Work Plan for Determining the Occurrence of Glyphosate, Its Transformation Product AMPA, Other Herbicide Compounds, and Antibiotics in Midwestern United States Streams, 2002

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CONTENTS

FIGURES

TABLES

CONVERSION FACTORS AND ABBREVIATIONS

Multiply	By	To obtain
cubic foot per second (ft^3/s)	0.02832	cubic meter per second
mile (mi)	1.609	kilometer
pound (lb)	453.6	grams
pound per acre (lb/acre)	1.121	kilogram per hectare
square mile (mi^2)	2.590	square kilometer
gallon (G)	3.785	liter (L)
milligrams per liter (mg/L)	1,000	micrograms per liter (µg/L)

Abbreviated Water-Quality Units

cubic meters per second (m^3/s)
micrograms per liter (µg/L)
milligrams per liter (mg/L)
milliliter (mL)
millimeter (mm)
microsiemens per centimeter at 25 degrees Celsius (µS/cm)

Miscellaneous Abbreviations and Acronyms
AMPA – amino methyl phosphonic acid
DEA – deethylatrazine
DDA – didealkylatrazine
DIA – deisopropylatrazine

GCS – GCMS analysis method for herbicides and herbicide transformation products
LCGY – LCMS analysis method for glyphosate, glufosinate, and AMPA
LC_{50} – contaminant concentration at which 50 percent of test organisms die
LCAA – LCMS analysis method for herbicide transformation products
LCAN – LCMS analysis method for antibiotics
LCEA – LCMS analysis method for herbicides and herbicide transformation products
LCMS – Liquid chromatography mass spectrometry
LOEC – lowest observed effect concentration
NASQAN – National Stream Quality Accounting Network
NAWQA – National Water-Quality Assessment
NOEC – no observed effect concentration

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Abstract

Changes in herbicide use in the Midwestern United States have been substantial over the last 5 years. Most significant is a tripling in the use of glyphosate (N-[phosphonomethyl]glycin). Over this same time period (1997–2001), atrazine use increased by 20 percent and acetochlor use increased by 10 percent, while cyanazine use decreased by 99 percent, alachlor use decreased by 70 percent, and metolachlor use decreased by 55 percent. Previous studies have documented that herbicide flushes occur in midwestern streams during runoff events for several weeks to several months following application, and that herbicide concentrations in midwestern streams during flushing events are related to rates of herbicide use.

The objective of this study is to determine the distribution of glyphosate and its primary transformation product aminomethylphosphonic acid (AMPA) in midwestern streams during post-application and harvest-season runoff events. Water samples will be collected in 2002 during two post-herbicide-application runoff events and one harvest-season runoff event from 53 sites on streams in the Midwestern United States. All samples will be analyzed at the U.S. Geological Survey Organic Geochemistry Research Laboratory in Lawrence, Kansas, for glyphosate and 20 other herbicides. Samples will also be analyzed for a glyphosate transformation product (AMPA) and 26 other herbicide transformation products, using GC/MS or HPLC/MS. Selected samples will be analyzed for 36 antibiotics or antibiotic transformational products. Results from this study will represent the first broad-scale investigation of glyphosate and AMPA in U.S. water resources.

INTRODUCTION

This report describes a plan of study to determine the distribution of glyphosate and its primary transformation product aminomethylphosphonic acid (AMPA) during runoff events in midwestern streams. The study will also determine if recent changes in herbicide use are affecting herbicide concentrations, and the distribution of didealkylatrazine (DDA), other herbicide transformation products, and selected antibiotics in midwestern streams.

Since 1989, the U.S. Geological Survey (USGS) Toxic Substances Hydrology Program has conducted reconnaissance studies of streams in the Midwestern United States (U.S.) to determine the geographic and seasonal distribution of herbicide compounds. Studies in 1989 and 1990 documented that large amounts of atrazine, cyanazine, alachlor, and metolachlor were flushed into streams during post-herbicide-application runoff events (Thurman and others, 1991, 1992; Goolsby and Battaglin, 1993). Additional studies in 1990-1992 using automatic samplers (Scribner and others, 1994) showed that the peak herbicide concentrations tend to occur during the first runoff event after herbicide application. Smaller herbicide concentration peaks continue to occur during runoff events for several weeks to several months following application. By late summer, herbicide concentrations are generally low (less than 0.05 µg/L) and tend to remain low until the process repeats during the following planting season.

Recent studies have focused on collecting water samples during these post-application runoff events. The same 53 stream sites have been sampled in 1989, 1990, 1994, 1995, and 1998. Results from these studies demonstrate that the concentrations of several herbicides in midwestern streams have decreased since 1989 (Battaglin and Goolsby, 1999; Scribner and others, 2000). In some cases, these decreases correspond to declines in herbicide application amounts, whereas in other cases, the reason for the decrease more likely is changes in application practices. These studies also found a widespread occurrence of herbicide transformation products in midwestern streams. In the 1998 stream reconnaissance, the median concentrations of acetochlor, alachlor, and metolachlor were all less than the median concentration of one or more of their transformation products. This concentration pattern would be expected later in the summer during low-flow conditions but was not expected during higher flows in spring and early summer (Battaglin and others, 2001).

Glyphosate (N-[phosphonomethyl]glycin) is a broad-spectrum nonselective systemic herbicide that was first used in the early 1970's. Glyphosate is among the most widely used herbicides in the world. Glyphosate has not been extensively monitored in the United States even though it is commonly used for agricultural, silvicultural, and nonagricultural weed control. In Germany, both glyphosate and its primary transformation product (AMPA) have been detected at low concentrations in rivers. Glyphosate, however, is both highly polar and water-soluble, making analysis for it in water samples difficult (Skark and others, 1998). In 1997 (the last year with available estimates), more than 50 million pounds of glyphosate were sold for agricultural and nonagricultural weed control (Aspelin and Grube, 1999).

