Fleeker, J. R.; Hall, J. C.; Herzog, D. P.; Rubio, F. M. Quantification of 2,4-D and related chlorophenoxy herbicides by a magnetic particle-based ELSA. *Bull. Environ. Contam. Toxicol.* **1994**, *52*, 538–545.
- (19) Rubio, F.; Veldhuis, L. J.; Clegg, B. S.; Fleeker, J. R.; Hall, J. C. Comparison of a direct ELISA and HPLC method for glyphosate determinations in water. *J. Agric. Food Chem.* **2003**, *51*, 691–696.
- (20) Graziano, N.; McGuire, M. J.; Roberson, A.; Adams, C.; Jiang, H.; Blute, N. 2004 National Atrazine Occurrence Monitoring Program Using the Abraxis ELISA Method. *Environ. Sci. Technol.* **2006**, *40*, 1163–1171.
- (21) Fare, T. L.; Itak, J. A.; Lawruk, T. S.; Rubio, F. M.; Herzog, D. P. Cross-reactivity analysis using a four-parameter model applied to environmental immunoassays. *Bull. Environ. Contam. Toxicol.* **1996**, *57* (3), 367–374.
- (22) Johnson, B. D.; Hall, J. C. Fluroxpyr- and triclopyr-specific enzyme-linked immunosorbent assays: Development and quantitation in soil and water. *J. Agric. Food Chem.* **1996**, *44*, 488–496.
- (23) Parnell, J. S.; Hall, J. C. Development of an enzyme-linked immunosorbent assay for the detection of metosulam. *J. Agric. Food Chem.* **1998**, *46*, 152–156.
- (24) Humphries, D.; Byrtus, G.; Anderson, A. M. *Glyphosate Residues in Alberta's Atmospheric Deposition, Soils and Surface Waters*; Alberta Research Council, Alberta Environment: Edmonton, Alberta, 2005; <http://environment.gov.ab.ca/info/library/6444.pdf> (accessed Apr 15, 2008).
- (25) Mallat, E.; Barcelo, D. Analysis and degradation study of glyphosate and of aminomethylphosphonic acid in natural waters by means of polymeric and ion-exchange solid-phase extraction columns followed by ion chromatography–post-column derivatization with fluorescence detection. *J. Chromatogr., A* **1998**, *823*, 129–136.
- (26) *Glyphosate HS Assay Kit 120T, PN 500081, Operating Manual*; Abraxis LLC: Warminster, PA; <http://www.abraxiskits.com/moreinfo/PN500081USER.pdf>.
- (27) Pillay, K. *AXYS Method MLA-054*; Axys Analytical Services: Sidney, BC, 2007.
- (28) *Groundwater and Drinking Water Consumer Factsheet on: GLYPHOSATE*; U.S. Environmental Protection Agency: Washington, D.C.; <http://www.epa.gov/safewater/dwh/c-soc/glyphosa.html> (accessed on Feb 7, 2008).
- (29) Scribner, E. A.; Battaglin, W. A.; Gilliom, R. J.; Meyer, M. T. *Concentrations of Glyphosate, Its Degradation Product, Aminomethylphosphonic Acid, And Glufosinate in Ground- And Surface-Water, Rainfall, And Soil Samples Collected in the United States, 2001–06: U.S. Geological Survey Scientific Investigations Report 2007–5122*; USGS: Reston, VA, 2007; <http://pubs.usgs.gov/sir/2007/5122/>.

- (30) Agriculture and Agri-Food Canada's National Agroclimate Information Service (NAIS); <http://www.agr.gc.ca/pfra/drought> (accessed on Apr 14, 2008).
- (31) *Environment Canada Weather Statement: Ontario Weather Review*; Environment Canada: Gatineau, Quebec, 2007; <http://www.on.ec.gc.ca/press.cfm?Year=2007&Lang=e> (accessed on Apr 14, 2008).
- (32) Kjaer, J.; Olsen, P.; Ullum, M.; Grant, R. Leaching of glyphosate and amino- methylphosphonic acid from Danish agricultural field sites. *J. Environ. Qual.* **2005**, *34* (2), 608–620.
- (33) Goolsby, D. A.; Thurman, E. M.; Pomes, M. L.; Meyer, M. T.; Battaglin, W. A. Herbicides and their metabolites in rainfall: origin, transport, and deposition patterns across the Midwestern and northeastern United States, 1990–1991. *Environ. Sci. Technol.* **1997**, *31*, 1325–1333.
- (34) Kuang, Z.; McConnell, L. L.; Torrents, A.; Merritt, D.; Tobash, S. Atmospheric Deposition of Pesticides to an agricultural watershed of the Chesapeake Bay. *J. Environ. Qual.* **2003**, *32*, 1611–1622.
- (35) McConnell, L. L.; LeNoir, J. S.; Datta, S.; Seiber, J. N. Wet deposition of current-use pesticides in the Sierra Nevada mountain range, California, USA. *Environ. Toxicol. Chem.* **1998**, *17*, 1908–1916.
- (36) Statistics Canada. 2006 Census of Agriculture. Farm data and farm operator data tables: Land-use, tenure, and land management practices. <http://www.statcan.ca/english/free pub/95-629XIE/2007000/landuse.htm#landuse> (accessed on Apr 15, 2008).
- (37) Environmental Protection Act. Ontario Regulation 535/05: Ethanol in Gasoline. http://www.e-laws.gov.on.ca/html/regs/english/elaws_regs_050535_e.htm (accessed on Jan 30, 2008).
- (38) *The Potential Impacts of Increased Corn Production for Ethanol in the Great Lakes-St. Lawrence River Region*; Great Lakes Commission for the U.S. Army Corps of Engineers Great Lakes & Ohio River Division: Cincinnati, OH, 2007; <http://www.glc.org/tributary/pubs/documents/EthanolPaper121807FINAL.pdf>.

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