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NATIONAL COUNCIL FOR AIR AND STREAM IMPROVEMENT

MEASUREMENT OF GLYPHOSATE, IMAZAPYR, SULFOMETURON METHYL, AND METSULFURON METHYL IN NEEDLE BRANCH STREAMWATER

SPECIAL REPORT NO. 13-01

JULY 2013

by
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Acknowledgments

This report was prepared by Jeff Louch, Principal Scientist at the NCASI West Coast Regional Center, with assistance from Ginny Allen, Senior Research Associate at the NCASI West Coast Regional Center. Ginny Allen also performed all the analytical work summarized herein, while Dean Hoy (Senior Research Associate), Marg Stewart (part-time Research Technician), and Terry Bousquet (Project Leader), all from NCASI's West Coast Regional Center, provided logistical support during sample collection. The document was prepared for publication by Karen Phelps, Office Manager at NCASI's West Coast Regional Center. Thanks also to Tina Garland, former Research Assistant at Oregon State University, for performing all the field sampling, and to Dr. George Ice, retired NCASI Fellow, for making this work possible.

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PRESIDENT'S NOTE

As part of its mission, NCASI is often called upon to provide analytical support for studies of significance to the forest products industry. In this vein, NCASI developed in-house capacity to measure multiple silvicultural herbicides in streamwater and analyzed samples collected after application of herbicides to an experimental woodland site (the Needle Branch drainage) in the Oregon Coast Range. This work was performed as part of NCASI's contributions to the Alsea Watershed Study Revisited, which was organized under the auspices of the Watersheds Research Cooperative at Oregon State University.

The purpose of this report is to make the results of this analytical work available to the broader constituency of study collaborators. Although the ultimate interpretation of the reported data will be the responsibility of these collaborators, the results presented in this report show that during storm events, the concentrations of herbicides found in streamwater were low (<1 ppb), and that peak concentrations were short-lived (<12 h). Because much of the data on biological responses to herbicides are based on experimental exposures to higher concentrations for much longer time periods, this information will be of use in addressing concerns about the impact of silvicultural herbicides on biota.

Ronald A. Yeske

July 2013

NOTE DU PRÉSIDENT

Dans le cadre de sa mission, NCASI est souvent appelé à fournir un soutien technique dans des études qui touchent l'industrie des produits forestiers. Ainsi, NCASI a développé des compétences internes qui lui permettent de mesurer la concentration de divers herbicides sylvicoles dans un cours d'eau, ce qui lui a permis d'analyser des échantillons prélevés après l'application d'herbicides dans une forêt expérimentale (bassin versant Needle Branch) située dans la chaîne côtière de l'Oregon. Ce travail représentait la contribution de NCASI à la mise à jour de l'étude sur le bassin versant Alsea, un projet mené sous les auspices de la *Watersheds Research Cooperative* de l'*Oregon State University*.

L'objectif du présent rapport est de diffuser les résultats de ce travail analytique au groupe élargi de chercheurs collaborateurs. Bien que l'interprétation finale des données rapportées soit sous la responsabilité de ces collaborateurs, les résultats présentés dans ce rapport montre que la concentration des herbicides dans les cours d'eau est faible durant une tempête (<1 ppb) et que les cours d'eau sont exposés aux concentrations les plus élevés pendant une courte période de temps (<12 h). Ces renseignements seront utiles pour répondre aux préoccupations concernant l'impact des herbicides sylvicoles sur le biote, car la plupart des données sur la réponse biologique aux herbicides reposent présentement sur des données expérimentales sur des cours d'eau exposés à des concentrations élevées d'herbicides pendant de plus grandes périodes de temps.

Ronald A. Yeske

Juillet 2013

MEASUREMENT OF GLYPHOSATE, IMAZAPYR, SULFOMETURON METHYL, AND METSULFURON METHYL IN NEEDLE BRANCH STREAMWATER

SPECIAL REPORT NO. 13-01
JULY 2013

ABSTRACT

Concentrations of dissolved glyphosate, aminomethylphosphonic acid (AMPA), imazapyr, sulfometuron methyl, and metsulfuron methyl were measured in streamwater collected during and after application of herbicides to a harvested commercial forestry site in the Oregon Coast Range. Samples were collected at three sites, one of which (NBH) was at the fish/no-fish interface in the middle of the harvest unit—that is, at the bottom of an area where there are no requirements to retain commercial trees or a riparian buffer. The other two sampling sites were downstream: one (NBU) at the bottom of the harvest unit and the other (NBL) well downstream. Application rates were 681 g/ac glyphosate (acid equivalent or a.e.), 85 g/ac imazapyr (a.e.), 64 g/ac sulfometuron methyl (active ingredient or a.i.), and 17 g/ac metsulfuron methyl (a.i.), and all herbicides were applied by helicopter in a single tank mix. Background interference from the sample matrix impacted analyte-specific method detection limits (MDLs) for all analytes, and sample-to-sample variability in this background often clouded interpretation of results. Sulfometuron methyl and metsulfuron methyl were not detected (ND) in any samples at their MDLs of 0.5 µg/L and 1 µg/L, respectively. Because of sample-to-sample variability in background interference, dissolved imazapyr could not be reliably quantified at concentrations <0.6 µg/L, a threshold that was not exceeded in any sample. Thus, imazapyr was also ND in all samples, including samples collected during application of herbicides. Likewise, AMPA was ND in all samples at 15 ng/L. However, a clear pulse of dissolved glyphosate manifested at NBH during the application (baseflow conditions). This pulse maximized at 40 to 60 ng/L dissolved glyphosate and persisted for two to three hours. An associated pulse was not detected (<20 ng/L) at the farthest downstream sampling site (NBL), while no glyphosate samples were collected during application at the mid-elevation site (NBU) due to a malfunction of its sampling equipment. Subsequent baseflow samples collected three days after treatment (DAT) showed ≈25 ng/L dissolved glyphosate at all three sites, and all sites were <20 ng/L at 19 DAT. Samples collected during the first storm event (8 DAT) showed a clear pulse of dissolved glyphosate at NBU, but not at NBH or NBL. The maximum concentration observed during this pulse at NBU was 115 ng/L, and the pulse persisted for about six hours. During the next storm event (10 DAT) a clear pulse of dissolved glyphosate manifested at NBH, but not at NBU or NBL. The maximum concentration observed was 42 ng/L, and this pulse persisted for about 10 hours. Results from all subsequent storm events showed dissolved glyphosate at <20 ng/L in all samples. A limited number of analyses on suspended sediment (SS) showed that SS in samples held *de minimis* masses of glyphosate and AMPA.

KEYWORDS

AMPA, glyphosate, herbicide, imazapyr, metsulfuron methyl, runoff, sulfometuron methyl, suspended sediment

RELATED NCASI PUBLICATIONS

Technical Bulletin No. 886 (October 2004). *The toxicity of silvicultural herbicides to wildlife: Vol. 2: Glyphosate and imazapyr*.

Technical Bulletin No. 631 (June 1992). *The effectiveness of buffer strips for ameliorating offsite transport of sediment, nutrients, and pesticides from silvicultural operations*.

Technical Bulletin No. 602 (February 1991). *The New Alsea Watershed Study*.

Technical Bulletin No. 430 (April 1984). *A guide to monitoring streamwater quality following forestry herbicide application*.

Special Report No. 07-01 (February 2007). *Measurement of glyphosate, hexazinone, imazapyr, and sulfometuron methyl in streamwater at the Texas Intensive Forestry Study sites*.