Glyphosate Toxicity

Glyphosate is a nonselective herbicide that inhibits aromatic amino acid biosynthesis. It is considered to be only slightly toxic to birds, fish, and aquatic invertebrates and does not bioaccumulate. The toxicity of chemicals to nontarget organisms can be compared using various endpoints including LC_{50} values (concentration that kills 50 percent of test organisms), NOEC (no observed effect concentration), and LOEC (lowest observed effect concentration). The 48-hour LC_{50} values for freshwater fish and aquatic invertebrates range from 55 to 780 mg/L (table 1). The LOEC's for freshwater green algal growth were less at 4.00 and 1.55 mg/L, respectively. Several studies examined the effect of glyphosate on soil bacteria and found no adverse effects on soil microbial biomass or on nitrification; but in fact, microbial activity

Table 1. Toxicity of technical-grade glyphosate to aquatic organisms

[LC_{50}, 50 percent lethal concentration; LOEC, lowest observed effect concentration; FETAX, Frog Embryo Teratogenesis Assay; NOEC, no observed effect concentration]

Organism	Endpoint	Value (mg/L)
Daphnia magna [a]	48-hour LC_{50}	780
Midge (*Chironomus plumosus*)[a]	48-hour LC_{50}	55
Bluegill sunfish [a, b]	48-hour LC_{50}	24–150
Rainbow trout [a, b]	48-hour LC_{50}	85–140
Fathead minnow [b]	48-hour LC_{50}	97
Freshwater green algae [c]	LOEC on growth	1.55, 4.00
Amphibian [d]	FETAX	5,407 (acid equivalent)
Coho salmon smolt [e]	NOEC osmoregulation and growth	2.78

[a] U.S. Environmental Protection Agency, 1993.
[b] Folmer and others, 1979.
[c] Saenz and others, 1997.
[d] Perkins and others, 2000.
[e] Mitchell and others, 1987.

increased with glyphosate as a carbon source (Stratton, 1990; Stratton and Stewart, 1991; Busse and others, 2001; Haney and others, 2002). The effect of glyphosate on amphibians was evaluated using the Frog Embryo Teratogenesis Assay (FETAX), and the resulting LC_{50} was 5,407 mg acid equivalent/L (Perkins and others, 2000). Mitchell and others (1987) found the NOEC for sublethal effects of glyphosate on coho salmon smolt osmoregulation and growth was 2.78 mg/L. Some of the surfactants in glyphosate formulations, particularly the ethylated amines and contaminants associated with them, are thought to be more toxic than glyphosate.

Changes in Herbicide Use

Several substantial changes in herbicide use have occurred since the last stream reconnaissance study in 1998. The most significant is the rapid increase in the use of glyphosate that corresponded with the introduction of "Roundup Ready" crops. Between 1997 and 2001 (the newest data available) agricultural glyphosate use in nine Midwestern States (Illinois, Indiana, Iowa, Kansas, Minnesota, Missouri, Nebraska, Ohio, and Wisconsin) more than tripled from 10.3 to 36.1 million pounds applied (fig. 1). Over this same time period, atrazine use increased from 43.7 to 52.2 million pounds, acetochlor use increased from 26.1 to 28.7 million pounds, and simazine use increased from 0.8 to 1.3 million pounds; while cyanazine use decreased from 15.7 to 0.05 million pounds, alachlor use decreased from 7.8 to 2.3 million pounds, metolachlor use decreased from 46.1 to 20.5 million pounds, and metribuzin use decreased from 1.2 to 0.2 million pounds (U.S. Department of Agriculture, 2002).

