

GLYPHOSATE AND ITS DEGRADATION PRODUCT AMPA OCCUR FREQUENTLY AND WIDELY IN U.S. SOILS, SURFACE WATER, GROUNDWATER, AND PRECIPITATION¹

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ABSTRACT: Glyphosate use in the United States increased from less than 5,000 to more than 80,000 metric tons/yr between 1987 and 2007. Glyphosate is popular due to its ease of use on soybean, cotton, and corn crops that are genetically modified to tolerate it, utility in no-till farming practices, utility in urban areas, and the perception that it has low toxicity and little mobility in the environment. This compilation is the largest and most comprehensive assessment of the environmental occurrence of glyphosate and aminomethylphosphonic acid (AMPA) in the United States conducted to date, summarizing the results of 3,732 water and sediment and 1,018 quality assurance samples collected between 2001 and 2010 from 38 states. Results indicate that glyphosate and AMPA are usually detected together, mobile, and occur widely in the environment. Glyphosate was detected without AMPA in only 2.3% of samples, whereas AMPA was detected without glyphosate in 17.9% of samples. Glyphosate and AMPA were detected frequently in soils and sediment, ditches and drains, precipitation, rivers, and streams; and less frequently in lakes, ponds, and wetlands; soil water; and groundwater. Concentrations of glyphosate were below the levels of concern for humans or wildlife; however, pesticides are often detected in mixtures. Ecosystem effects of chronic low-level exposures to pesticide mixtures are uncertain. The environmental health risk of low-level detections of glyphosate, AMPA, and associated adjuvants and mixtures remain to be determined.

(KEY TERMS: glyphosate; AMPA; water quality; surface water; groundwater; precipitation.)

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INTRODUCTION

Problem

Commercial glyphosate [*N*-(phosphonomethyl)glycine] formulations have been used worldwide for decades, but glyphosate is seldom included in environmental monitoring programs (Gilliom *et al.*, 2006; Loos *et al.*, 2010; U.S. Department of Agriculture,

2011), due in part to difficulties in quantifying this polar and water-soluble compound at environmentally relevant concentrations (Skark *et al.*, 1998; Sanchis *et al.*, 2011). In the early 2000s, scientists at the U.S. Geological Survey (USGS) began developing analytical methods (Lee *et al.*, 2002) and conducting reconnaissance studies (Scribner *et al.*, 2003; Battaglin *et al.*, 2005) for the occurrence of glyphosate and aminomethylphosphonic acid (AMPA) in anticipation of growing gaps in scientific understanding due to (1)

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the widespread agricultural and nonagricultural use of glyphosate, (2) the rapid increase in glyphosate use starting in 1997 corresponding to the introduction of genetically modified glyphosate-resistant crops like soybeans and corn, and (3) the absence of information on the environmental occurrence of glyphosate and AMPA. These USGS scientists continued to develop new analytical methods and began a series of studies to determine the fate of glyphosate and AMPA in the environment. Researchers from Canada also have noted the need for methods to monitor glyphosate due to its increasing use (Byer *et al.*, 2008).

Study Objective

The objective of this investigation was to broadly summarize glyphosate and AMPA occurrence and concentration in water and sediment samples collected in 2001 through 2010 from diverse hydrologic settings and a wide geographic range of locations in the United States (U.S.). The data also are used to identify, in which hydrologic settings glyphosate and AMPA are more or less likely to occur and to a limited degree the temporal patterns of their occurrence or concentrations over the study period. The data used in this analysis were collected by a series of studies (Scribner *et al.*, 2003, 2007; Kolpin *et al.*, 2004, 2006; Battaglin *et al.*, 2005, 2009; Baker *et al.*, 2006; McCarthy *et al.*, 2011; Coupe *et al.*, 2012), most, but not all of which were designed to determine the fate of glyphosate and AMPA or other pesticides in the environment.

Glyphosate Use

Herbicides containing glyphosate are used in more than 130 countries on more than 100 crops (Monsanto, 2009). Glyphosate was first registered for use in the U.S. in 1974 in Roundup[®] and is the most heavily used pesticide for agriculture, and the second most heavily used pesticide for home and garden and commercial/industrial sectors in the U.S. Glyphosate use in U.S. agriculture has increased dramatically from ~3,180 metric tons of active ingredient in 1987 to ~82,800 metric tons in 2007 (Figure 1) (Kiely *et al.*, 2004; Grube *et al.*, 2011). Glyphosate accounted for about 40% of all herbicide use (by weight of active ingredient) in the U.S. in 2007. Similar increases in glyphosate use also have occurred in Canada (Struger *et al.*, 2008). The majority of this increase is the result of glyphosate use on soybean, cotton, canola, and corn crops that have been genetically modified to tolerate this glyphosate (e.g., Roundup[®] Ready crops) (Cerdeira and Duke, 2006; Young, 2006). About 80% of all genetically modified crops planted worldwide are designed to

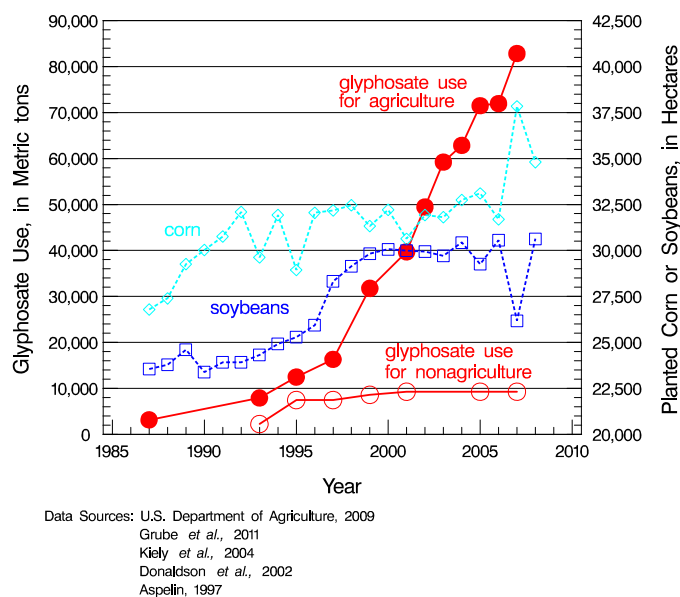


FIGURE 1. Use in U.S. of Glyphosate and Planted Hectares of Corn and Soybeans, 1987-2008.

tolerate glyphosate (Dill *et al.*, 2008), hence these crops comprise the “overwhelming majority” of herbicide-resistant crops (Benbrook, 2012). Glyphosate is typically (but not always) applied “post-emergence” or after crops and weeds have emerged from the soil, and may be applied more than once during a growing season. Glyphosate use also has increased due to increased use of “no-till” farming practices on crops that are not genetically modified to tolerate glyphosate (Horowitz *et al.*, 2010). Glyphosate loading rates (total use in a county divided by county land area) are largest in the corn and soybean producing region of the Midwest, along the Mississippi River alluvial floodplain, and in parts of California and Florida (Figure 2).

Glyphosate is popular with farmers for a number of reasons. Some studies indicate that the planting of glyphosate-tolerant crops in U.S. agriculture has saved farmers money and reduced the total pounds of herbicides applied (Gianessi and Sankula, 2003; Gianessi, 2008). Another reason for its popularity is the perception that glyphosate is an “environmentally benign” herbicide (Giesy *et al.*, 2000; Duke and Powles, 2008) that has low toxicity and little mobility or persistence in the environment. However, other studies indicate that glyphosate-resistant weeds can become a problem in areas where glyphosate-based crop production systems are used (Owens, 2008; Powles, 2008).