MEASURE DU GLYPHOSATE, DE L'IMAZAPYR, DU SULFOMÉTURON DE MÉTHYLE ET DU METSULFURON-MÉTHYLE DANS DES COURS D'EAU DU BASSIN VERSANT NEEDLE BRANCH

RAPPORT SPÉCIAL N^{O} 13-01
JUILLET 2013

RÉSUMÉ

La concentration de glyphosate dissous, d'acide aminométhylphosphonique (AMPA), d'imazapyr, de sulfométuron de méthyle et de metsulfuron-méthyle a été mesurée dans des cours d'eau avant et après l'application d'herbicides dans une forêt commerciale récoltée de la chaîne côtière de l'Oregon. Trois sites ont été échantillonnés dont l'un (NBH) se trouvait à l'interface « poisson/pas de poisson » dans le centre de l'unité de récolte, c'est-à-dire dans le bas d'un endroit où l'on n'exigeait pas de conserver des arbres à valeur commerciale ou d'instaurer une zone tampon. Les deux autres sites se trouvaient en aval : le site NBU était situé au bas de l'unité de récolte et le site NBL se trouvait beaucoup plus loin en aval. Les doses d'application étaient les suivantes: 681 g/acre de glyphosate (équivalent acide ou éa), 85 g/acre d'imazapyr (éa), 64 g/acre de sulfométuron de méthyle (ingrédient actif ou ia) et 17 g/acre de metsulfuron-méthyle (ia). Tous les herbicides ont été mélangés dans un réservoir et ont été appliqués à l'aide d'un hélicoptère. L'effet de fond de la matrice d'échantillonnage a eu un impact sur les limites de détection de la méthode (LDM) des composés à analyser, et ce, pour tous les composés, et la variabilité des échantillons causée par cet effet de fond a souvent embrouillé l'interprétation des résultats. Le sulfométuron de méthyle et le metsulfuron-méthyle n'ont pas été détectés (ND) dans aucun des échantillons (limites de détection : 0,5 µg/L et 1 µg/L, respectivement). En raison de la variabilité des échantillons causée par l'effet de fond, l'imazapyr dissous n'a pas pu être quantifié de façon fiable à des concentrations <0,6 µg/L (un seuil qui n'a jamais été dépassé par aucun des échantillons). L'imazapyr n'a pas été détecté (ND) dans aucun des échantillons, y compris dans les échantillons recueillis durant l'application des herbicides. À 15 ng/L, l'AMPA n'a également pas été détecté (ND) dans aucun des échantillons. Par contre, le glyphosate dissous a été clairement détecté au site NBH durant l'application des herbicides (conditions de débit de base). Le glyphosate dissous a été mesuré à une concentration maximale de 40 à 60 ng/L pendant 2 à 3 heures. Le glyphosate dissous n'a pas été détecté (<20 ng/L) au site d'échantillonnage le plus en aval (NBL), tandis qu'aucun échantillon n'a été prélevé pour le glyphosate au site de moyenne altitude (NBU) en raison d'une défaillance de l'appareil d'échantillonnage. Trois jours après le traitement (3 JAT), des échantillons ont été prélevés en conditions de débit de base et ont révélé une concentration de glyphosate dissous d'environ 25 ng/L à tous les sites. Dix-neuf (19) jours après le traitement (19 JAT), la concentration était inférieure à 20 ng/L à tous les sites. Le glyphosate dissous a été clairement détecté dans les échantillons prélevés au site NBU, mais pas dans ceux prélevés aux sites NBH et NBL. Le glyphosate dissous a été mesuré à une concentration maximale de 115 ng/L pendant une période d'environ six heures. Durant la deuxième tempête (10 JAT), le glyphosate dissous a été clairement détecté dans les échantillons prélevés au site NBH, mais pas dans les échantillons prélevés aux sites NBU et NBL. Le glyphosate dissous a été mesuré à une concentration maximale de 42 ng/L pendant une période d'environ 10 heures. Tous les échantillons prélevés au cours des tempêtes subséquentes ont révélé que le glyphosate dissous était présent à une concentration inférieure à 20 ng/L. Les résultats de quelques analyses sur les sédiments en suspension (SS) ont montré que les échantillons contenaient une quantité *de minimis* de glyphosate et d'AMPA.

MOTS-CLÉS

AMPA, glyphosate, herbicide, imazapyr, metsulfuron-méthyl, ruissellement, sédiments en suspension, sulfométuron de méthyle

AUTRES PUBLICATIONS DE NCASI

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Bulletin technique n° 631 (juin 1992). *The effectiveness of buffer strips for ameliorating offsite transport of sediment, nutrients, and pesticides from silvicultural operations*.

Bulletin technique n° 602 (février 1991). *The New Alsea Watershed Study.*

Bulletin technique n° 430 (avril 1984). *A guide to monitoring streamwater quality following forestry herbicide application*.

Rapport spécial n° 07-01 (février 2007). *Mesure du glyphosate, de l'hexazinone, de l'imazapyr et du sulfométuron de méthyle dans les cours d'eau des sites de l'étude de la foresterie intensive au Texas.* (seul le résumé est en français)

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MEASUREMENT OF GLYPHOSATE, IMAZAPYR, SULFOMETURON METHYL, AND METSULFURON METHYL IN NEEDLE BRANCH STREAMWATER

1.0 INTRODUCTION

The Alsea Watershed Study Revisited is part of the Watersheds Research Cooperative (WRC; http://watershedsresearch.org), of which NCASI is a member. One component of the study is characterizing potential impacts on biota resulting from the use of herbicides, and the work reported herein focused on a harvest unit in the Needle Branch drainage. As part of this effort, the analytical laboratory at NCASI's West Coast Regional Center was tasked with measuring concentrations of multiple herbicides in samples collected from Needle Branch by WRC researchers affiliated with Oregon State University. The herbicides applied were glyphosate (the active ingredient in Accord®, Roundup®, Touchdown®, and Vision®), imazapyr (the active ingredient in Arsenal®, Assault®, and Chopper®), metsulfuron methyl (the active ingredient in Escort®), and sulfometuron methyl (the active ingredient in Oust®).

This report provides the results of this analytical work, which also included determinations of aminomethylphosphonic acid (AMPA), a metabolite of glyphosate. Section 4 gives a general discussion of concentration results, while Section 5 provides a more concise summary of those results. For the interested reader, Appendix C contains a detailed discussion of analytical results on an analyte-specific basis. The purpose of this report is to present these analytical results; placing the data into context will be an ongoing activity.

2.0 OVERVIEW

2.1 Study Site and Herbicide Treatments

Three herbicide monitoring stations were established in the Needle Branch drainage (Figure 2.1). The lowest elevation station (NBL) is near the mouth of Needle Branch and was the site of most water quality monitoring conducted in the original Alsea Watershed Study (conducted from 1959 to 1973). The middle elevation or upper station (NBU) is at the bottom of the first harvest unit and was established several years prior to the 2009 harvest to provide data on water quality impacts immediately below the harvest unit. At this location, Needle Branch is a small, fish-bearing stream requiring a forested riparian management area of 50 ft (from the ordinary high water mark) on both sides of the stream and with minimal basal area retention requirements. The highest elevation station (NBH; H stands for an H-flume installed to monitor discharge) is at the fish/no-fish interface; there was no riparian buffer above NBH (forested riparian management areas are not required around most no-fish stream reaches in Oregon). A no-spray buffer is required around streams like Needle Branch, including above the no-fish/fish interface.

Prior to replanting, the upper portion of the study site (122 acres above NBU) received an aerial site-release application of herbicides. All herbicides were applied in a single tank mix at rates of 48 oz/ac of Accord® XRT II (glyphosate), 12 oz/ac Chopper® Gen 2 (imazapyr), and 4 oz/ac Sulfomet® Extra (sulfometuron methyl and metsulfuron methyl), corresponding to 681 g/ac acid equivalents (a.e.) of glyphosate, 85 g/ac a.e. of imazapyr, 64 g/ac active ingredient (a.i.) of sulfometuron methyl, and 17 g/ac a.i. of metsulfuron methyl. The tank mix was applied by helicopter using a 32 ft boom with 35 D7 nozzles (no spinner) set at a 20° angle producing 22 psi. The application was initiated at 11:37 AM and completed at 1:18 PM on August 22, 2010.