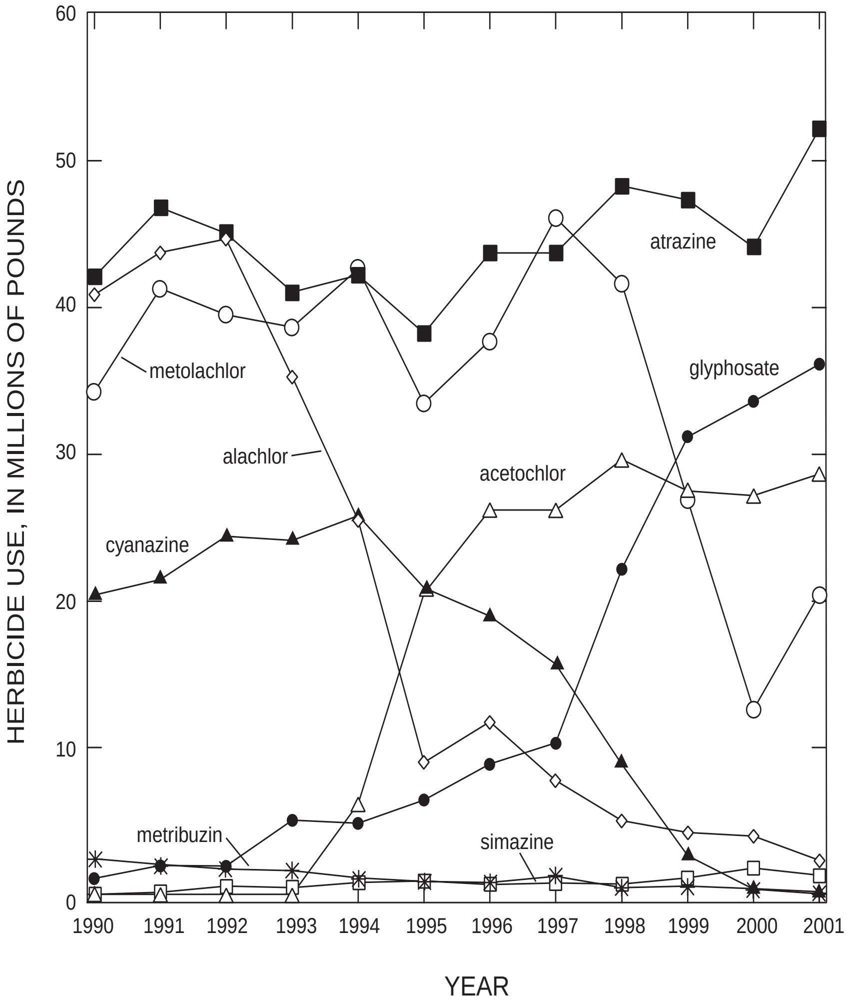

Figure 1. Herbicide use estimates in millions of pounds of active ingredient applied.

Assuming that these trends in herbicide use continued through 2002, we suspect glyphosate and its primary transformation product AMPA will be detected in a majority of midwestern streams during 2002 post-application runoff events. We also suspect that when compared with the distribution of concentrations measured in the 53 midwestern streams in 1998, those measured in 2002 will be about the same for atrazine and acetochlor and significantly lower for alachlor, cyanazine, and metolachlor.

Herbicide Transformation Products

The half-life of glyphosate in soils typically found in the Midwestern United States is approximately 25–40 days. Glyphosate can be transformed (degraded) in soil, water, and plants to AMPA. Glyphosate loses an acetic acid moiety to form the AMPA transformation product. AMPA retains the phosphate group and many of the physical properties of glyphosate. AMPA is soluble and easily dissolved but will precipitate in the presence of iron and calcium after formation of insoluble phosphate salts (Royal Society of Chemistry, 1998). AMPA is most likely more persistent than glyphosate and also should be monitored as part of any study of glyphosate.

The 1998 reconnaissance (Battaglin and others, 2001) and other recent investigations (Thurman and others, 1994; Boyd, 2000; Kalkhoff and others, 1998; Phillips and others, 1999; Porter and others, 2001) have documented the widespread occurrence of herbicide transformation products in water. Still, scientists have not analyzed natural water samples for many of the herbicide transformation products that are likely to occur in the environment. Thurman and others (1994) showed that both the deethylatrazine (DEA) and deisopropylatrazine (DIA) transformation products of atrazine are commonly found in soils, ground water, and surface water of the Midwestern United States. Both DEA and DIA form by losing either an ethyl or isopropyl group—which is a common degradation pathway in soil. It is hypothesized that the continued transformation of DEA and DIA with the loss of either the remaining ethyl or isopropyl group will result in didealkylatrazine (DDA). Its occurrence was suspected in the past, but methods were not available until now to isolate and identify DDA. DDA retains the chlorine atom and probably retains some of the herbicidal activity and toxicity of the parent atrazine. No published data exist for the occurrence of DDA in surface or ground water. The authors hypothesize that both AMPA and DDA will be detected in most midwestern streams.

Antibiotics in Streams

Recent studies have shown antibiotics to be present in U.S. streams (Kolpin and others, 2002). A number of the antibiotics found are those used in livestock production, but the transport mechanisms of antibiotics to streams are not yet understood. Samples collected during runoff events in midwestern streams will help to identify the dominant source of antibiotics in streams. If point sources such as sewage-treatment discharges or confined animal feeding operation (CAFO) discharges are the dominant source, then concentrations should be much lower during runoff events than during lower flow conditions. If nonpoint sources (such as manure applications to fields) are dominant, then concentrations should be about the same or higher during runoff events. Because manure applications also take place during the post-harvest period, fall and early winter storms may be “flushing” events for antibiotics.

WORK PLAN

Approximately 180 samples will be collected during the 2002 reconnaissance study. Samples will be collected from 53 sites (table 2) on small to medium-sized midwestern streams. All 2002 reconnaissance samples will be sent to the USGS Organic Geochemistry Research Laboratory (OGRL) in Lawrence, Kansas, and analyzed for 21 herbicides, 27 herbicide transformation products, and 36 antibiotics (table 3). The analytical reporting limits for all compounds are at or below known biologically significant levels (Battaglin and Fairchild, 2002).

Objectives and Hypotheses

The principal objective of this study is to determine the distribution of glyphosate and its primary transformation product AMPA in midwestern streams during runoff events. The proposed study also provides a plan to determine (1) if recent changes in

Table 2. Midwestern streams to be sampled in 2002, and streamflow conditions for samples collected in 1998

[mi^2, square miles; ft^3/s, cubic feet per second]