Glyphosate is also commonly used by homeowners and for other nonagricultural purposes. The nonagricultural use of glyphosate has increased from 2,270 metric tons in 1993 to 9,300 metric tons in 2007 (Figure 1) (Aspellin, 1997; Kiely *et al.*, 2004; Grube *et al.*, 2011). Urban glyphosate use can result in contamination of areas downstream from wastewa-

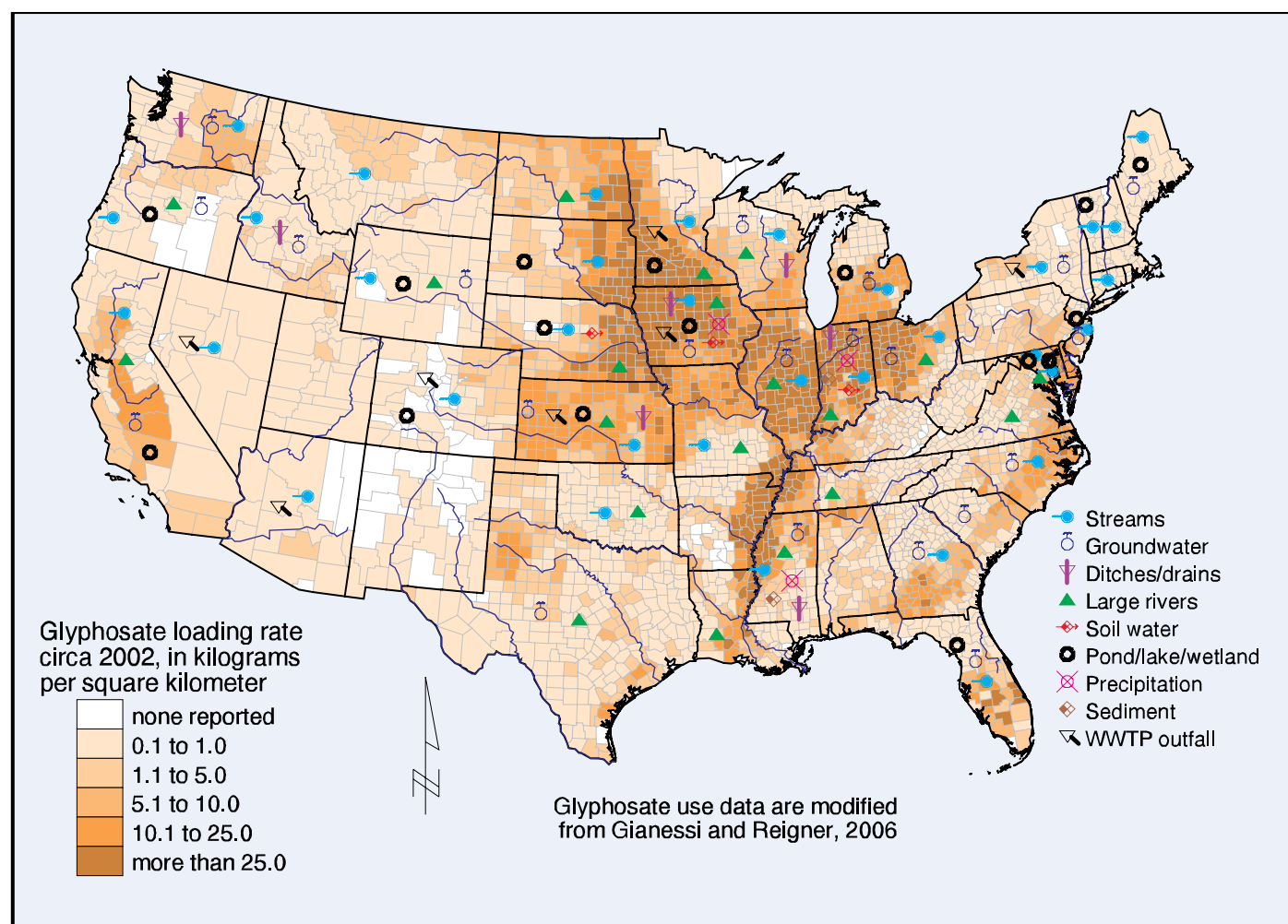


FIGURE 2. Glyphosate Loading Rate, Circa 2002 (the most recent year for which county-scale estimates of glyphosate sales are publicly available), and State in Which Various Hydrologic Settings Were Sampled and Analyzed for Glyphosate and AMPA (actual site locations are not shown). WWTP, wastewater treatment plant.

ter treatment plants (WWTPs) or storm sewers (Kolpin *et al.*, 2006; Botta *et al.*, 2009; Hanke *et al.*, 2010). Although pesticide application amounts in urban areas are generally less than in agricultural regions, those applications are frequently on or near impervious surfaces and can result in substantial pesticide inputs to urban drainage systems (Blanchoud *et al.*, 2007; Wittmer *et al.*, 2011).

Toxicity

Glyphosate is a nonselective contact herbicide that kills plants by inhibiting the synthesis of aromatic amino acids needed for protein formation (Franz *et al.*, 1997). Glyphosate is no more than slightly toxic to birds, fish, and aquatic invertebrates and exhibits low oral and dermal acute toxicity to humans (U.S. Environmental Protection Agency, 1993). Glyphosate showed little effect on soil microbial com-

munities (Haney *et al.*, 2000; Busse *et al.*, 2010) and limited effect on riverine microbial communities at exposures of about 10 $\mu\text{g/l}$ (Pesce *et al.*, 2009).

Recent studies, however, have documented the potential for sublethal and other toxic effects of glyphosate and its adjuvant formulations. A recent study suggests that glyphosate exposure can delay periphytic colonizations, reduce diatom abundance, and enhance the development of cyanobacteria in shallow lakes (Vera *et al.*, 2010). Some research suggests that glyphosate, at environmentally realistic concentrations, can act synergistically with parasites to reduce fish survival (Kelly *et al.*, 2010). Glyphosate also seems to cause malformations by interfering with retinoic acid signaling in *Xenopus laevis* (Paganelli *et al.*, 2010). At least one study has suggested that glyphosate-based herbicides are “info-disruptors” that can interfere with chemical communications between male and female spiders (Griesinger *et al.*, 2011). Other research suggests that glyphosate can

negatively impact microbial activity in the root zone of glyphosate-resistant soybeans (Zobiolo *et al.*, 2010) resulting in reduced plant growth and reduced resistance to pathogen colonization. Interestingly, glyphosate exposure appeared to reduce *Batrachochytrium dendrobatidis* (Bd) caused mortality in exposed wood frogs, presumably having a greater adverse effect on the pathogen than the host (Gahl *et al.*, 2011). Glyphosate is on the U.S. Environmental Protection Agency list of pesticide active ingredients that will be tested for potential hormonal effects under its Endocrine Disruptor Screening Program (U.S. Environmental Protection Agency, 2009).

AMPA acid is generally considered to be less toxic, or of no greater toxicological concern, than glyphosate (FAO, 1997; Giesy *et al.*, 2000), however, few studies have done direct comparisons of the toxicity of glyphosate and AMPA on non-target species. From the review by Giesy *et al.* (2000), AMPA was equally toxic as glyphosate to green algae (*Scenedesmus subspicatus*), equally toxic to birds (*Colinus virginianus*), equally toxic to terrestrial mammals (rat), slightly more toxic to aquatic invertebrates (*Daphnia magna*), and substantially less toxic to fish (*Oncorhynchus mykiss*). AMPA was relatively toxic to fungus (*Glomus intraradices*) but less so than glyphosate (Wan *et al.*, 1998). AMPA was found to have a clastogenic effect in human lymphocytes and otherwise demonstrated genotoxicity using the Comet assay (Mañas *et al.*, 2009). The tolerances established by the U.S. Environmental Protection Agency under 40 CFR Part 180 for commodities such as grains or livestock are “expressed in terms of glyphosate, including its metabolites and degradates” (U.S. Environmental Protection Agency, 1993, 2011).