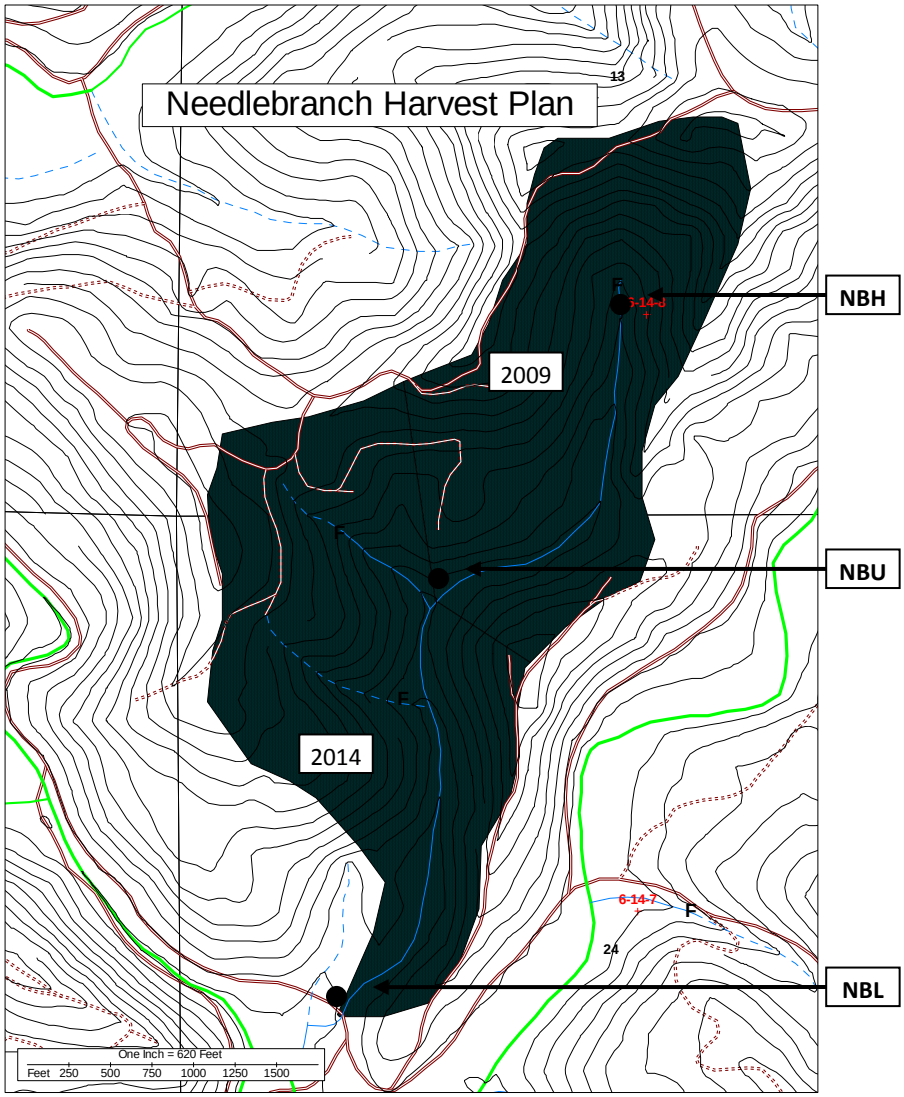

Figure 2.1 Herbicide Monitoring Stations Established in Needle Branch [NBH = bottom of no-fish stream reach; NBU = bottom of harvest and spray unit; NBL = main gauge near mouth of watershed]

2.2 Sample Collection and Sample Count

Streamwater samples were collected during post-application storm events at all three herbicide monitoring stations (henceforth sampling sites) using ISCO autosamplers (see Section 3.1 for details). The samplers were manually triggered when a storm was predicted, and the sampling frequency was adjusted based on the predicted intensity and duration of each storm. Although these were subjective decisions, sampling frequency was highest (one sample per hour) when a high intensity storm was predicted and was reduced (to as low as one sample every six hours) when a low intensity event was predicted. Longer events required multiple triggerings of these autosamplers, and the sampling frequency was often adjusted between cycles. During each sampling event, all samplers were programmed to initiate collection at the same time using the same sample collection rate. In addition to the storm event samples collected using the ISCO samplers, baseflow grab samples were manually collected approximately every week.

The ISCO samplers were also used to collect samples every hour during aerial application of the herbicides. Specifically, collection was initiated at 9:00 a.m., and the samplers were programmed to collect additional samples every hour. Thus, three samples were collected before the application was initiated at 11:37 a.m. and sampling continued for nominally 20 hours after application.

Table 2.1 summarizes the final sample count per sampling site (Section 3.1). Samples for determination of imazapyr, sulfometuron methyl, and metsulfuron methyl were preserved at pH 7 at the time of collection. Samples for determination of AMPA and glyphosate were not preserved until frozen for long-term storage.

Table 2.1 Number of Samples Collected from Each Sampling Site as Part of Each Sampling Event

Sampling Dates				Unpreserved Samples			pH 7 Preserved Samples		
Start	End	DAT[a]	Event	NBL	NBU	NBH	NBL	NBU	NBH
08/22/10	08/23/10	0	Baseflow[b]	24	4[c]	24	24	24	1[d]
08/25/10		3	Baseflow	1	1	1	1	1	1
08/30/10	09/01/10	8-10	Storm	45	48	46	0[e]	48	48
09/10/10		19	Baseflow	1	1	1	1	1	1
09/14/10		23	Baseflow	1	1	1	1	1	1
09/15/10	09/21/10	24-30	Storm	53	54	54	54	54	54
09/24/10		33	Baseflow	1	1	1	1	1	1
10/01/10		40	Baseflow	1	1	1	1	1	1
10/08/10		47	Baseflow	1	1	1	1	1	1
10/08/10	10/10/10	47-49	Storm	0[f,g]	14[g]	14[g]	19	19	19
10/14/10		53	Baseflow	1	1	1	1	1	1
10/22/10		61	Baseflow	1	1	1	1	1	1
10/23/10	10/25/10	62-64	Storm	11[h]	23	24	23	23	24
11/05/10		75	Baseflow	1	1	1	1	1	1
11/18/10	11/19/10	88-89	Storm	11	15	15	15	14	15
11/20/10		90	Baseflow	1	1	1	1	1	1
12/03/10		103	Baseflow	1	1	1	1	1	1
12/10/10	12/12/10	110-112	Storm	27	6[i]	9[i]	36	25	30
				182	175	196	182	218	202

[a] DAT = days after treatment (application of herbicides).
[b] ISCO samplers collected one sample per hour starting three hours prior to application of herbicides during baseflow conditions.
[c] ISCO sampler collected samples #1-3 then malfunctioned; additional grab sample collected ≈24 h after application of herbicides.
[d] ISCO sampler malfunctioned; single grab sample collected ≈24 h after application of herbicides.
[e] ISCO sampler malfunctioned.
[f] ISCO sampler malfunctioned during first portion of storm event.
[g] samples collected by ISCO samplers during second portion of storm event not retained.
[h] ISCO sampler malfunctioned after collecting 10 samples; additional grab sample collected at end of storm event.
[i] Reduced number of NBU and NBH samples retained during first portion of storm event; none retained from second portion.

As shown in Table 2.1, in multiple instances sampler malfunction led to either no samples or a reduced number of samples being collected during a specific storm event. In addition, some samples collected during later storm events were not retained. Specifically, only a subset of the samples for determination of AMPA and glyphosate (unbuffered samples) collected during the 10/08/2010 to 10/10/2010 and 12/10/2010 to 12/12/2010 storm events was retained. This decision was informed by

analytical results showing that AMPA and glyphosate were not detected in earlier storm events. This was also the basis for terminating collection of samples for determination of imazapyr, sulfometuron methyl, and metsulfuron methyl after the 12/10/2010 to 12/12/2010 storm event.

2.3 Analytical Strategy

The goals of this study were to characterize how herbicide concentrations in streamwater varied during storm events and the concentrations that might manifest in baseflow. Considering the relatively small numbers involved, baseflow samples were analyzed in chronological order until all herbicides were no longer detected.

The strategy used in analysis of samples collected during storm events was to analyze samples closest to the peak in the site-specific hydrograph (stage level at the site-specific flume) first, and then work outwards toward the leading and trailing edges of the hydrograph peak until either a clear maximum in herbicide concentration manifested or it became obvious that such a maximum would not manifest. This process started with the first storm event and was terminated once it became clear that herbicide concentrations in samples collected during storm events had dropped to non-detectable levels.

As noted, samples were also collected immediately before and during application of the herbicides. Samples collected at NBH were analyzed first, beginning with samples collected prior to application and continuing until concentrations had returned to the site-specific pre-application background or it became obvious that no herbicide was going to be detected.

For efficiency, all analyses for determination of AMPA and glyphosate were completed prior to initiating determinations of imazapyr, sulfometuron methyl, and metsulfuron methyl.

3.0 MATERIALS AND METHODS

3.1 Sample Handling and Preservation

Two ISCO pump samplers (Model #6712) were installed at each of the three sampling sites (Figure 2.1). Samples for determination of imazapyr, metsulfuron methyl, and sulfometuron methyl were collected in one set of bottles (1 L ISCO polypropylene sector bottles) containing 5 mL of a 2M phosphate buffer solution. Thus, the samples were buffered at pH 7 at the time of collection (NCASI 2007; Fischer, Michael, and Gibbs 2008). Samples for determination of AMPA and glyphosate were collected in a separate set of bottles (i.e., by the other ISCO sampler) that did not contain buffer. All samplers were manually programmed to trigger at set times based on predicted storms (Section 2.2). Each sampler could collect 24 samples and most storms were monitored using an hour interval between samples. Sampling frequency was reduced when multiple storms or prolonged events were forecast. In addition to the storm event samples collected using the ISCO samplers, baseflow grab samples were manually collected on a nominally weekly basis.

Samples collected by the ISCO samplers were removed from the field as soon as possible (usually within 48 hours of collection) and delivered to the NCASI West Coast Regional Center laboratory (approximately one hour away from the Needle Branch sites). On receipt, nominal 800 mL aliquots of the pH 7 preserved samples were transferred to 1 L high density polyethylene (HDPE) bottles and frozen whole (i.e., without filtration). Two nominal 400 mL sample splits were generated for a subset of these whole, pH preserved samples; one was spiked with imazapyr, metsulfuron methyl, and sulfometuron methyl prior to freezing.