Map no.	State	ID	Station name	Drainage area (mi^2)	Sampling dates and flow, in ft^3/s			
					Pre-emergence		Post-emergence	
1	IA	05411850	Turkey River near Eldorado	640	not sampled in 1998			
2	IA	05421000	Wapsipinicon River at Independence	1,048	5/27	595	6/20	1,300
3	IA	05455100	Old Mans Creek near Iowa City	201	6/10	209	6/30	840
4	IA	05472500	N. Skunk River near Sigourney	730	5/21	1,140	6/10	1,810
5	IA	05474000	Skunk River at Augusta	4,303	5/26	15,600	6/18	14,400
6	IA	05480500	Des Moines River at Fort Dodge	4,190	5/16	7,880	6/12	5,190
7	IA	05484500	Raccoon River at Van Meter	3,441	5/17	6,330	6/10	9,160
8	IA	06606600	Little Sioux River at Correctionville	2,500	5/27	1,400	6/18	2,760
9	IA	06607200	Maple River at Mapleton	669	5/29	891	6/9	889
10	IA	06609500	Boyer River at Logan	871	5/22	1,070	6/9	2,890
11	IL	03378000	Bonpas Creek at Browns	228	5/23	834	7/7	499
12	IL	03381495	Little Wabash River at Carmi	3,088	5/23	9,340	7/9	4,020
13	IL	05439500	S. Branch Kishwaukee R. at Fairdale	387	5/20	1,620	7/29	61
14	IL	05526000	Iroquois River near Chebanse	2,091	6/10	1,340	7/8	8,490
15	IL	05540500	Dupage River near Shorewood	324	6/9	284	7/29	166
16	IL	05569500	Spoon River at London Mills	1,072	5/20	1,270	7/1	1,780
17	IL	05576500	Sangamon River at Riverton	2,618	5/26	6,800	7/9	4,070
18	IL	05587000	Macoupin Creek near Kane	868	not sampled in 1998			
19	IL	05592100	Kaskaskia River near Cowden	1,330	5/20	1,400	7/8	2,700
20	IL	05594000	Shoal Creek near Breese	735	5/22	768	7/8	761
21	IN	03275000	Whitewater River near Alpine	522	5/26	727	7/8	652
22	IN	03302800	Blue River at Fredericksburg	283	5/27	447	7/8	490
23	IN	03328500	Eel River near Logansport	789	5/21	701	6/30	688
24	IN	03333450	Wildcat Creek near Jerome	146	5/21	126	6/30	563
25	IN	03335000	Wildcat Creek near Lafayette	794	5/20	883	7/1	1,130
26	IN	03351000	White River near Nora	1,219	5/28	1,260	7/1	1,780
27	IN	03362500	Sugar Creek near Edinburgh	474	5/26	1,610	7/20	1,490
28	IN	03371500	E. Fork White River near Bedford	3,861	5/28	14,300	7/6	6,050
29	KS	06885500	Black Vermillion River near Frankfort	410	5/5	80	6/9	162
30	KS	06890100	Delaware River near Muscotah	431	5/5	111	6/10	100
31	MN	05317000	Cottonwood River near New Ulm	1,280	5/26	525	7/21	673
32	MN	05476000	Des Moines River at Jackson	1,220	5/26	339	8/24	267
33	MN	05320270	Little Cobb River near Beauford	130	5/18	166	8/17	2.8
34	MO	06817700	Nodaway River near Graham	1,320	6/18	5,030	8/19	282
35	NE	06803000	Salt Creek at Roca	167	5/15	470	6/10	148
36	NE	06804000	Wahoo Creek at Ithaca	271	5/15	118	6/8	495
37	NE	06880800	W. Fork Big Blue River near Dorchester	1,206	5/23	247	6/10	231
38	NE	06815000	Big Nemaha River at Falls City	1,340	5/26	333	6/8	438

Table 2. Midwestern streams to be sampled in 2002, and streamflow conditions for samples collected in 1998—Continued

[mi^2, square miles; ft^3/s, cubic feet per second]

Map no.	State	ID	Station name	Drainage area (mi^2)	Sampling dates and flow, in ft^3/s			
					Pre-emergence		Post-emergence	
39	NE	06882000	Big Blue River at Barneston	4,447	5/15	1,110	6/9	1,170
40	NE	06884000	Little Blue River near Fairbury	2,350	5/12	261	6/8	239
41	OH	03157000	Clear Creek near Rockbridge	89	6/10	36	6/30	84
42	OH	03219500	Scioto River near Prospect	567	6/2	128	6/29	1,040
43	OH	03223000	Olentangy River at Claridon	157	6/9	13	6/29	3,770
44	OH	03230500	Big Darby Creek at Darbyville	534	6/3	208	6/29	11,400
45	OH	03234500	Scioto River at Higby	5,131	6/4	2,620	7/8	5,030
46	OH	03240000	Little Miami River near Oldtown	129	6/10	97	6/30	191
47	OH	03267900	Mad River at Eagle City	310	6/10	228	6/30	454
48	OH	04185000	Tiffin River at Stryker	410	6/1	98	7/7	40
49	OH	04186500	Auglaize River near Fort Jennings	332	6/17	670	7/8	751
50	WI	04087240	Root River at Racine	190	6/1	98	8/3	5
51	WI	05340500	St. Croix River at St. Croix Falls	6,240	6/3	4,220	8/5	1,690
52	WI	05407000	Wisconsin River at Muscoda	10,400	6/16	12,900	8/7	8,890
53	WI	05430500	Rock River at Afton	3,340	6/17	2,140	7/21	1,830

Table 3. Physical properties and chemical compounds to be determined on samples collected in 2002

[ft^3/s, cubic feet per second; µS/cm, microsiemens per centimeter at 25 degrees Celsius; °C, degrees Celsius; mg/L, milligrams per liter; µg/L, micrograms per liter]