Some studies indicate that commercial glyphosate formulations can be more toxic than pure glyphosate due to the toxicity and (or) action of the surfactants and other adjuvants used (Giesy *et al.*, 2000; Edginton *et al.*, 2004; Bringolf *et al.*, 2007; Mesnage *et al.*, 2012; Moore *et al.*, 2012). Surfactants such as polyethoxylated tallowamine (POEA) are added to some commercial glyphosate formulations to enhance its efficacy. The Roundup® formulation was more toxic than glyphosate or AMPA for all taxa tested (Giesy *et al.*, 2000). Some formulations of POEA were toxic to *Daphnia magna*, inhibiting growth and causing mortality at concentrations less than 100 µg/l (Brausch *et al.*, 2007). Effects on the development and survival of amphibians have been observed at various levels of glyphosate and POEA exposure (Lajmanovich *et al.*, 2003; Edginton *et al.*, 2004; Howe *et al.*, 2004; Cauble and Wagner, 2005; Relyea, 2005a, b, 2012; Dinehart *et al.*, 2009; Mann *et al.*, 2009; Jones *et al.*, 2010; King and Wagner, 2010; Lenkowski *et al.*, 2010; Williams and Semlitsch, 2010; Moore *et al.*, 2012).

Glyphosate and several glyphosate formulations have a cytotoxic effect on human cells, and endocrine disruption, specifically inhibition of estrogen synthesis, has been demonstrated (Richard *et al.*, 2005; Benachour *et al.*, 2007; Benachour and Seralini, 2009; Mesnage *et al.*, 2012). Glyphosate formulations also may cause birth defects or adverse reproductive effects in vertebrates or contribute to a variety of human diseases (Daruich *et al.*, 2001; Dallegrave *et al.*, 2003, 2007; Paganelli *et al.*, 2010; Samsel and Seneff, 2013).

Environmental Fate

Glyphosate is a polar, amphoteric compound that binds strongly to soils, but also is very water soluble (more than 10,000 mg/l at 25°C). Glyphosate has a soil half-life that ranges from 2 to 215 days, and an aquatic half-life that ranges from 2 to 91 days (Giesy *et al.*, 2000; Grunewald *et al.*, 2001; National Pesticide Information Center, 2008; Vera *et al.*, 2010). Glyphosate degrades in the environment, primarily by microbial processes, to AMPA. AMPA also is very water soluble, and it degrades more slowly than glyphosate (Grunewald *et al.*, 2001). AMPA has a soil half-life that ranges from 60 to 240 days and an aquatic half-life that is comparable to that of glyphosate (Giesy *et al.*, 2000; Bergstrom *et al.*, 2011). AMPA ultimately degrades to inorganic phosphate, ammonium, and CO₂ (Borggaard and Gimsing, 2008), a process that can result in substantial increases in total phosphorus in aquatic systems (Vera *et al.*, 2010). Glyphosate also can be degraded by bacteria to sarcosine but this process has not been well documented in soils (Borggaard and Gimsing, 2008). AMPA also can be formed by the degradation of phosphonic acids found in some household and industrial detergents and cleaning products (Skark *et al.*, 1998; Nowack, 2003) making outfall from WWTPs and septic tanks a potential source of AMPA in some areas (Kolpin *et al.*, 2006; Botta *et al.*, 2009). However, phosphonic acids are strongly adsorbed to sediments and suspended particles, and recalcitrant to biological or non-biological degradation (HERA, 2004).

METHODS

Study Sites and Sample Collection

A total of 3,732 environmental samples collected from 38 states and the District of Columbia were included in this analysis. The hydrologic settings sampled include groundwater, streams (having drain-

TABLE 1. Number of Samples, Percentage Detections, and Median and Maximum Glyphosate and AMPA Concentrations by Hydrologic Setting.

Hydrologic Setting	Number of Samples	Percentage and (number) with Glyphosate Detections	Median Glyphosate in $\mu\text{g/l}$ or $\mu\text{g/kg}$	Maximum Glyphosate in $\mu\text{g/l}$ or $\mu\text{g/kg}$	Percentage and (number) with AMPA Detections	Median AMPA in $\mu\text{g/l}$ or $\mu\text{g/kg}$	Maximum AMPA in $\mu\text{g/l}$ or $\mu\text{g/kg}$
All sites	3,732	39.4 (1,470)	<0.02	476	55.0 (2,052)	0.04	397
Streams	1,508	52.5 (791)	0.03	73	71.6 (1,079)	0.20	28
Groundwater	1,171	5.8 (68)	<0.02	2.03	14.3 (168)	<0.02	4.88
Ditches and drains	374	70.9 (265)	0.20	427	80.7 (302)	0.43	397
Large rivers	318	53.1 (169)	0.03	3.08	89.3 (284)	0.22	4.43
Soil water	116	34.5 (40)	<0.02	1.00	65.5 (76)	0.06	1.91
Lakes, ponds, and wetlands	104	33.7 (35)	<0.02	301	29.8 (31)	<0.02	41
Precipitation	85	70.6 (60)	0.11	2.50	71.8 (61)	0.04	0.48
Soil and sediment	45	91.1 (41)	9.6	476	93.3 (42)	18.0	341
WWTP outfall	11	9.09 (1)	<0.02	0.30	81.8 (9)	0.45	2.54

age areas less than 10,000 km²), rivers (having drainage areas greater than 10,000 km²), ditches and drains (both tile and surface), lakes, ponds, and wetlands, precipitation, WWTP outfalls, soil water, and sediment (Figure 2, Table 1). The most samples were collected from streams (1,508) followed by groundwater (1,171); ditches and drains (374); rivers (318); soil water (116); lakes, ponds, and wetlands (104); precipitation (85); sediment (45); and WWTP outfalls (11).

Analytical Methods

In 2000-2002, the USGS developed an analytical method (Lee *et al.*, 2002) that used online solid-phase extraction (SPE), and liquid chromatography/mass spectrometry (LC/MS) for determination of concentrations of glyphosate and AMPA in water samples with a reporting level of 0.1 $\mu\text{g/l}$ for both compounds. The method was modified, beginning in April 2004, to use isotope dilution and online SPE and liquid chromatography/tandem mass spectrometry (LC/MS/MS), which improved sensitivity and lowered the reporting level to 0.02 $\mu\text{g/l}$, for both compounds (Meyer *et al.*, 2009). The lower analytical reporting level made it possible for environmental researchers to gain a better understanding of the fate and transport of glyphosate and AMPA. In a few samples (seven for glyphosate and five for AMPA) concentrations less than the 0.02 $\mu\text{g/l}$ reporting level were measured and reported.

Statistical Methods

When glyphosate or AMPA concentrations were less than the reporting level, those concentrations were set to zero for the purposes of calculating detection frequencies, the total glyphosate concentration,

or other statistics; and to the reporting level for plotting. The total glyphosate concentration was calculated as the sum of glyphosate and AMPA concentrations. The relative percent difference between two concentration values (e.g., laboratory duplicates) was calculated as the absolute value of the difference between the two concentrations divided by the maximum of the two concentrations, that quantity multiplied by 100. Estimates of the instantaneous total glyphosate daily flux were calculated for samples at selected sites. Instantaneous daily fluxes in grams per day (or in some cases kilograms per day) for the date of sample collection were estimated as the product of the total glyphosate concentration (micrograms per liter), daily mean discharge (cubic feet per second), and 2.4463 (a units conversion). Instantaneous daily total glyphosate fluxes were estimated as zero on days when both glyphosate and AMPA were not detected in a sample. The Wilcoxon signed-rank test was used to determine if differences between groups of data are statistically significant (Helsel and Hirsch, 2002). The percentage AMPA (%AMPA) was calculated as shown below (Equation 1) where [AMPA] and [glyphosate] are their respective concentrations in water. %AMPA was set to zero (0.01 $\mu\text{g/l}$ for plotting purposes) when glyphosate was detected and AMPA was not, and not calculated when both glyphosate and AMPA were not detected. This ratio gives some insight into sources, fate, and transport of glyphosate and AMPA in the environment.

$$\% \text{AMPA} = ([\text{AMPA}] / ([\text{glyphosate}] + [\text{AMPA}])) \times 100 \quad (1)$$

Quality Assurance Samples

A total of 1,018 quality assurance (QA) samples were collected and analyzed in conjunction with the

3,732 environmental (ENV) samples described here. QA samples consisted of 514 laboratory duplicates (or ~14% of ENV samples although some were duplicates of other QA samples), 288 field replicates (~7.7% of ENV samples), 188 field blanks (~5.0% of ENV samples), and 28 field spikes. Glyphosate was not detected in any of the 188 field blanks. AMPA was detected in 2 of 188 field blanks, both surface water sites, at concentrations of 0.02 and 0.04 $\mu\text{g/l}$ (both of these samples had a reporting level of 0.02 $\mu\text{g/l}$).