Once in the laboratory, multiple splits of the unbuffered samples were generated, including a 20 mL split for isotope analysis and sometimes[1] a 400 mL split for determination of suspended sediments (SS). These samples were forwarded to researchers at Oregon State University. Subsequently, nominally 180 mL of whole, unbuffered sample was filtered (0.7 µm glass fiber filter) and the filtrate was collected in a 250 mL HDPE bottle and frozen. Separate 180 mL splits of a subset of samples were spiked with glyphosate and AMPA prior to filtration and freezing. Additional volumes of whole (unfiltered) samples were frozen when there was visual indication of SS, and some splits of whole samples were also spiked with glyphosate and AMPA prior to freezing.

3.2 Determination of Dissolved Glyphosate and AMPA

Sample extracts were prepared as described by Hanke, Singer, and Hollender (2008). Appendix A provides notes on the procedures used, sources of standards, and so on. Briefly, after thawing, 80 mL of sample filtrate in a 125 mL HDPE bottle was adjusted to pH 1 with 6M hydrochloric acid (HCl) and set aside for one hour. After this, 6M potassium hydroxide (KOH) was added to achieve pH ≥2.5. Working one sample at a time and as rapidly as possible, 10 mL of 40mM borate buffer followed by 10 mL of 6.5mM 9-fluorenylmethylchloroformate (FMOC-Cl in acetonitrile) were added to initiate derivatization of glyphosate and AMPA. Samples were held at 35°C for two hours, then 1 mL formic acid was added and each sample was filtered through a 0.45 µm nylon membrane into a 250 mL HDPE bottle holding 4 mL of 1M ethylenediaminetetraacetic acid (EDTA). The original 125 mL HDPE bottle was rinsed three times with nominally 33 mL laboratory water (a total of 100 mL laboratory water); each rinse was put through the nylon membrane and collected with the sample. The derivatized sample was loaded onto a conditioned solid phase extraction (SPE) cartridge at nominally 10 mL/min and the cartridge was then dried for 30 minutes. After this drying step, 3.5 mL dichloromethane (DCM) was pulled through the SPE cartridge and the cartridge was dried for an additional 15 minutes. Finally, the dried SPE cartridge was gravity eluted with 4 mL methanol and all eluant was collected in a 15 mL polypropylene conic tube. The contents of this tube were concentrated to 200 µL at 50°C under a gentle stream of nitrogen and 800 µL of reagent water was added to obtain a final extract of 1 mL 80:20 water:methanol. A syringe filter was used to transfer this extract to an autosampler vial and the final extract was held for analysis.

All final extracts were analyzed using high performance liquid chromatography (HPLC) with fluorescence (FLUOR) detection in place of the HPLC-mass spectrometric (LC/MS-MS) analysis used by Hanke, Singer, and Hollender (2008). HPLC separations were performed using a Phenomenex Luna NH_2 column with a gradient elution (see Appendix A for details). Fluorescence was monitored using excitation at 264 nm and emission at 315 nm.

All quantifications were made versus an external calibration generated using purchased pre-derivatized glyphosate-FMOC and AMPA-FMOC (see Appendices A and B). The calibration range for the associated instrumental calibration (ICAL) was 1.2 to 1200 ng/mL (extract concentration) of underivatized glyphosate (a.e.) and AMPA. Assuming an initial sample volume of 80 mL and a final extract volume of 1 mL, this corresponds to a calibration range of 15 to 15,000 ng/L (ppt as a.e. glyphosate) in samples. The experimentally determined study-specific method detection limits (MDLs) were 3.8 ng/L for AMPA and 18 ng/L for glyphosate (see Appendix C, Section 1.2.2 for discussion of these MDLs). Thus, for glyphosate the MDL was actually higher than the lower calibration level (LCL) of the ICAL.

[1] Decisions to generate splits for determination of SS were made by the field sampler based on visual inspection.

As noted, Appendix A provides additional detail on this analytical procedure. In addition, Appendix B discusses various stand-alone experiments executed to characterize the performance of specific aspects of the procedure. Results from these experiments are discussed in Appendix B and are noted where appropriate herein.

3.3 Determination of Glyphosate and AMPA on Suspended Sediment

A limited number of whole unfiltered samples were also analyzed. The first step was to filter 80 mL of whole sample (in a 125 mL HDPE bottle) using a 0.7 µm glass fiber filter (GFF). The resulting filtrate was treated as described in Section 3.2. The wet filter with any solids was collected and placed back into the original 125 mL HDPE bottle to which 80 mL 0.5M KOH and a Teflon™-coated stir bar had been added. The sample was then sonicated for one hour. After sonication, the bottle was shaken to disintegrate the GFF and the pH was adjusted to <9 using 6M HCl. Immediately thereafter, 10 mL of 40mM borate buffer followed by 10 mL of 6.5mM FMOC-Cl (in acetonitrile) were added to initiate derivatization of glyphosate and AMPA, and the remainder of the analysis was performed as described for filtrates.

Appendix A provides additional detail on the analytical procedure used to analyze SS collected via filtration, and Appendix B gives some results characterizing the performance of the procedure. Based on results presented in Appendix B, the solids analysis was limited to 10 mg of Needle Branch SS per derivatization (or 125 ppm SS in a 80 mL sample; see Appendix B, Section 2.0), so the lowest point in the instrumental calibrations (Section 3.2) corresponded to a sample concentration of nominally 0.12 mg/kg (ppm on dry solids), or 15 ng/L in an 80 mL sample regardless of the SS concentration. MDLs for AMPA and glyphosate on solids were not determined.

3.4 Determination of Dissolved Imazapyr, Sulfometuron Methyl, and Metsulfuron Methyl

Dissolved imazapyr, sulfometuron methyl, and metsulfuron methyl were determined using a basic approach described by multiple researchers (Wells and Michael 1987; Powely and deBernard 1998; Rodriguez and Orescan 1998) and modified by NCASI. Details of this analytical method have been presented elsewhere (NCASI 2007). Briefly, a 200 mL volume of thawed sample was filtered (0.45 um nylon membrane filter) and the filtrate was adjusted to pH ≤2.3 by addition of dilute phosphoric acid. Immediately following acidification, the sample was pulled through a reverse-phase SPE cartridge. This cartridge was dried and then placed on top of a conditioned strong anion exchange (SAX) SPE cartridge. Analytes were eluted from the reversed-phase SPE cartridge and through the SAX cartridge using 50 mL of methanol. The methanol was collected and taken to dryness. The residue was made up in exactly 1.0 mL of water:acetonitrile (80:20, v:v), filtered through a 0.45 µm membrane filter into an autosampler vial, and held for analysis.

All extracts were analyzed by HPLC with an ultraviolet absorbance detector (HPLC/UV). The instrumental conditions used in these analyses are summarized in Table 3.1. [Note that the chromatographic elution was optimized for analysis of Needle Branch samples, and thus was different than the elution used historically (NCASI 2007).]

All quantifications were made versus an external calibration spanning the range of 0.125 to10 µg/mL (extract concentration) of each herbicide (imazapyr a.e.). Assuming an initial sample volume of 200 mL and a final extract volume of 1 mL, this corresponds to a calibration range of 0.625 to 50 µg/L (ppb as a.i.) in samples. The experimentally determined study-specific MDLs were 0.2 µg/L for imazapyr (a.e.), 0.5 µg/L for sulfometuron methyl, and 1.0 µg/L for metsulfuron methyl (see Appendix C, Section 2.2.2 for a discussion of these MDLs).

Table 3.1 Instrumental Conditions Used in HPLC/UV Analysis of Final Extracts for Determination of Dissolved Imazapyr, Sulfometuron Methyl, and Metsulfuron Methyl

Column	Phenomenex® Luna® phenyl-hexyl 5 µm, 17.5%, 250 x 4.6 mm				
Guard Cartridge	Phenomenex® Security Guard® system 2 Phenomenex® Phenyl(phenylpropyl) cartridges (4 mm L x 3 mm ID)				
Column temperature	35 ±1°C				
Injection volume	25 µL				
Autosampler temperature	20 ±1°C				
Detector	UV/Vis, 235 and 195 nm				
Flow rate	1 mL/min				
		Percent			
Mobile phase gradient	min	0.024M H_3PO_4	Methanol	Curve	Comment
	0	72	28		Start data acquisition.
	20	72	28	linear	
	25	50	50	convex 4	
	50	50	50	linear	End data acquisition.
	54	6	94	linear	Flush.
	64	6	94	linear	
	68	72	28	linear	Re-equilibrate.
	75	72	28	linear	

4.0 DISCUSSION

4.1 Method Performance and Analytical Caveats

Measured analyte concentrations are tabulated in Appendices D through H, and Appendix C provides a thorough discussion of these results on an analyte-specific basis. The Appendix C discussions are summarized here prior to providing a more general discussion of concentration results.