Property or compound	Method	Reporting limit
Physical properties		
streamflow	meter or rating	0.01 ft^3/s
specific conductance	meter	1.0 µS/cm
pH	electrometric	0.1 unit
water temperature	thermometer or thermister	0.1 °C
Herbicides		
acetochlor	GCS	0.05 µg/L
alachlor	GCS	0.05 µg/L
ametryn	GCS	0.05 µg/L
atrazine	GCS, LCEA	0.05 µg/L
cyanazine	GCS, LCEA	0.05 µg/L
dimethenamid	GCS	0.05 µg/L
diuron	LCEA	0.05 µg/L
flufenacet	GCS	0.05 µg/L
fluometuron	LCEA	0.05 µg/L
glyphosate	LCGY	0.10 µg/L
glufosinate	LCGY	0.10 µg/L
linuron	LCEA	0.05 µg/L
metolachlor	GCS	0.05 µg/L
metribuzin	GCS	0.05 µg/L

Table 3. Physical properties and chemical compounds to be determined on samples collected in 2002—Continued

[ft^3/s, cubic feet per second; µS/cm, microsiemens per centimeter at 25 degrees Celsius; °C, degrees Celsius; mg/L, milligrams per liter; µg/L, micrograms per liter]

Property or compound	Method	Reporting limit
Herbicides—Continued		
pendimethalin	GCS	0.05 µg/L
prometon	GCS	0.05 µg/L
prometryn	GCS	0.05 µg/L
propachlor	GCS	0.05 µg/L
propazine	GCS, LCEA	0.05 µg/L
simazine	GCS, LCEA	0.05 µg/L
terbutryn	GCS	0.05 µg/L
Herbicide transformation products		
acetochlor ESA	LCAA	0.05 µg/L
acetochlor OXA	LCAA	0.05 µg/L
acetochlor SAA	LCAA	0.05 µg/L
alachlor ESA	LCAA	0.05 µg/L
alachlor OXA	LCAA	0.05 µg/L
alachlor SAA	LCAA	0.05 µg/L
AMPA	LCGY	0.10 µg/L
cyanazine acid	LCEA	0.05 µg/L
cyanazine amide	GCS, LCEA	0.05 µg/L
deethylcyanazine	LCEA	0.05 µg/L
deethylcyanazine acid	LCEA	0.05 µg/L
deethylcyanazine amide	LCEA	0.05 µg/L
deethylatrazine	GCS, LCEA	0.05 µg/L
deethylhydroxyatrazine	LCEA	0.05 µg/L
deisopropylatrazine	GCS, LCEA	0.05 µg/L
demethylfluometuron	LCEA	0.05 µg/L
desiopropylhydroxyatrazine	LCEA	0.05 µg/L
didealkylatrazine	LCEA	0.05 µg/L
dimethenamid ESA	LCAA	0.05 µg/L
dimethenamid OXA	LCAA	0.05 µg/L
flufenacet ESA	LCAA	0.05 µg/L
flufenacet OXA	LCAA	0.05 µg/L
hydroxyatrazine	LCEA	0.05 µg/L
metolachlor ESA	LCAA	0.05 µg/L
metolachlor OXA	LCAA	0.05 µg/L
propachlor ESA	LCAA	0.05 µg/L
propachlor OXA	LCAA	0.05 µg/L
Antibiotics		
Beta lactams		
amoxicillin	LCAN	0.20 µg/L
ampicillin	LCAN	0.10 µg/L
cefotaxime	LCAN	0.10 µg/L
cloxacillin	LCAN	0.10 µg/L
oxacillin	LCAN	0.10 µg/L
penicillin G	LCAN	0.10 µg/L
penicillin V	LCAN	0.10 µg/L

Table 3. Physical properties and chemical compounds to be determined on samples collected in 2002—Continued

[ft^3/s, cubic feet per second; µS/cm, microsiemens per centimeter at 25 degrees Celsius; °C, degrees Celsius; mg/L, milligrams per liter; µg/L, micrograms per liter]

Property or compound	Method	Reporting limit
Antibiotics–Continued		
Macrolides		
erythromycin	LCAN	0.10 µg/L
lincomycin	LCAN	0.05 µg/L
roxithromycin	LCAN	0.10 µg/L
trimethoprim	LCAN	0.05 µg/L
tylosin	LCAN	0.10 µg/L
virginiamycin	LCAN	0.10 µg/L
Quinolines		
ciprofloxacin	LCAN	0.05 µg/L
clinafloxacin	LCAN	0.05 µg/L
flumequine	LCAN	0.05 µg/L
lomefloxacin	LCAN	0.05 µg/L
norfloxacin	LCAN	0.05 µg/L
ofloxacin	LCAN	0.05 µg/L
oxolinic acid	LCAN	0.05 µg/L
sarafloxacin	LCAN	0.05 µg/L
Sulfonamides		
sulfachlorpyridazine	LCAN	0.05 µg/L
sulfadiazine	LCAN	0.05 µg/L
sulfadimethoxine	LCAN	0.05 µg/L
sulfamerazine	LCAN	0.05 µg/L
sulfamethazine	LCAN	0.05 µg/L
sulfamethoxazole	LCAN	0.05 µg/L
sulfathiazole	LCAN	0.05 µg/L
Tetracyclines		
chlorotetracycline	LCAN	0.10 µg/L
doxycycline	LCAN	0.10 µg/L
minocycline	LCAN	0.20 µg/L
oxytetracycline	LCAN	0.10 µg/L
tetracycline	LCAN	0.10 µg/L
Tetracycline transformation products		
anhydrochlortetracycline	LCAN	0.10 µg/L
anhydrotetracycline	LCAN	0.20 µg/L
demeclocycline	LCAN	0.10 µg/L

herbicide use are affecting herbicide concentrations in midwestern streams, (2) the distribution of DDA and other herbicide transformation products with limited environmental data, and (3) the distribution of selected antibiotics.