In 514 laboratory duplicate sample pairs, the presence or absence of glyphosate and AMPA was confirmed in 96% of the sample pairs. Glyphosate was detected in both samples in 198 sample pairs and in one of the two samples in 18 sample pairs. The relative percent differences in these 216 sample pairs ranged from 0 to 100, and median and mean percent differences were 10.0 and 20.1, respectively. The absolute difference in measured concentrations between environmental samples and laboratory duplicates ranged from 0 to 58 $\mu\text{g/l}$, and median and mean differences were 0.03 and 0.38 $\mu\text{g/l}$, respectively. AMPA was detected in both samples in 273 sample pairs and in one of the two samples in 19 sample pairs. The relative percent differences in these 292 sample pairs ranged from 0 to 100, and median and mean percent differences were 9.9 and 19.2, respectively. The absolute difference in measured detections ranged from 0 to 55 $\mu\text{g/l}$, and median and mean absolute difference were 0.03 and 0.29 $\mu\text{g/l}$, respectively.

In 288 field replicate sample pairs, the presence or absence of glyphosate was confirmed in 98% of sample pairs, whereas the presence or absence of AMPA was confirmed in 97% of sample pairs. Glyphosate was detected in both samples in 70 sample pairs and in one of the two samples in 6 sample pairs. The relative percent differences in these 76 sample pairs ranged from 0 to 100, and median and mean percent differences were 17.0 and 25.2, respectively. The absolute difference in measured detections between environmental samples and laboratory duplicates ranged from 0 to 27 $\mu\text{g/l}$, and median and mean absolute differences were 0.04 and 0.79 $\mu\text{g/l}$, respectively. AMPA was detected in both samples in 113 sample pairs and in one of the two samples in 9 sample pairs. The relative percent differences in these 122 sample pairs ranged from 0 to 100, and median and mean percent differences were 14.6 and 23.1, respectively. The absolute difference in measured detections ranged from 0 to 26 $\mu\text{g/l}$, and median and mean absolute differences were 0.03 and 0.37 $\mu\text{g/l}$, respectively. For both laboratory duplicates and field replicates, differences larger than 1 $\mu\text{g/l}$ were rare and were typically observed in samples with high (greater than 5 $\mu\text{g/l}$) concentrations of glyphosate or AMPA. Results

from 28 field spike samples were not analyzed for this report.

RESULTS

A total of 3,732 water or sediment samples were collected from 1,341 sites in 38 states and the District of Columbia. Glyphosate was detected at least once in samples from 289 sites, whereas AMPA was detected at least once at 384 sites. Glyphosate was detected in 1,470 of 3,732 or 39.4% of all environmental samples, and AMPA was detected in 2,052 of 3,732 or 55.0% of all environmental samples (Table 1). The median and maximum glyphosate concentrations in all samples were <0.02 and 476 $\mu\text{g/l}$, respectively. The median and maximum AMPA concentrations in all samples were 0.04 and 397 $\mu\text{g/l}$, respectively. Glyphosate was detected in more than 50% of samples of sediment, ditches and drains, precipitation, large rivers, and streams and in less than 40% of samples of lakes, ponds, and wetlands; soil water; WWTP outfalls; and groundwater (Table 1, Figure 3). AMPA was detected in more than 50% of samples of soil and sediment, large rivers, WWTP outfalls, ditches and drains, precipitation, streams, and soil water; and in less than 30% of samples of lakes, ponds, and wetlands; and groundwater (Table 1, Figure 3). It was uncommon for glyphosate to be detected without AMPA, happening in only 2.3% of all samples. AMPA was detected without glyphosate in 17.9% of all samples. Both

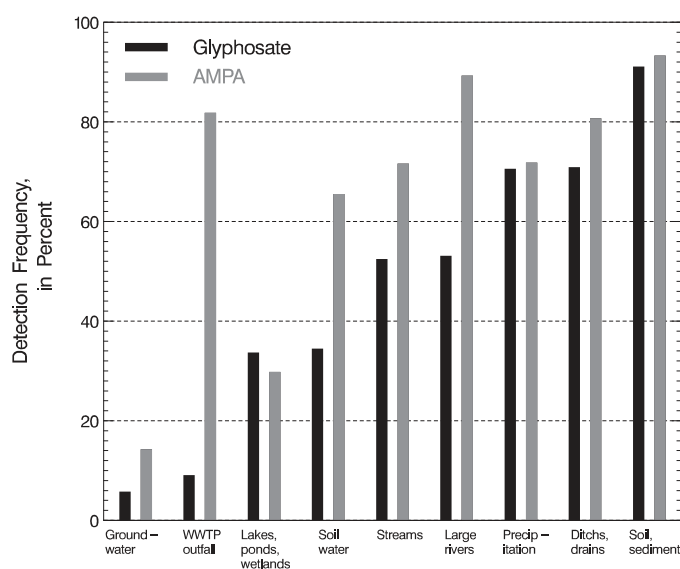


FIGURE 3. Detection Frequencies for Glyphosate and AMPA by Hydrologic Setting.

glyphosate and AMPA were detected in 37.1% of all samples, and neither glyphosate nor AMPA was detected in 42.7% of all samples.

Soil and Sediment, and Soil Water

A total of 45 soil and sediment samples were collected from seven sites in Indiana and Mississippi (Figure 2). Glyphosate and AMPA were detected at least once in samples from all seven sites. Both glyphosate and AMPA were detected in more than 90% of sediment samples with concentrations frequently exceeding 10 $\mu\text{g/kg}$ (Figures 3 and 4). The median and maximum glyphosate concentrations in these samples were 9.6 and 476 $\mu\text{g/kg}$, respectively, whereas the median and maximum AMPA concentrations were 18 and 341 $\mu\text{g/kg}$, respectively. The median %AMPA ratio (in 42 samples) was 65% with an interquartile range of 55-78% (Figure 5).

A total of 116 soil water samples were collected from 13 sites in Indiana, Iowa, and Nebraska. Glyphosate was detected at least once in samples from nine sites, whereas AMPA was detected at least once at 12 sites. Glyphosate was detected in 34.5% and AMPA in 66.5% of soil water samples (Figures 3 and 4). The median and maximum glyphosate concentrations in these samples were <0.02 and 1.0 $\mu\text{g/l}$, respectively, whereas the median and maximum AMPA concentrations were 0.06 and 1.91 $\mu\text{g/l}$, respectively (Table 1). The median %AMPA ratio (in 79 samples) was 89% with an interquartile range of 76-100% (Figure 5).

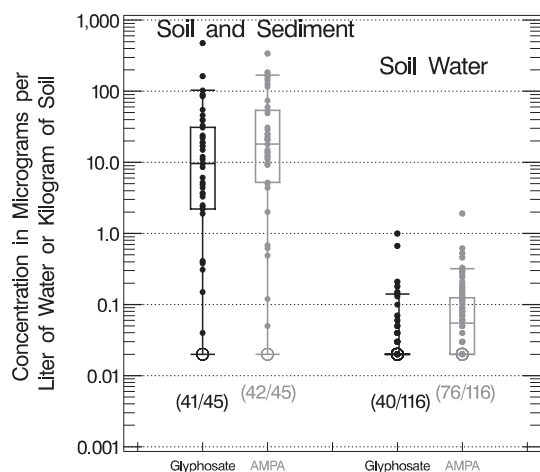


FIGURE 4. Boxplot-Dot Plots Showing Concentrations of Glyphosate and AMPA for Soil and Sediment and Soil Water Samples (numbers in parentheses are number of detections/number of samples, open circle is reporting level).