4.1.1 *Glyphosate and AMPA*

The MDLs cited in Section 3.2 were determined via replicate analyses of a single baseflow sample collected at NBL immediately prior to application of herbicides in August 2010. As noted in Appendix C, Section 1.2.2, the resulting MDLs for both AMPA and glyphosate reflect the impact of background interference at sample concentrations equivalent to ≈2 ng/L AMPA and ≈13 ng/L glyphosate (a.e.). Ultimately, it was shown that the interference impacting both analytes varied from sample to sample. The interference affecting AMPA was as high as ≈7 ng/L (as AMPA) in some samples (Appendix C, Section 1.2.5), while the interference impacting glyphosate reached ≈40 ng/L (Appendix C, Section 1.2.4). Under these circumstances, the cited MDLs provide academic measures of method performance; that is, true analyte-specific MDLs were impacted by the presence of variable background interference such that the MDLs varied from sample to sample.

More importantly, the inability to discriminate these interferences means that all concentrations from NCASI's analyses carry some high bias unless each result is background subtracted. However, because the levels of interference affecting both AMPA and glyphosate were shown to vary on a sample-specific basis, background subtraction was not defensible. Compounding this dilemma, study-specific quality assurance (QA) showed losses of both analytes over the analytical process (Appendix C, Section 1.2.3).

Recovery of AMPA from the analysis was on the order of 80% (20% loss). However, all measured AMPA concentrations were low enough (<12 ng/L) to be impacted by background interference. The level of this interference was as high as ≈7 ng/L (as AMPA) in some samples, indicating that all measured concentrations could carry >50% high bias. Thus, the AMPA concentrations in Appendix E are considered to carry a net high bias (Appendix C, Section 1.2.5). Recovery was on the order of 90% (10% loss) for glyphosate, and measured glyphosate ranged from ≈18 (i.e., not detected) to ≈150 ng/L. As noted, the interference impacting glyphosate was shown to range from ≈13 to ≈40 ng/L. Thus, even at the highest concentrations found in this study, high bias almost certainly outweighed low bias, meaning that glyphosate concentrations in Appendix D are also considered to carry a net high bias (Appendix C, Section 1.2.4).

In the broadest sense, this situation reflects the limitations of LC/FLUOR analysis vs. LC/MS (or LC/MS-MS) analysis (Hanke, Singer, and Hollender 2008). More specifically, an LC/MS-MS analysis has greater potential to discriminate interference from chromatographic co-elutors as a result of better selectivity (mass spectrometry vs. fluorescence). Thus, when sample splits were submitted for confirmatory analysis by LC/MS-MS, glyphosate concentrations from NCASI's LC/FLUOR analysis were shown to be high biased by anywhere from 6.6 to 42 ng/L, corresponding to a 25 to 100% high bias on a sample-specific basis (Appendix C, Section 1.2.6). Because the LC/FLUOR analysis is actually more sensitive than an LC/MS-MS analysis, all of NCASI's AMPA results were less than the LC/MS-MS reporting limit and so could not be confirmed by the LC/MS-MS analysis.

These factors are discussed in greater detail in subsequent sections.

4.1.2 *Imazapyr, Sulfometuron Methyl, and Metsulfuron Methyl*

The MDLs cited in Section 3.3 were determined via replicate analyses of a single baseflow sample collected at NBL and, as discussed in Appendix C, Section 2.2.2, the resulting MDLs for imazapyr, sulfometuron methyl, and metsulfuron methyl reflect the impact of background interference at sample concentrations equivalent to ≈0.1 µg/L imazapyr (a.e.), ≈0.2 µg/L sulfometuron methyl (a.i.), and ≈0.4 µg/L metsulfuron methyl (a.i.). As with AMPA and glyphosate (Section 4.1.1), results showed that the magnitudes of the analyte-specific interferences varied from sample to sample (Appendix C, Section 2). However, sulfometuron methyl and metsulfuron methyl were not detected in any sample at concentrations above the MDLs cited in Section 3.3, so the issues around background interference and bias are moot.

Imazapyr was detected in only a handful of samples and all measured concentrations were low enough (≤0.4 µg/L) to be impacted by background interference, which results showed to be as high as ≈0.2 µg/L in some samples (Appendix C, Section 2.2.4). Because this interference was known to vary from sample to sample, background subtraction was not performed. Imazapyr recovery was ≈80% at the concentrations found in samples (≤0.4 µg/L), indicating that high bias due to background interference almost certainly overwhelmed low bias due to losses incurred during analysis.

The situation with imazapyr is generally analogous to that for AMPA and glyphosate (Section 4.1.1), and there are laboratories that use LC/MS-MS for determination of imazapyr (as well as sulfometuron methyl and metsulfuron methyl). However, no LC/MS-MS confirmatory analyses were performed for these analytes. Thus, all that can be said is that all reported imazapyr concentrations (Appendix F) carry unknown high bias.

These factors are discussed in greater detail in subsequent sections.

4.2 Sample Collection and Stage Data

Figure 4.1 shows stage (water height at the flume) data for NBL covering the period over which most samples were collected. (Although a limited number of samples collected after 10/27/2010 were

analyzed and results are reported in the appropriate appendices, these samples are not shown in the figure.) The figure also shows when storm event and baseflow samples were collected for determination of herbicides. As noted in Section 2.2, samples were collected at all three sites at the same time; that is, each sample point shown in Figure 4.1 represents a sample collected at NBH, NBU, and NBL.

NBL stage data clearly reflect the impact of each storm event. More importantly, the figure also shows that the sample collection regimen effectively sampled each storm event.

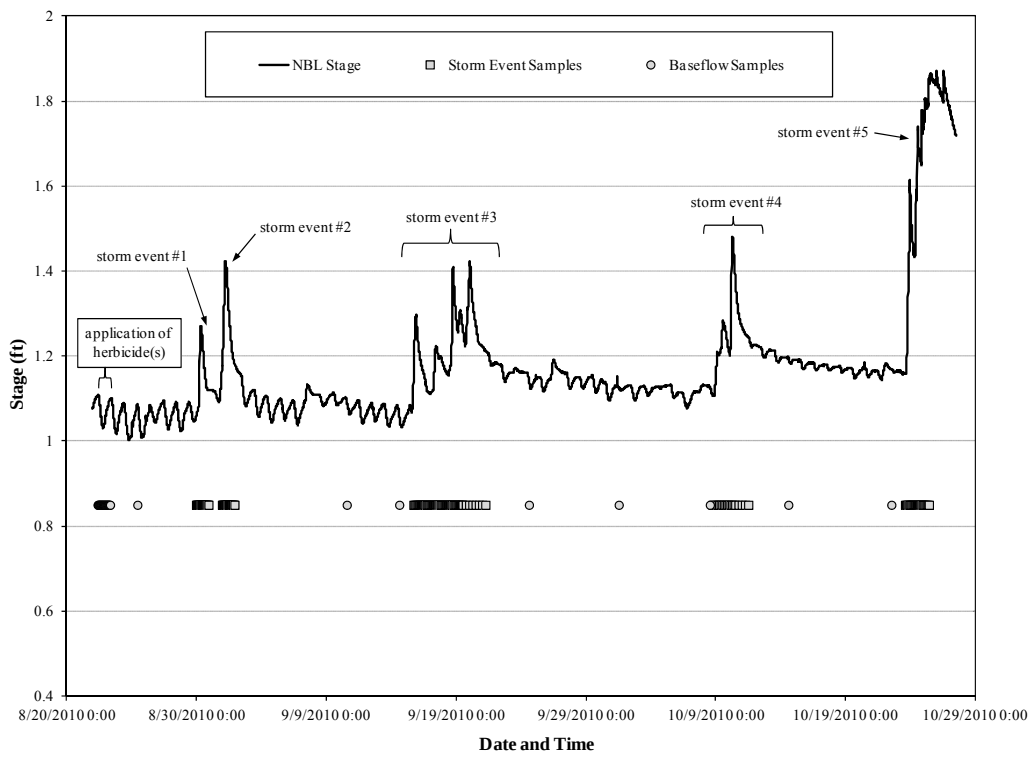

Figure 4.1 Stage Level at NBL from 08/22/2010 through 10/27/2010 with Identification of All Sampling Events

4.3 Herbicides in Streamwater during Application

Aerial application of the herbicides was initiated at ≈11:00 a.m. on 8/22/2010. As noted, each ISCO sampler was programmed to collect a sample at 9:00 a.m. and every hour thereafter. Dissolved glyphosate results from this sampling are presented in Figure 4.2, and show a clear pulse (or spike) in dissolved glyphosate at NBH during application of the herbicides. Because there are measured values reflecting site-specific background immediately prior to application of glyphosate, issues regarding background subtraction (Section 4.1.1) are potentially moot, suggesting that the mean of these event- and site-specific background values can be subtracted from the associated event- and site-specific results. This pre-application background averaged 16.7 ±1.9 ng/L (n = 3)[2] as glyphosate, giving a background-corrected maximum concentration of 45 ng/L. However, it is possible that the

[2] This background is higher than the mean obtained from replicate analyses of both refrigerated and frozen blank control sample, which averaged 13.0 ng/L and 12.8 ng/L, respectively (Appendix C, Table C1.3), again showing that the background interference acting on glyphosate was variable.

background was dynamic even over the limited period of time during which these samples were collected (e.g., the background might vary diurnally), so the background-corrected result could still carry unknown bias. Thus, it is simpler to accept the uncorrected result (62 ng/L) as a high-biased estimate of the maximum concentration. In any case, results show that the maximum concentration manifested in the first sample collected after application was initiated and that concentrations dropped to pre-application background in nominally six hours. That is, the pulse of dissolved glyphosate lasted no more than six hours and the maximum concentration persisted for no more than two to three hours.