Specific hypotheses:

1. Glyphosate will be detected at a majority of the stream sites in post-emergence runoff samples, but not in the majority of harvest-season runoff samples.
2. AMPA will occur with a frequency and in concentrations similar to that of acetochlor ESA. The frequency of AMPA detection will be larger in harvest-season runoff samples than in post-application runoff samples.
3. The ratio of AMPA to glyphosate will be greater than one in pre-emergence and harvest-season samples but less than one in post-emergence samples.
4. The AMPA in midwestern streams is from transformation of glyphosate and not transformation of detergents in sewage-treatment plants.
5. When compared to concentrations measured in 1998 samples, those measured in post-application runoff in 2002 will be about the same for atrazine and acetochlor and significantly lower for alachlor, cyanazine, and metolachlor.
6. Didealkylatrazine (DDA) will be detected in most midwestern streams in concentrations similar to that of de-ethylatrazine.
7. The herbicide transformation product to parent ratios for alachlor, cyanazine, and metolachlor will be larger in 2002 than in 1998 because of declining use of these products.
8. Selected antibiotics will be detected in about one-half of the 53 midwestern streams.
9. Because of the timing of spring manure applications, antibiotic concentrations will be higher in the pre-emergence runoff samples than in the post-emergence runoff samples or harvest-season runoff samples.

Sampling Sites

The sampling sites are representative of unregulated streams that drain small, agriculturally dominated watersheds in the Midwestern United States. All but 2 of the 53 stream sites sampled in this study have been sampled in the prior reconnaissance studies (Goolsby and others, 1994; Scribner and others, 1998). The Turkey River near Eldorado site (05411850) is about 15 miles downstream from the previously sampled site at Spillville (05411600). The Little Cobb River near Beauford site (05320270) was sampled in 1998 but not in the previous reconnaissance studies, which had sampled the Rock River at Luverne (06483000). The locations of the sites to be sampled and their drainage basins are shown in figure 2. Sampling stations, drainage areas, 1998 sampling dates, and streamflow are given in table 2.

Sampling Schedule

Three samples will be collected at each site. The first set of samples will be collected after pre-emergence herbicides have been applied (usually after planting but before crop plants emerge in May or June) and following a precipitation event that produces a significant increase in streamflow. Ideally, streamflow should represent runoff conditions with flow at or above the 50th percentile (50 percent exceeds streamflow for the period of record, published in annual USGS Water Resources Data reports). These samples will be referred to as “pre-emergence runoff samples.” The second set of samples will be collected after post-emergence herbicides like glyphosate have been applied (usually to the foliage of weeds in June or July), again following a precipitation event that produces runoff conditions and streamflows at or above the 50th percentile. These samples will be referred to as “post-emergence runoff samples.” The third set of samples will be collected during crop harvest (usually September to November), and following a precipitation event that produces runoff conditions and rising streamflows. These samples will be referred to as “harvest-season runoff samples.”