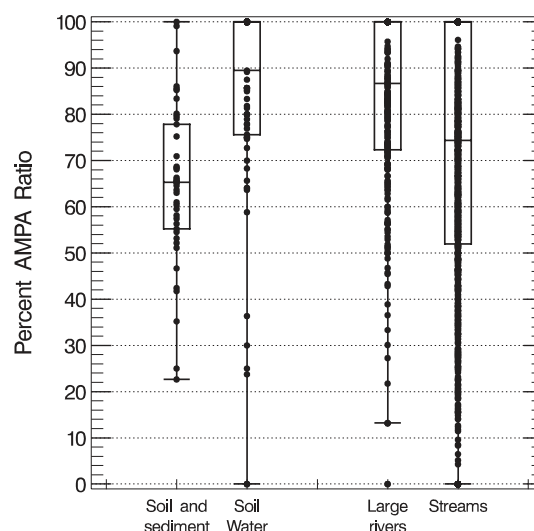


FIGURE 5. Boxplot-Dot Plots Showing the Percent AMPA Ratio for Soil and Sediment, Soil Water, Large River (drainage basin area 10,000 km^2 or greater), and Stream Samples.

Large Rivers and Streams

A total of 318 large river (drainage basin area 10,000 km^2 or greater at the sampling site) samples were collected from 47 sites in California, Iowa, Illinois, Indiana, Kansas, Louisiana, Maryland, Minnesota, Missouri, Mississippi, North Dakota, Nebraska, Ohio, Oklahoma, Oregon, Tennessee, Texas, Virginia, Wisconsin, and Wyoming. Glyphosate was detected at least once in samples from 32 sites, whereas AMPA was detected at least once at 42 sites. Glyphosate was detected in 53.1% and AMPA in 89.3% of large river samples (Figures 3 and 6). The median and maximum glyphosate concentrations in these samples were 0.03 and 3.08 $\mu\text{g/l}$ respectively, whereas the median and maximum AMPA concentrations were 0.22 and 4.43 $\mu\text{g/l}$, respectively (Table 1). The median %AMPA ratio (in 285 samples) was 87% with an interquartile range of 72-100% (Figure 5).

A total of 1,508 stream (drainage basin area less than 10,000 km^2 at the sampling site) samples were collected from 358 sites in Arizona, California, Colorado, Connecticut, District of Columbia, Florida, Georgia, Iowa, Idaho, Illinois, Indiana, Kansas, Maryland, Maine, Michigan, Minnesota, Missouri, Mississippi, Montana, North Dakota, Nebraska, New Hampshire, New Jersey, New York, Nevada, Ohio, Oklahoma, Oregon, South Dakota, Virginia, Vermont, Washington, Wisconsin, and Wyoming. Glyphosate was detected at least once in samples from 155 sites, whereas AMPA was detected at least once at 210 sites. Glyphosate was detected in 52.5% and AMPA in 71.6% of stream samples (Figures 3 and 6). The median and maximum glyphosate concentrations in

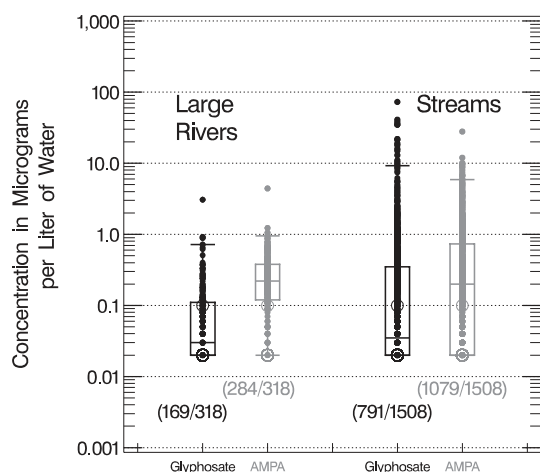


FIGURE 6. Boxplot-Dot Plots Showing Concentrations of Glyphosate and AMPA for Large River (drainage basin area 10,000 km² or greater) and Stream Water Samples (numbers in parentheses are number of detections/number of samples, open circle is reporting level).

these samples were 0.03 and 73 µg/l, respectively, whereas the median and maximum AMPA concentrations were 0.20 and 28 µg/l, respectively (Table 1). The median %AMPA ratio (in 1,116 samples) was 74% with an interquartile range of 52-100% (Figure 5).

Ditches and Drains; and Lakes, Ponds, and Wetlands

A total of 374 ditch, tile, or surface drain samples were collected from 32 sites in Iowa, Idaho, Indiana, Kansas, Mississippi, Washington, and Wisconsin. Glyphosate was detected at least once in samples from 23 sites, whereas AMPA was detected at least once at 24 sites. Glyphosate was detected in 70.9%, and AMPA in 80.7% of ditch or drain samples (Figures 3 and 7). The median and maximum glyphosate concentrations in these samples were 0.20 and 427 µg/l respectively, whereas the median and maximum AMPA concentrations were 0.43 and 397 µg/l, respectively (Table 1). The median %AMPA ratio (in 316 samples) was 63% with an interquartile range of 29-85% (Figure 8).

A total of 104 lake, pond, or wetland samples were collected from 65 sites in California, Colorado, District of Columbia, Florida, Iowa, Kansas, Maryland, Maine, Michigan, Minnesota, Nebraska, New Jersey, Oregon, South Dakota, Vermont, and Wyoming. Glyphosate and AMPA were detected at least once in samples from 27 sites. Glyphosate was detected in 33.7% and AMPA in 29.8% of lake, pond, or wetland samples (Figures 3 and 7). The median and maximum glyphosate concentrations in these samples

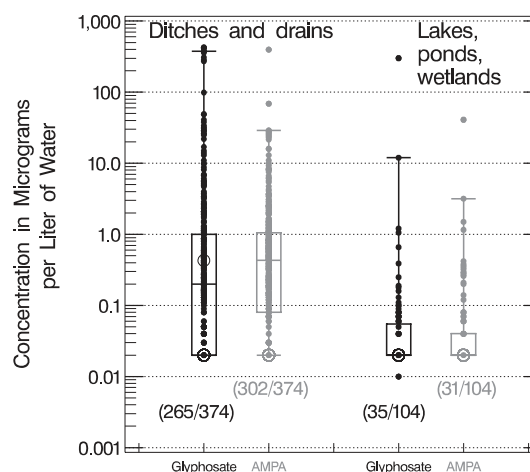


FIGURE 7. Boxplot-Dot Plots Showing Concentrations of Glyphosate and AMPA for Ditch and Drain Samples and for Lake, Pond, and Wetland Samples (numbers in parentheses are number of detections/number of samples, open circle is reporting level).

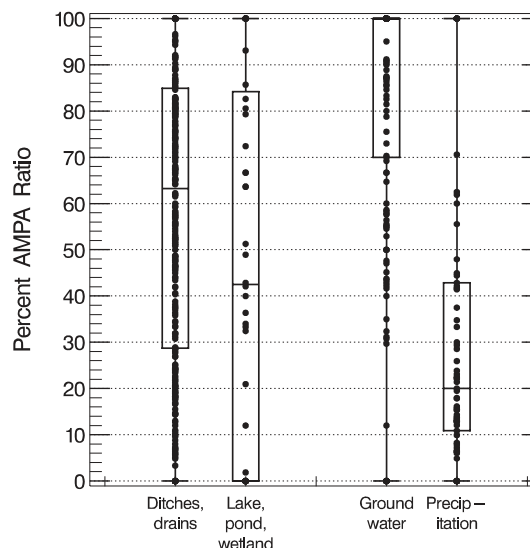


FIGURE 8. Boxplot-Dot Plots Showing the Percent AMPA Ratio for Ditches and Drains; Lake, Pond, and Wetland; Groundwater; and Precipitation Samples.

were <0.02 and 301 µg/l, respectively, whereas the median and maximum AMPA concentrations were <0.02 and 41 µg/l, respectively. The median %AMPA ratio (in 44 samples) was 42% with an interquartile range of 0-84% (Figure 8).