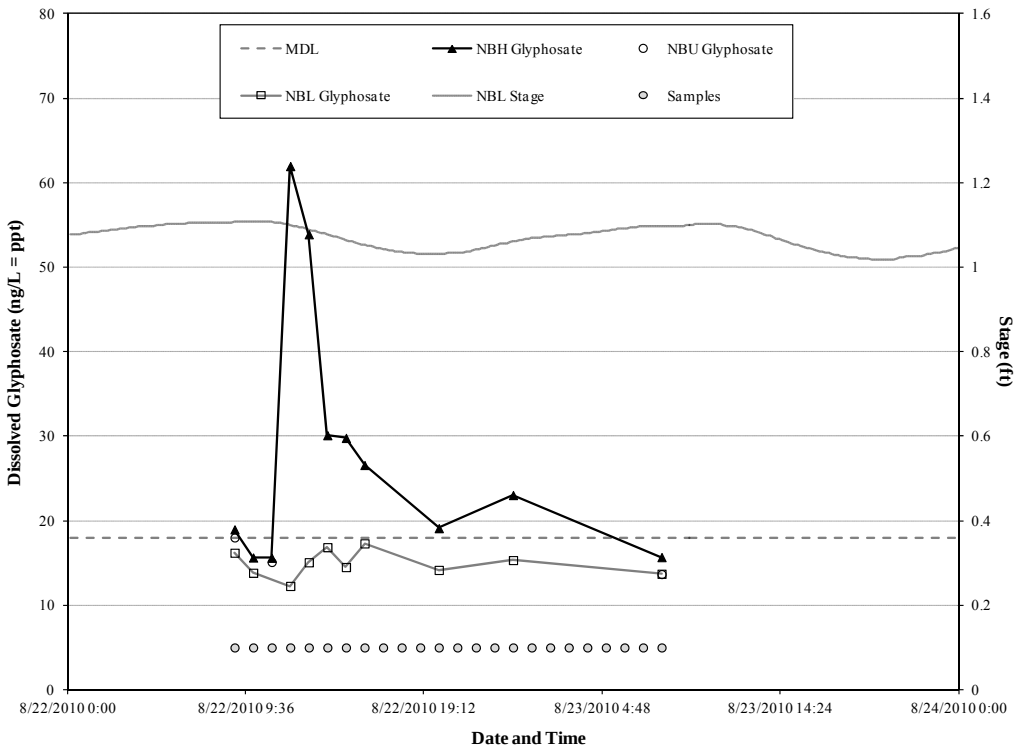

Figure 4.2 Dissolved Glyphosate in Streamwater (baseflow) Collected at NBH, NBU, and NBL during Application of Herbicides [all concentrations plotted regardless of MDL]

Dissolved glyphosate at NBL remained at concentrations at or below the MDL (i.e., all reported concentrations were <18 ng/L) during the application period. The ISCO sampler at NBU malfunctioned after collecting the first three samples, which reflect pre-application background only. These three samples gave concentrations nominally equivalent to those found in the corresponding NBH and NBL samples (Figure 4.2), as did a sample collected at NBU ≈20 hours after application.

As might be expected, AMPA was not detected in any sample collected during application (i.e., all samples returned <4 ng/L dissolved AMPA). As noted in Section 4.1.2, sulfometuron methyl and metsulfuron methyl were not detected in any samples collected during this study.

On the other hand, as shown in Figure 4.3, some baseflow samples collected at NBU during the application gave results for dissolved imazapyr exceeding the MDL (0.2 µg/L), which was based on measurements made in the blank control (a pre-application baseflow sample collected at NBL). However, the highest concentration found in any of these samples was 0.31 µg/L (reported as 0.3 µg/L in Appendix F), less than twice the mean site-specific pre-application background signal (≈0.2 µg/L; Figure 4.3) and well below the LCL of the ICAL, which was 0.6 µg/L (Section 3.4). As

discussed in Appendix C, Section 2.2.4, these factors suggest that all concentrations shown in Figure 4.3 reflect variability in the site-specific background interferent known to be present, not the presence of dissolved imazapyr. The fact that these results (Figure 4.3) do not show a clear pulse in dissolved imazapyr as was seen for glyphosate (Figure 4.2) is additional evidence that imazapyr was not present in these samples.

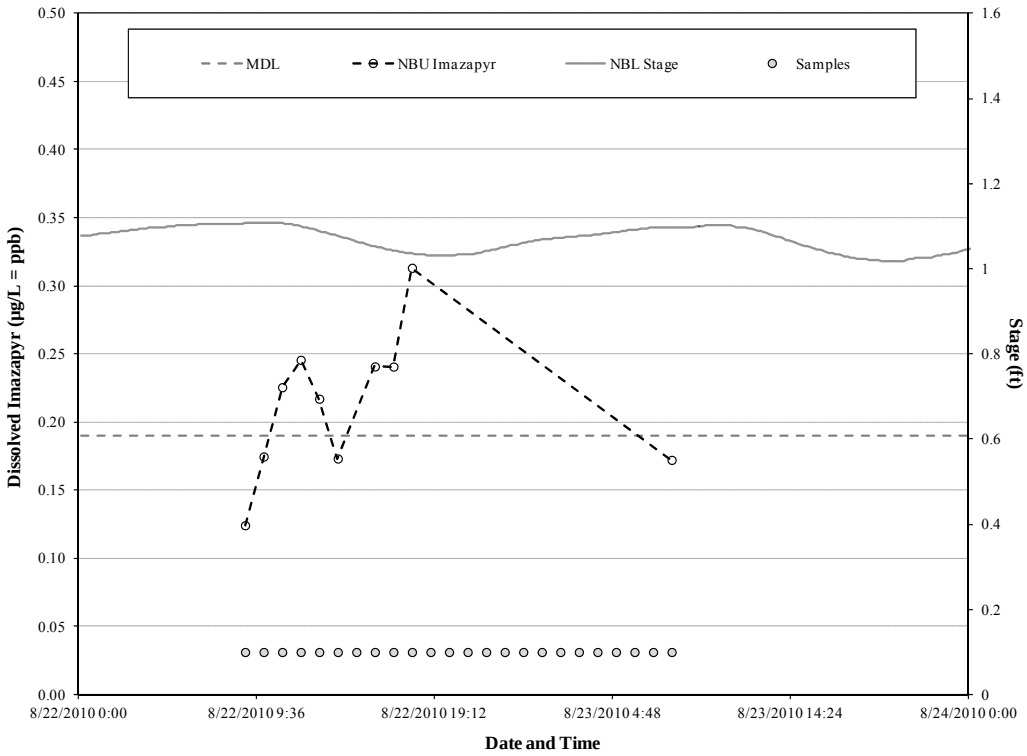

Figure 4.3 Dissolved Imazapyr in Streamwater (baseflow) Collected at NBU during Application of Herbicides [all concentrations plotted regardless of MDL]

Unfortunately, the ISCO sampler at NBH malfunctioned during this sampling episode, so there are no imazapyr data for NBH. Based on the results at NBU, it was decided that there was no purpose in analyzing the NBL samples. Thus, results shown in Figure 4.3 are the only data indicating whether dissolved imazapyr manifested in baseflow during the application, and they support the absence of imazapyr during this period. Ultimately, however, given the uncertainties around variability in the background interferent and the fact that all measured concentrations were less than the ICAL LCL, the most defensible statement concerning these samples is that dissolved imazapyr was <0.6 µg/L (i.e., the ICAL LCL) in all of them.