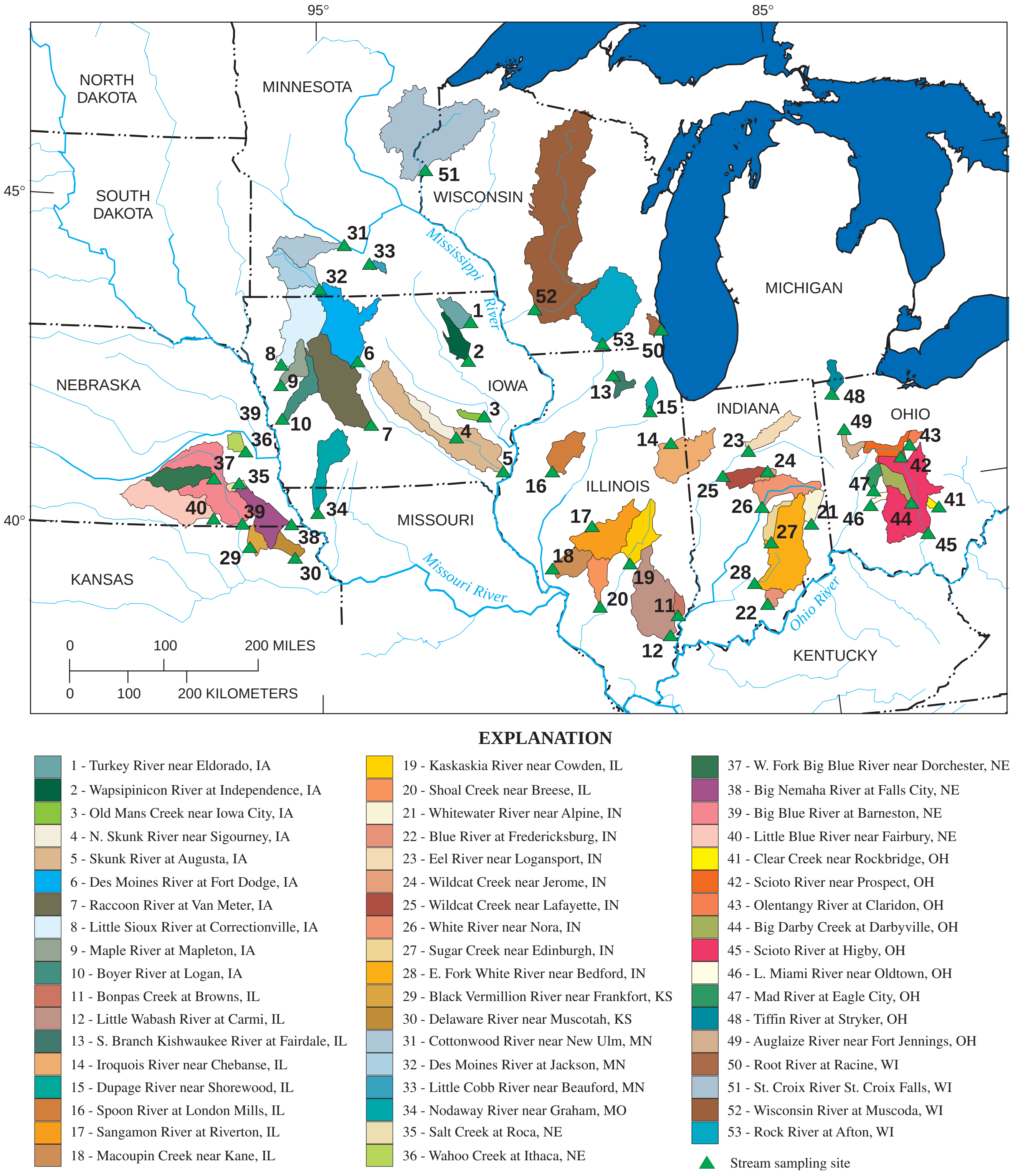

Figure 2. Locations of sampling sites and associated contributing drainage areas.

Sampling Procedures

Samples will be collected using methods described in Shelton (1994). The equal-width-increment sampling method (Edwards and Glysson, 1988) will be used for all samples. The step-by-step sampling procedure is given in the Appendix. All pesticide samples will be filtered through 0.7-mm pore-size baked glass-fiber filters, using an aluminum plate filter holder and a ceramic piston fluid-metering pump with all Teflon tubing, into precleaned 125-mL amber glass bottles. Eight 125-mL amber glass bottles from each site will be filled and sent to the ORGL. Sample bottles will be clearly labeled with waterproof marker or preprinted labels. The minimum information on the label will be the project code, site id, site name, date and time of sample collection, sampler name(s), and the following phrase "USGS 2002 RECON." Field measurements for specific conductance, pH, and temperature will be taken for all samples and a discharge will be obtained by direct measurement, from a rating curve or estimated from a nearby USGS gaging station.

Analytical Procedures

Glyphosate and AMPA. Samples are analyzed for glyphosate, glufosinate, and AMPA by using a liquid chromatography mass spectrometry (LCMS) method as described in Lee and others (2002a). For the LCGY analysis, 10-mL water samples are derivatized in the laboratory and extracted with solid-phase extraction (SPE). The analytes (table 3) are separated by LC and identified with an MS in electrospray negative-ion (ES–NEG) mode operating in selected-ion mode. The concentration of each sample is determined by using labeled glyphosate as an internal standard and a standard-addition replicate using the following equation:

$$C = [\ (A_c/A_i)/(A_{csa}/A_{isa} - A_c/A_i)] \times SA_c \times DF$$

where

C is concentration of the selected compound in the sample,

Ac is area of peak of the ion for the selected compound,

A_i is area of peak for the internal standard,

A_{csa} is area of the peak for the selected compound in the standard-addition sample,

A_{isa} is the area of the peak for the internal standard in the standard-addition sample,

SA_c is concentration of the standard addition, and

DF is dilution factor.

Other Herbicides and Transformation Products. The other herbicide compounds and transformation products (table 3) are analyzed using three methods. The LCEA analysis (Lee and others, 2002b) uses SPE and atmospheric chemical ionization (APCI) positive LCMS. The LCAA analysis (Lee and others, 2001) uses SPE and ES–NEG–LCMS. The GCS analysis (Zimmerman and Thurman, 1999; Kish and others, 2000) uses SPE and gas chromatography mass spectrometry (GCMS). All three methods use 123-mL samples and include surrogate and internal standards. Mass spectrometers are operated in selected-ion mode, and concentrations of detected analytes are calculated from calibration curves.

Antibiotics. A method (LCAN) has been developed for the trace analysis (concentrations between 0.05 and 2.0 µg/L) of five classes of antibiotics consisting of seven beta lactams (BLs), six macrolides (MCs), eight quinolines (QUs), seven sulfonamides (SAs), and eight tetracyclines (TCs) (table 3). These antibiotics are commonly are used by humans, livestock, and pets or are agricultural feed additives. Some of these antibiotics are suspected to leach into ground and surface water. Three slightly different sample preparation methods are used for BLs and MCs, QUs and SAs, and TCs. All samples are extracted with SPE and determined by LCMS with positive-ion electrospray using methods similar to Lindsey and others (2001). Surrogate and internal standards are used in the analysis of 10-mL samples, and concentrations of detected analytes are calculated from calibration curves acquired with the samples.