Groundwater and Precipitation

A total of 1,171 groundwater samples were collected from 807 sites in California, Delaware, Florida, Georgia, Iowa, Idaho, Illinois, Indiana, Kansas, Mary-

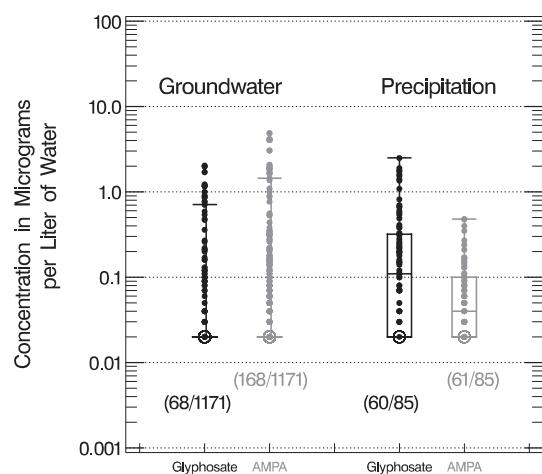


FIGURE 9. Boxplot-Dot Plots Showing Concentrations of Glyphosate and AMPA for Groundwater and Precipitation Samples (numbers in parentheses are number of detections/number of samples, open circle is reporting level).

land, Maine, Michigan, Mississippi, North Carolina, New Jersey, New York, Ohio, Oregon, South Carolina, Texas, Washington, Wisconsin, and Wyoming. Glyphosate was detected at least once in samples from 32 sites, whereas AMPA was detected at least once at 57 sites. Glyphosate was detected in 5.8% and AMPA in 14.3% of groundwater samples (Figures 3 and 9). The median and maximum glyphosate concentrations in these samples were <0.02 and $2.03 \mu\text{g/l}$, respectively, whereas the median and maximum AMPA concentrations were <0.02 and $4.88 \mu\text{g/l}$, respectively (Table 1). The median %AMPA ratio (in 179 samples) was 100% with an interquartile range of 70-100% (Figure 8).

A total of 85 precipitation samples were collected from three sites in Iowa, Indiana, and Mississippi. Glyphosate and AMPA were detected at least once in samples from all three sites. Glyphosate was detected in 70.6% and AMPA in 71.8% of precipitation samples (Figures 3 and 9). The median and maximum glyphosate concentrations in precipitation samples were 0.11 and $2.50 \mu\text{g/l}$, respectively, whereas the median and maximum AMPA concentrations were 0.04 and $0.48 \mu\text{g/l}$, respectively (Table 1). The median %AMPA ratio (in 69 samples) was 20% with an interquartile range of 11-43% (Figure 8).

Temporal Patterns

Most of the samples analyzed in this study were not collected with the intention of identifying temporal patterns or trends. No sites had results from all years and most sites only had results from one or two years. A change in the laboratory reporting level in 2004 also complicates the interpretation of temporal

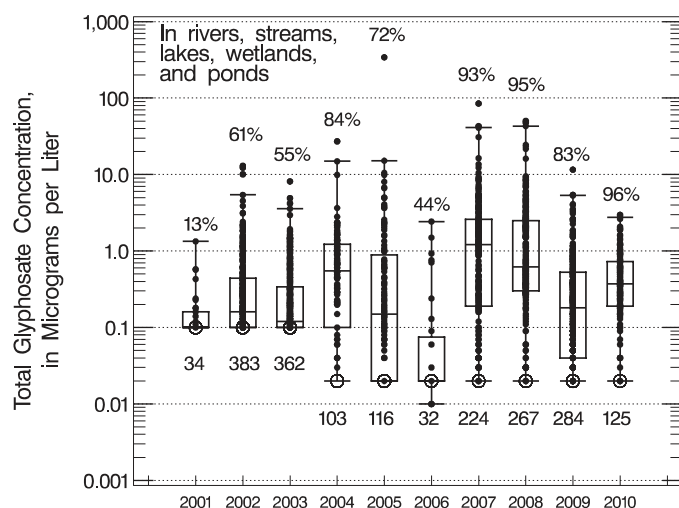


FIGURE 10. Boxplot-Dot Plots Showing Number of Samples (number below boxplots), Percentage Detections (number above boxplots), and Total Glyphosate Concentrations, by Year 2001-2010, for Surface Water Samples from Rivers, Streams, Lakes, Wetlands, and Ponds (open circle is reporting level).

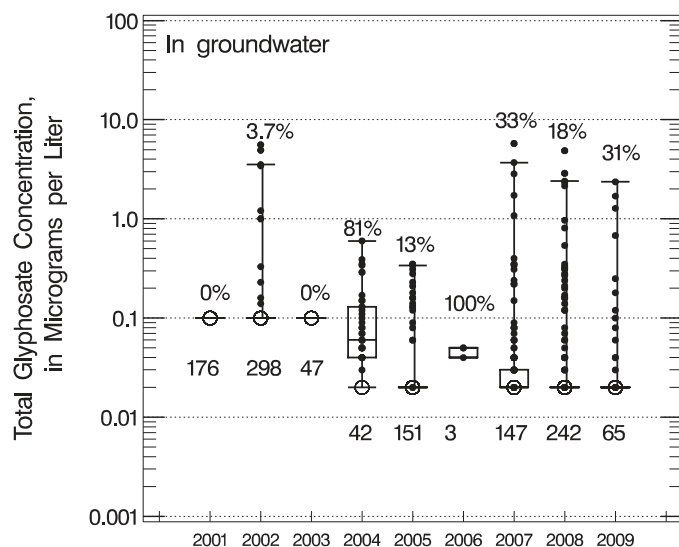


FIGURE 11. Boxplot-Dot Plots Showing Number of Samples (number below boxplots), Percentage Detections (number above boxplots), and Total Glyphosate Concentrations, by Year 2001-2009, for Groundwater Samples (open circle is reporting level).

patterns of glyphosate and AMPA occurrence. A plot of the total glyphosate concentration (sum of glyphosate and AMPA) by year for all surface water samples (Figure 10) provides limited indication of increases in detection frequency and median concentration. A plot of the total glyphosate concentration by year for all groundwater samples (Figure 11) provides no indication of increases in detection frequency or concentration.

At six stream sites and three river sites, there were multiple samples from multiple years, both early (prior to 2006) and late (2006 and later) during the period of study. Streamflow data were acquired for these sites and used to calculate estimates of instantaneous daily total glyphosate flux on dates when samples were collected (Figure 12).

The Wilcoxon signed-rank test was used to gain a measure of the statistical significance of differences in streamflows, total glyphosate concentrations, and instantaneous daily total glyphosate fluxes between the early period (2001-2005) and the later period (2006-2010). Results (Table 2) indicated that stream-

flow was significantly ($p < 0.05$) larger for the late period samples at two sites; significantly smaller for the late period at two sites; larger, but not statistically significantly larger at three sites; and smaller, but not significantly smaller at two sites. Hence, there was a mix of changing streamflow conditions at the nine sites. In contrast, total glyphosate concentrations were significantly larger for the late period samples at five sites, and larger, but not significantly larger at the other four sites. Instantaneous daily total glyphosate fluxes were significantly larger for the late period samples at four sites, larger, but not significantly larger at three sites; and smaller, but