4.4 Dissolved Herbicides in Baseflow

All samples returned non-detects for sulfometuron methyl (MDL = 0.5 µg/L) and metsulfuron methyl (MDL = 1 µg/L), so these herbicides were not detected in any baseflow sample. Thus, the only statement that can be made concerning these herbicides is that dissolved concentrations in baseflow never exceeded the herbicide-specific MDLs.

In the first set of post-application baseflow samples, collected three days after application of herbicides (days after treatment, or DAT), imazapyr was detected at 0.2 µg/L at NBH but was not detected (<0.2 µg/L) at NBU or NBL. The second set of baseflow samples was collected 19 DAT,

and imazapyr was detected at 0.2 µg/L at NBU but was not detected (<0.2 µg/L) at NBH or NBL. Thereafter, imazapyr was not detected in any baseflow sample collected at any site out to 33 DAT, at which point analysis of baseflow samples for determination of imazapyr, sulfometuron methyl, and metsulfuron methyl was discontinued. As discussed in Appendix C, Section 2.2.4, none of these results can be taken as definitive evidence for the presence of dissolved imazapyr and, ultimately, the most defensible statement that can be made is that dissolved imazapyr was <0.6 µg/L in all of these samples.

Measured concentrations of dissolved glyphosate in baseflow samples were also low, ranging from non-detect (<18 ng/L) to 34 ng/L (Appendix D). These results are shown in Figure 4.4, which also shows results from the LC/MS-MS confirmation analysis performed on selected samples (LC/MS-MS results from Appendix C, Table C1.5).

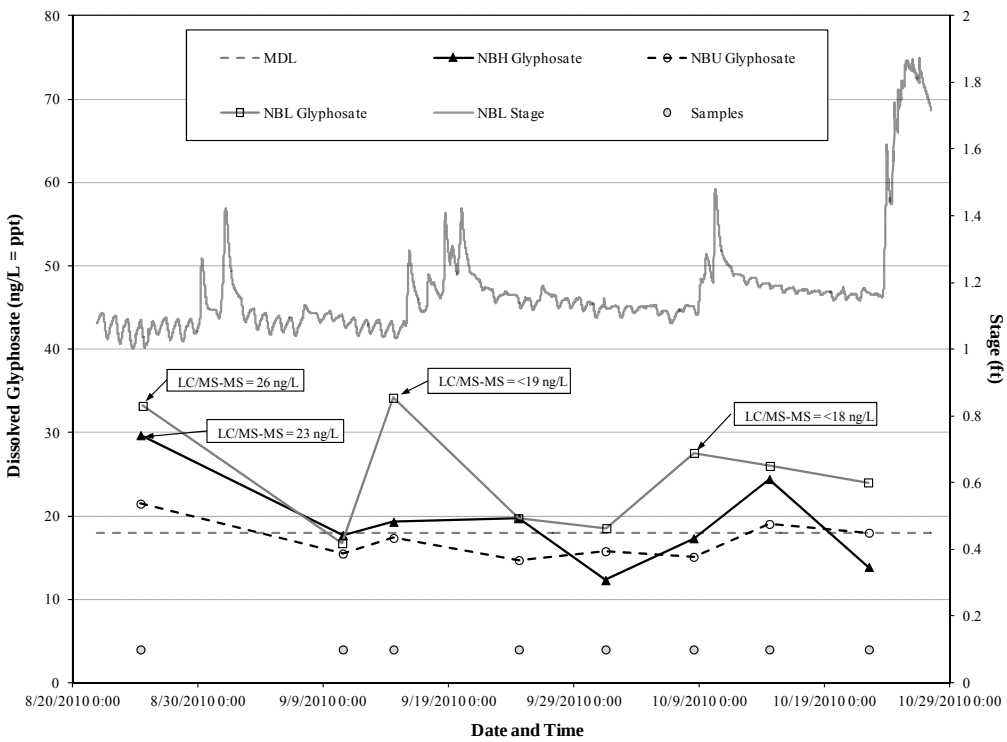

Figure 4.4 Dissolved Glyphosate in Baseflow Samples Collected at NBH, NBU, and NBL with Results from LC/MS-MS Confirmations [all concentrations plotted regardless of MDL]

Figure 4.4 shows that LC/MS-MS analyses always returned lower concentrations than those found by NCASI's analyses, again illustrating that NCASI's results are high biased (Section 4.1.1). The LC/MS-MS results also confirm the presence of dissolved glyphosate in the first baseflow samples collected at NBH and NBL after application of herbicides, which were collected on 8/25/2010 (3 DAT). Based on LC/MS-MS results, dissolved glyphosate was present at nominally 25 ng/L in these baseflow samples (3 DAT). NCASI's results show that dissolved glyphosate concentrations dropped to <20 ng/L by the second baseflow sampling (9/10/2010, 19 DAT). Although some of NCASI's results were >20 ng/L in subsequent baseflow samples, the LC/MS-MS results effectively show that dissolved glyphosate remained at concentrations <20 ng/L in baseflow samples collected after 9/10/10 (19 DAT).

Overall, these results show that dissolved glyphosate in baseflow was ≈25 ng/L for a few days immediately following application of herbicides and dropped to <20 ng/L by 19 DAT, by which time two storm events had impacted the study site.

The first baseflow samples collected at NBH, NBU, and NBL following application of herbicides returned 6 to 7 ng/L dissolved AMPA. All subsequent baseflow samples from NBH and NBU returned non-detects (i.e., reported results <4 ng/L). On the other hand, AMPA concentrations in subsequent NBL baseflow samples were variable, ranging from <4 to 8 ng/L.

These results suggest the presence of up to 6 to 7 ng/L dissolved AMPA in baseflow for a few days immediately following application of herbicides, and that concentrations at NBH and NBL dropped to <4 ng/L by 19 DAT. They also suggest that dissolved AMPA in NBL baseflow remained at concentrations in the range of 4 to 8 ng/L out to 75 DAT (Appendix E). However, all measured AMPA concentrations at all three sites were low enough to be biased by background interference, which is known to be as high as 7 ng/L (as AMPA) in samples (Section 4.1.1), and all were well below the 15 ng/L ICAL LCL. Ultimately, because of the uncertainties concerning sample-to-sample variability in the background signal and the fact that all measured concentrations were below the ICAL LCL, the most defensible conclusion to be drawn from these results is that dissolved AMPA was <15 ng/L in all post-application baseflow samples (see Appendix C, Section 1.2.5, for additional discussion).

4.5 Dissolved Herbicides in Streamwater during First and Second Storm Events after Application

Figure 4.5 shows dissolved glyphosate results for samples collected at all three sites during the first two post-application storm events. These storms occurred on 8/30/2010 (8 DAT) and 9/1/2010 (10 DAT). The results clearly show pulses of dissolved glyphosate at NBU during the 8/30/2010 storm event and at NBH during the 9/1/2010 storm event.

Although it cannot be proven, the absence of any observable pulse at NBH during the first storm event might be attributed to triggering the autosamplers too late (i.e., the highest concentrations at NBH could have manifested before sample collection was initiated). Certainly, the absence of a pulse at NBH during this first storm event is inconsistent with results obtained during the second storm event, which showed a clear pulse at NBH and only suggestions of pulses at the other two sites. Regardless, results from the second storm event suggest that any glyphosate pulse at NBH during the first storm event would have shown a maximum concentration on the order of twice the concentration seen at NBU; that is, the maximum concentration would have been around 300 ng/L. However, this estimate does not account for the potential impact of background interference on measured glyphosate (Section 4.1.1) and, ultimately, the apparent behavior of glyphosate at NBH during the first storm event remains inexplicable.

A number of the samples represented in Figure 4.5 were submitted for analysis by LC/MS-MS, and results from these confirmation analyses are included in the figure (data from Appendix C, Table C1.5). Comparing NCASI's results to those obtained via LC/MS-MS analysis confirms that NCASI's results are high biased, and that the absolute magnitude of this bias is sample specific. Thus, the LC/MS-MS results show that the maximum concentration at NBU during the 8/30/2010 storm event was 115 ng/L, not 149 ng/L (NCASI result biased high by 34 ng/L). LC/MS-MS results also show that the maximum concentration at NBH during the 8/30/2010 storm event was 42 ng/L instead of the 84 ng/L from NCASI's analysis (NCASI result biased high by 42 ng/L).

Of greater significance, the samples showing the highest concentrations found by NCASI at NBL during the two storm events, 51 ng/L and 48 ng/L on 8/30/2010 and 9/1/2010, respectively, returned non-detects at 19 ng/L by the LC/MS-MS analysis. This supports the conclusion that dissolved

glyphosate was <20 ng/L in all NBL samples collected during these two storm events. In addition, the LC/MS-MS analysis of the sample with the second highest concentration found by NCASI at NBU during the 9/1/2010 storm event also returned a non-detect (<18 ng/L) from the LC/MS-MS analysis. This supports the conclusion that dissolved glyphosate was <20 ng/L in all NBU samples collected during the 9/10/2010 storm event.