Quality Assurance

Quality assurance of sample collection and handling will consist of 15 concurrent replicate samples and 4 field blank samples (table 4). In the laboratory, 10 percent or more of the samples will be split and analyzed in replicate (including sample preparation) to yield data on the precision of the analysis. These same samples are also analyzed as spikes for

Table 4. Schedule for quality-assurance samples

[CR, current replicates; FB, field blanks;--, none]

State	ID	Station name	Pre-emergence sample	Post-emergence sample	Harvest-season sample
IA	05411600	Turkey River near Eldorado	CR	--	--
IA	05484500	Raccoon River at Van Meter	--	CR	--
IA	06607200	Maple River at Mapleton	--	--	FB
IL	03378000	Bonpas Creek at Browns	CR	--	--
IL	03381495	Little Wabash River at Carmi	--	CR	--
IL	05592100	Kaskaskia River near Cowden	--	--	CR
IN	03275000	Whitewater River near Alpine	FB	--	--
IN	03362500	Sugar Creek near Edinburgh	--	CR	--
IN	03371500	E. Fork White River near Bedford	--	--	CR
KS	06885500	Black Vermillion River near Frankfort	CR	--	--
MN	05476000	Des Moines River at Jackson	--	CR	--
MO	06817700	Nodaway River near Graham	FB	--	--
NE	06880800	W. Fork Big Blue River nr Dorchester	--	CR	--
NE	06882000	Big Blue River at Barneston	--	--	CR
OH	03219500	Scioto River near Prospect	CR	--	--
OH	03267900	Mad River at Eagle City	--	CR	--
OH	04185000	Tiffin River at Stryker	--	--	CR
WI	04087240	Root River at Racine	CR	--	--
WI	05430500	Rock River at Afton	--	FB	--

determining accuracy. Method blanks are prepared and analyzed with each batch of samples. Standard reference materials (SRMs), if available, are routinely used as Laboratory Control Standards (LCS).

DATA ANALYSIS METHODS AND REPORTS

Results from this study will represent the first broad-scale investigation of glyphosate and AMPA in Midwestern United States streams. Analytical results from this study will be published in a USGS open-file report. Analysis of the results of this study will be published in one or more journal articles to be prepared in 2003 and 2004. Some ancillary data in addition to streamflow will be collected in association with the water-quality data in order to better understand the information provided. The amount of ancillary data collected will be limited by resources but at a minimum will include estimates of corn and soybean planting date, monthly precipitation and temperature, crops planted (will not be available until 2002 Census of Agriculture data are available), and pesticide use (may only be State level data).

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APPENDIX

APPENDIX – Detailed sample collection and processing instructions

Determining where and when to sample

1. Determine which sites are to be sampled in your State (table 2).
2. Determine which sites need quality-assurance samples collected (table 4).
3. Follow news on progress of planting and weather (Battaglin will provide weekly data during the growing season, or you can monitor at http://usda.mannlib.cornell.edu/reports/nassr/field/weather/) accessed May, June, and July 2002.
4. Collect the first set of surface-water samples (pre-emergence runoff) after pre-emergence herbicides have been applied (usually May or June), when corn is at least 50 percent planted, and following a precipitation event that produces a significant increase in streamflow. Ideally, the sample should represent runoff conditions with streamflow at or above the 50th percentile, which is equal to the "50 percent exceeds" streamflow value for the period of record, published for most sites in annual USGS Water Resources Data reports.
5. Continue to follow news on local progress of plant development and weather.
6. Collect the second set of surface-water samples (post-emergence runoff) after post-emergence herbicides, particularly glyphosate, have been applied (usually June or July), when soybeans are nearing 100 percent emerged, and following a precipitation event that produces runoff conditions and streamflow at or above the 50th percentile.
7. Collect the third set of surface-water samples (harvest-season runoff) during or after crop harvest (usually September, October, or November) following a precipitation event that produces runoff conditions and a rise in streamflow at the site. Streamflow does not have to be at or above the 50th percentile.

Sample processing

1. Preclean all equipment with a Liquinox/tap-water solution, rinse with tap water, deionized water, then methanol, and then air dry.
2. Collect and composite a minimum of ~2 liters of water using equal-width-increment sampling into a large precleaned glass carboy.
3. Make field measurements of pH, temperature, and specific conductance, in the stream if possible, or on the composite sample.
4. Filter 100 mL of sample water through a 0.7-mm pore-size baked glass-fiber filter to condition, and then filter 1.0 L of sample water into eight 125-mL baked amber glass bottles.
5. Label all sample bottles clearly with waterproof marker or preprinted labels (a paper label completely covered with clear packing tape is preferred). The minimum information on the label for bottles sent to the ORGL is the project code, site ID, site name, date and time of sample collection, sampler name(s), and the phrase "USGS 2002 RECON" as shown below:

Project code	DBD
station id	05465500
stream name/location	Iowa River at Wapello, IA
date and time	6-18-02 @ 1015
sampler name/initials	Joe Sample and Jane Stream
study label	USGS 2002 RECON

6. Glass bottles will be placed in foam packers to help prevent breakage. The eight bottles from each site will then be placed in a plastic bag, which will help keep them dry in the coolers and keep all bottles from the same site together. All samples are to be kept chilled on ice and shipped within 2 days to the ORGL in Lawrence, Kansas.
7. Notify Bill Battaglin (303-236-4882 extension 256) wbattagl@usgs.gov with the date and time of sample collection and physical properties as soon as possible after samples are shipped.

Quality-assurance samples

Concurrent replicate and field blank samples will be collected during all three sampling periods (table 4). The intent of these samples is to help determine analytical precision. The concurrent replicate sample will require collecting and compositing a larger volume of water from the sites than normally needed. The replicates will be processed in the same manner as the normal samples using the same filter if possible. Record the same site name and ID for both samples but a time that is 1 minute later for the replicate sample. Both samples will be sent to the ORGL in Lawrence, Kansas, for herbicide analysis. The field blank samples will consist of organic-free water that is processed through all of the sample collection and filtration equipment after it has been cleaned as described above. These samples will be labeled as a field blank and sent to the ORGL in Lawrence, Kansas, for analysis.