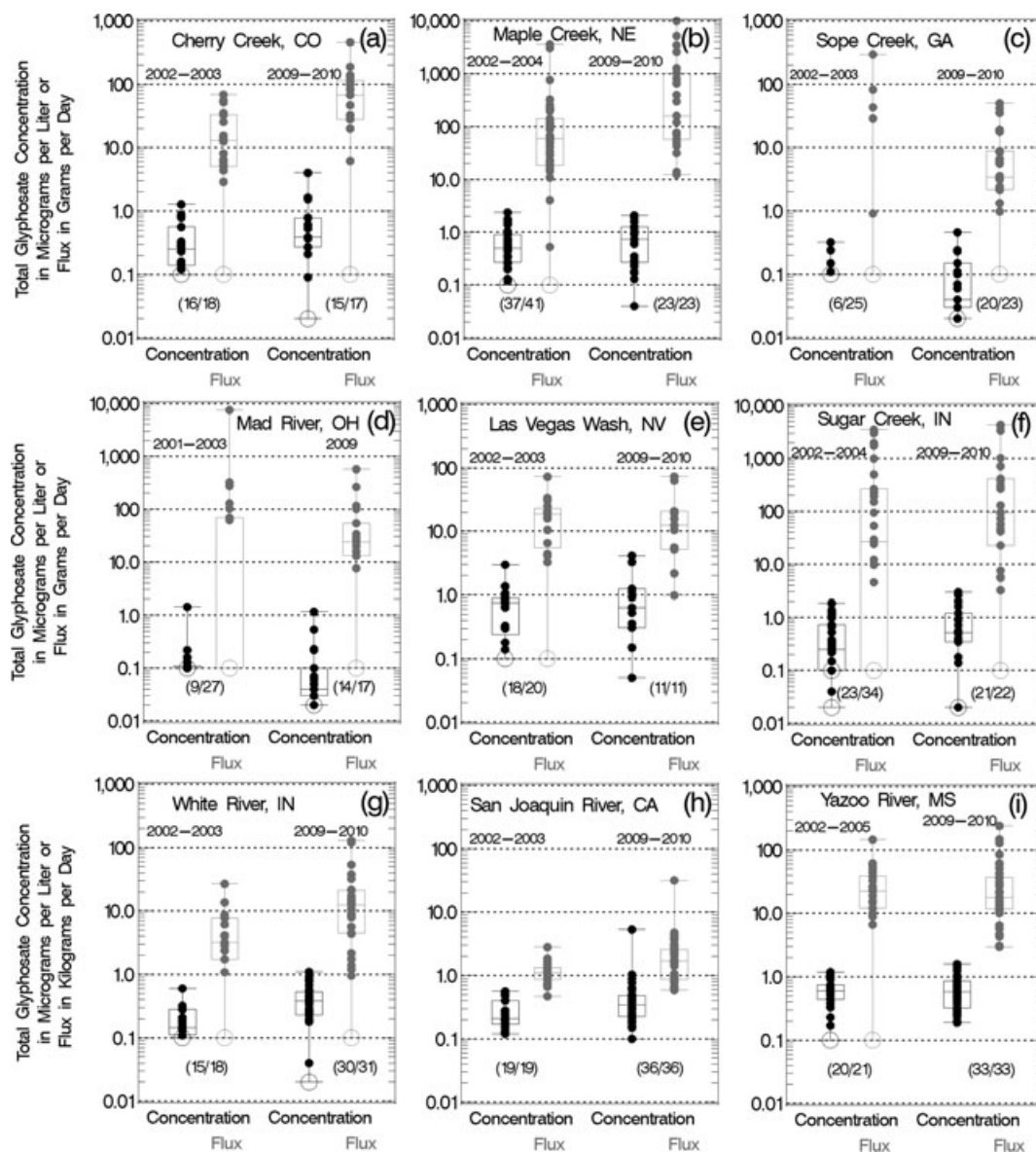


FIGURE 12. Boxplot-Dot Plots Showing Total Glyphosate Concentrations and Instantaneous Daily Total Glyphosate Fluxes for Early and Late Samples from (a) Cherry Creek, Colorado; (b) Maple Creek, Nebraska; (c) Sope Creek, Georgia; (d) Mad River, Ohio; (e) Las Vegas Wash, Nevada; (f) Sugar Creek, Indiana; (g) White River, Indiana; (h) San Joaquin River, California; and (i) Yazoo River, Mississippi (numbers in parentheses are number of detections/number of samples, open circle is reporting level).

TABLE 2. Wilcoxon Signed-Rank Test *p*-Values and Direction of the Differences between Early (2001-2005) and Late (2006-2010) Values of Streamflow, Total Glyphosate Concentration, and Instantaneous Daily Total Glyphosate Flux at Nine Sites (bold values indicate that differences are statistically significant at the $p \leq 0.05$ level).

Site	Streamflow		Concentration		Instantaneous Flux	
	<i>p</i> Value	2006-2010 Values Are	<i>p</i> Value	2006-2010 Values Are	<i>p</i> Value	2006-2010 Values Are
Cherry Creek, Colorado	<0.001	Larger	0.08	Larger	0.003	Larger
Maple Creek, Nebraska	<0.001	Larger	0.206	Larger	0.002	Larger
Sope Creek, Georgia	0.227	Larger	0.001	Larger	0.001	Larger
Mad River, Ohio	0.003	Smaller	0.039	Larger	0.078	Larger
Las Vegas Wash, Nevada	<0.001	Smaller	0.591	Larger	0.698	Smaller
Sugar Creek, Indiana	0.731	Smaller	0.017	Larger	0.142	Larger
White River, Indiana	0.583	Larger	0.001	Larger	0.003	Larger
San Joaquin River, California	1.0	Larger	0.034	Larger	0.074	Larger
Yazoo River, Mississippi	0.729	Smaller	0.936	Larger	0.887	Smaller

not significantly smaller at two sites, both of which have smaller streamflow in the later period (Table 2).

DISCUSSION

Results described here indicate that glyphosate and AMPA are mobile and occur widely in the environment. It was uncommon for glyphosate to be detected without AMPA, happening in only 2.3% of all samples, whereas AMPA was detected without glyphosate in 17.9% of all samples. Glyphosate was detected in 52.5% of stream and 53.1% of large river samples, whereas AMPA was detected in 71.6% of stream and 89.3% of large river samples (Table 1, Figure 3). Glyphosate and AMPA were detected in very large rivers such as the Mississippi with drainage areas in the millions of square kilometers and in headwaters streams with drainage areas less than 10 km². These detection frequencies are greater than those determined from samples collected in 2007 from urban and rural streams in Ontario, 33 and 32%, respectively (Byer *et al.*, 2008). The detection frequencies also are much greater than those identified by Struger *et al.* (2008) in Ontario streams, 21% for glyphosate and 3% for AMPA in 502 samples, however, the analytical reporting level for the method used in that study were substantially higher (5 µg/l for glyphosate and 20 µg/l for AMPA) than the reporting levels used in this study, emphasizing the importance of low reporting levels for targeted analytes and their degradates in environmental occurrence studies.

Most observed concentrations of glyphosate were well below existing health benchmarks and levels of concern for humans or wildlife, and none exceeded the U.S. Environmental Protection Agency's Maximum Contaminant Level of 700 µg/l or Canadian

short-term (27,000 µg/l) and long-term (800 µg/l) freshwater aquatic life standards (Canadian Council of Ministers of the Environment, 2012). Median glyphosate concentrations in all hydrologic settings (other than sediment and precipitation) were less than or equal to 0.2 µg/l and median AMPA concentration in all hydrologic settings (other than sediment) were less than or equal to 0.45 µg/l. In isolated samples glyphosate concentrations in surface water approached a level (about 400 µg/l) that could be of concern for the survival of some amphibian species (King and Wagner, 2010), but only if the surfactants and other adjuvants used in glyphosate formulations were also present. While concentrations of glyphosate and AMPA were below the levels of concern for humans or wildlife, pesticides (and other environmental contaminants) are often detected in mixtures, and the ecosystem effects of chronic low-level exposures to pesticide mixtures are uncertain. Hence, the environmental health risk of these low-level detections of glyphosate, AMPA, and the potential associated adjuvants and mixtures remains to be determined.

One likely reason for the high detection frequencies is simply the widespread and increasing use of products containing glyphosate in the U.S. The extensive use of tile/subsurface drains in many agricultural regions in the U.S. is another factor that could contribute to the higher than expected frequency of detection of glyphosate and AMPA in U.S. streams and rivers. Others have suggested that glyphosate transport via tile drains could be significant (Stone and Wilson, 2006), and both glyphosate and AMPA were detected frequently and often at elevated concentrations in ditch and drain samples analyzed in this study (Figures 3 and 7). The widespread use of glyphosate for nonagricultural purposes and the frequent occurrence of glyphosate and AMPA in precipitation, and the discharge of AMPA by WWTP and septic tanks also could contribute to frequent detections in surface waters.

Another potentially important reason why glyphosate occurrence is more common than would be expected is that surfactants and other adjuvants are almost always included with glyphosate in commercial products, or added as “tank-mixtures” just prior to application. Several studies have demonstrated that the mobility of typically immobile pesticides can be increased in the presence of surfactants (Grant *et al.*, 2011). Surfactants can increase the apparent water solubility of a pesticide, influence biodegradation, and effect soil structure and related adsorption and desorption processes (Katagi, 2008). The type and concentration of the surfactant is important and there is typically a critical concentration at which surfactant micelles form. When below this level, surfactants may act to increase the sorption of a pesticide to soils, whereas when above this level they would decrease the sorption to soils and increase mobility (Haigh, 1996).

The detection of glyphosate in 5.8% and AMPA in 14.3% of groundwater samples (Figures 3 and 9) was about what was expected. While most prior reviews of the occurrence or expected occurrence of glyphosate and AMPA suggested that both compounds were “unlikely to leach into groundwater” due to their strong adsorptive characteristics (U.S. Environmental Protection Agency, 1993; Giesy *et al.*, 2000; Cerdeira and Duke, 2006; Borggaard and Gimsing, 2008), at least one (Vereecken, 2005) suggested some potential for movement after heavy rainfall in the presence of preferential flow paths. Also, one recent study (Sanchis *et al.*, 2011), which used methods that had comparably low analytical limits of quantification (~ 10 ng/l), found glyphosate in 41% of groundwater samples from Catalonia, Spain. The detection frequencies for glyphosate and AMPA in this study, which includes shallow and deep wells, and wells from nonagricultural areas, are similar to those determined for other high use herbicides such as acetochlor, atrazine, alachlor, metolachlor, and their degradates in Iowa groundwater (Kolpin *et al.*, 2000). One prior study (U.S. Environmental Protection Agency, 2002) had detected glyphosate in less than 0.1% of groundwater systems used as drinking water supplies, however, the samples were collected between 1992 and 1997, well before the rapid increase in glyphosate use, and the analytical reporting level for glyphosate was 6 $\mu\text{g/l}$.

The detection of glyphosate and AMPA in more than 70% of the precipitation samples (Figure 3) was not expected due to their low vapor pressures and strong adsorptive characteristics, however, spray drift from such a heavily used pesticide is always possible (Giesy *et al.*, 2000). Other herbicides with similar use patterns such as atrazine and metolachlor also commonly occur in precipitation in agricultural areas (Goolsby *et al.*, 1997; Vogel *et al.*, 2008; Schummer *et al.*, 2010). A more detailed analysis of the occur-

rence of glyphosate and AMPA in these precipitation samples and associated air samples was recently provided by Chang *et al.* (2011), who indicated that both spray drift and wind erosion are important sources of glyphosate to the atmosphere and that precipitation is very effective at removing glyphosate and AMPA from the atmosphere.

The %AMPA values provide some information on the source, fate, and transport of glyphosate in the environment with lower values suggesting recent or proximal input of glyphosate and higher values suggesting more residence time or distance between input and the measured occurrence. More than 75% of %AMPA values from sediment, soil water, large river, stream, and groundwater samples (Figures 5 and 8) were greater than 50 indicating that AMPA tends to occur at higher concentrations than glyphosate in these environmental settings. Coupe *et al.* (2012) suggest that the timing of rainfall runoff events relative to glyphosate and the amount of glyphosate and AMPA in the soil reservoir from previous applications controls %AMPA values in surface water. Larger %AMPA values are expected when rainfall runoff events occur later in the season or when there is a larger reservoir of available AMPA than glyphosate in the soil reservoir or when there is sufficient travel distance/residence time between source applications and transport to surface water to allow for the degradation of glyphosate to AMPA. Coupe *et al.* (2012) also suggest that the %AMPA values should increase with increases in drainage area. In this study, %AMPA values from ditch and drain; and lake, pond, and wetland samples both ranged from 0 to 100% with median values of 63 and 42%, respectively. This result suggests that these site types span a wide range of hydrologic conditions, but that more often than with streams or rivers, they are closely connected to the source applications (in time or space). Groundwater samples had the highest %AMPA values (Figure 8) with a median value of 100 indicating that water in this hydrologic setting is the farthest (in residence time or space) from the source application. Greater sorption of glyphosate relative to AMPA in soils also may contribute to the higher %AMPA values in groundwater samples. More than 75% of %AMPA values from precipitation samples (Figure 8) were less than 50 indicating that glyphosate tends to occur at higher concentrations than does AMPA in this environmental setting.

Although most of the samples analyzed in this study were not collected with the intention of identifying temporal patterns or trends, six stream sites and three river sites had multiple samples from multiple years, both early (prior to 2006) and late (2006 and later) during the period of study. Total glyphosate concentrations were larger in 2006–2010 than in 2001–2005 at all nine sites and those differences are

statistically significant ($p < 0.05$) at five of the sites. And while streamflow was larger in 2006-2010 at five sites and smaller in 2006-2010 at four sites, instantaneous daily flux values were larger in 2006-2010 at seven of nine sites, and four sites indicate statistically significant increases, and no sites indicate statistically significant decreases (Figure 12, Table 2).

CONCLUSIONS

This investigation is the largest and most comprehensive assessment of the environmental occurrence of glyphosate and AMPA in the U.S. conducted to date, summarizing the results of 3,732 environmental water and sediment samples and 1,018 quality assurance samples collected between 2001 and 2010 from 38 states and the District of Columbia. The results indicate that glyphosate and AMPA are mobile, occur widely in the environment, and have both agricultural and urban sources. It was uncommon for glyphosate to be detected without AMPA, occurring in only 2.3% of all samples, whereas AMPA was detected without glyphosate in 17.9% of all samples. Glyphosate and AMPA occurred widely in surface water with one or both compounds being detected at least once at 59% of 470 sites. Glyphosate and AMPA were detected with similar frequency in large rivers such as the Mississippi with drainage areas equal to or greater than 10,000 km² and in smaller streams with drainage areas less than 10,000 km². Glyphosate and AMPA occurred less widely in groundwater or soil water with one or both compounds being detected at least once at 8.4% of 820 sites. Glyphosate was detected in more than 50% of soil and sediment samples, and water samples from ditches and drains, precipitation, large rivers, and streams. Glyphosate was detected in less than 40% of water samples from lakes, ponds, and wetlands; soil water; and groundwater. AMPA was detected in more than 50% of soil and sediment samples, and water samples from large rivers, ditches and drains, precipitation, streams, and soil water. AMPA was detected in less than 30% of water samples from lakes, ponds, and wetlands; and groundwater. AMPA was detected more frequently than glyphosate in all hydrologic settings except lakes, ponds, and wetlands. These differences in detection frequencies for glyphosate and AMPA are likely due to differences in source proximity, water travel time, water residence time, degradation processes, and other natural processes.

The %AMPA values confirm that AMPA is detected at higher concentrations than glyphosate in most

hydrologic settings, with groundwater and soil water samples having the highest values; and precipitation and lake, pond, and wetland samples having the lowest values. These results indicate that the glyphosate in precipitation and wetland, pond, and lake water samples was more closely associated with source applications or has had less opportunity to degrade than did the glyphosate occurring in the other hydrologic settings, and that glyphosate reaching soil water and groundwater had the most opportunity to degrade. Median glyphosate concentrations in all hydrologic settings (other than sediment) were less than or equal to 0.2 µg/l and median AMPA concentration in all hydrologic settings (other than sediment) were less than or equal to 0.45 µg/l, emphasizing the importance of low limits of detection for targeted analytes and their degradates in environmental occurrence studies.

Although most samples analyzed in this study were not collected with the intention of identifying temporal patterns or trends, results from nine surface water sites that had multiple samples from both the early (2001-2005) and late (2006-2010) study periods provide an indication of increases in glyphosate and AMPA detection frequency, median concentrations, and instantaneous daily fluxes. Finally, the results indicate that glyphosate and AMPA frequently add to the chronic low-level exposures to mixtures of pesticides and pesticide degradation products that plants and animals experience in a wide range of ecosystems in the U.S.

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