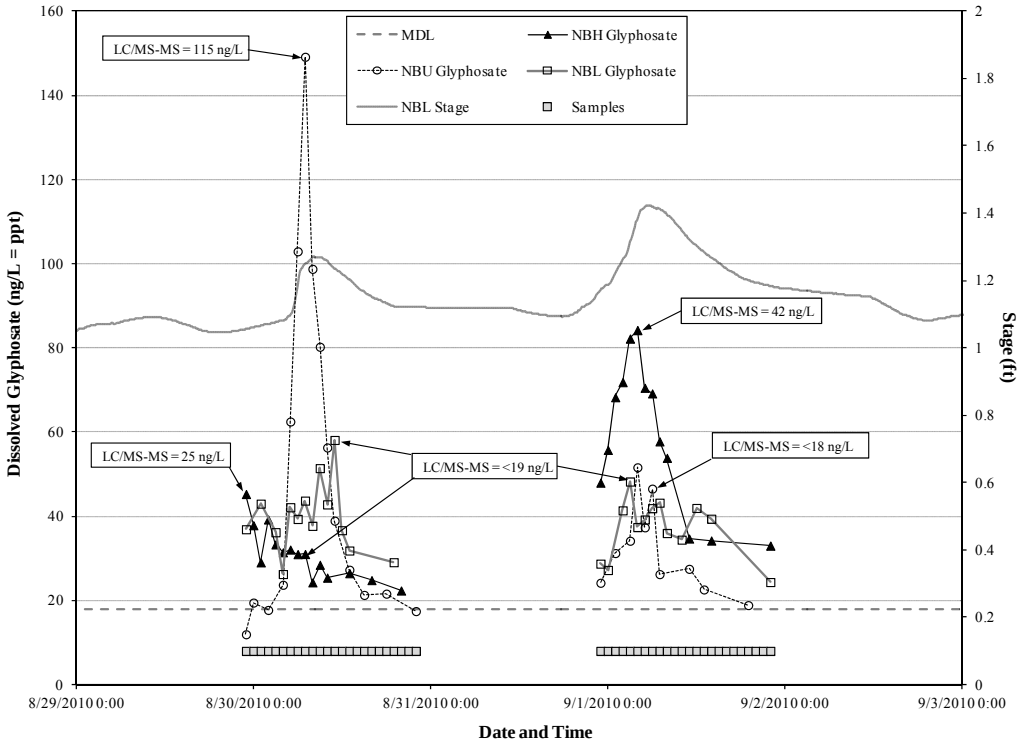

Figure 4.5 Dissolved Glyphosate at NBH, NBU, and NBL during First Two Storm Events after Application of Herbicides with Results from LC/MS-MS Confirmations [all concentrations plotted regardless of MDL]

Figure 4.6 shows results for dissolved AMPA from the same samples shown in Figure 4.5. Many returned non-detects (<4 ng/L), indicating that the sample-specific result was no greater than the mean background found in the frozen blank control. In addition, a majority of the measured concentrations were less than three times the mean background found in the same frozen blank control (2.4 ng/L; Appendix C, Table C1.3), and all were less than the 15 ng/L ICAL LCL (thus all measured AMPA concentrations must be considered estimates; Appendix C, Section 1.2.5). Beyond this, there was no clear pulse in dissolved AMPA at any site during either storm event, with the possible exception of NBH during the second storm event. This observation alone suggests that there was no measurable AMPA in any of these samples and that all that was being measured was the background interferent. Thus, the results shown in Figure 4.6 could be interpreted as demonstrating that this interferent varied from site to site during these storm events. However, without additional data this is only speculation. Ultimately, the most defensible statement regarding dissolved AMPA is that concentrations in streamwater collected at all three sites during these two storm events were <15 ng/L (Appendix C, Section 1.2.5).

As noted, all samples returned non-detects for sulfometuron methyl (MDL = 0.5 µg/L) and metsulfuron methyl (MDL = 1 µg/L), so these herbicides were not detected in any samples collected

during any storm event. Thus, the only statement that can be made concerning these herbicides is that dissolved concentrations in streamwater influenced by storm runoff never exceeded herbicide-specific MDLs.

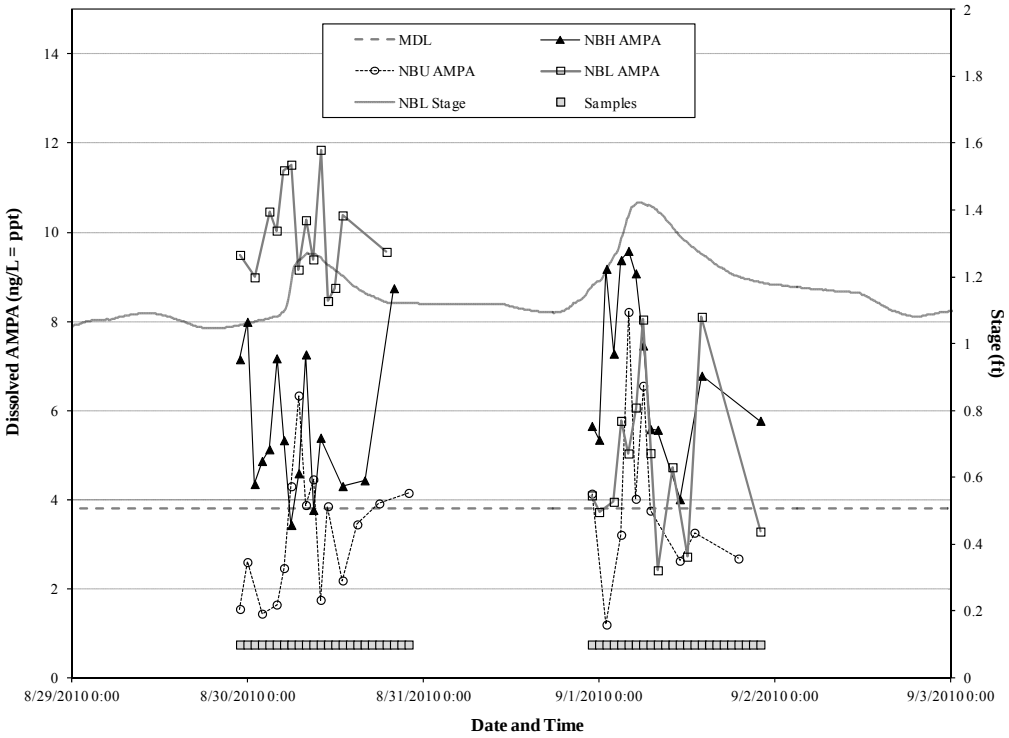

Figure 4.6 Dissolved AMPA at NBH, NBU, and NBL during First Two Storm Events after Application of Herbicides [all concentrations plotted regardless of MDL]

Measured imazapyr concentrations in samples collected at NBH and NBU during the first two post-application storm events ranged from <0.2 µg/L (i.e., not detected above the mean background found in the blank control) to 0.4 µg/L (Appendix E). Thus, all measured dissolved imazapyr concentrations were low enough to be biased by the background interferent known to be present in all samples (Section 4.1.2), and were also below the 0.6 µg/L ICAL LCL. In addition, results showed no clear imazapyr pulse as was observed for glyphosate (Figure 4.5), indicating that the measured concentrations reflected variability in the background signal. Overall, these results can be taken as evidence of the absence of measurable dissolved imazapyr in streamwater at NBH and NBU during these storm events (the ISCO sampler at NBL malfunctioned during these sampling events). However, as with dissolved AMPA, this is a hypothesis, and the most defensible conclusion is that dissolved imazapyr was always <0.6 µg/L in these storm event samples (see Appendix C, Section 2.2.4 for additional discussion).

4.6 Dissolved AMPA and Glyphosate in Streamwater during Third Storm Event after Application

Figure 4.7 shows results for dissolved glyphosate in samples collected at all three sites during the third post-application storm event. This storm started on 9/15/2010 (24 DAT) and continued through 9/21/2010 (30 DAT). These results show no evidence for the kind of pulse in dissolved glyphosate seen during the first two post-application storm events (Figure 4.5) and, with a handful of exceptions, all concentrations were less than three times the mean concentration found in the frozen blank

control. This suggests that these measured concentrations reflect the variable background interferent known to be present in samples. This is supported by results from the LC/MS-MS confirmation analysis, which returned non-detects (<20 ng/L) for the two samples submitted. This outcome is significant, as one of these samples (from NBH) returned the highest concentration (62 ng/L) from NCASI's analysis of any sample from this storm event. Overall, these results support the statement that dissolved glyphosate was <20 ng/L in streamwater at all three sites during the third storm event.

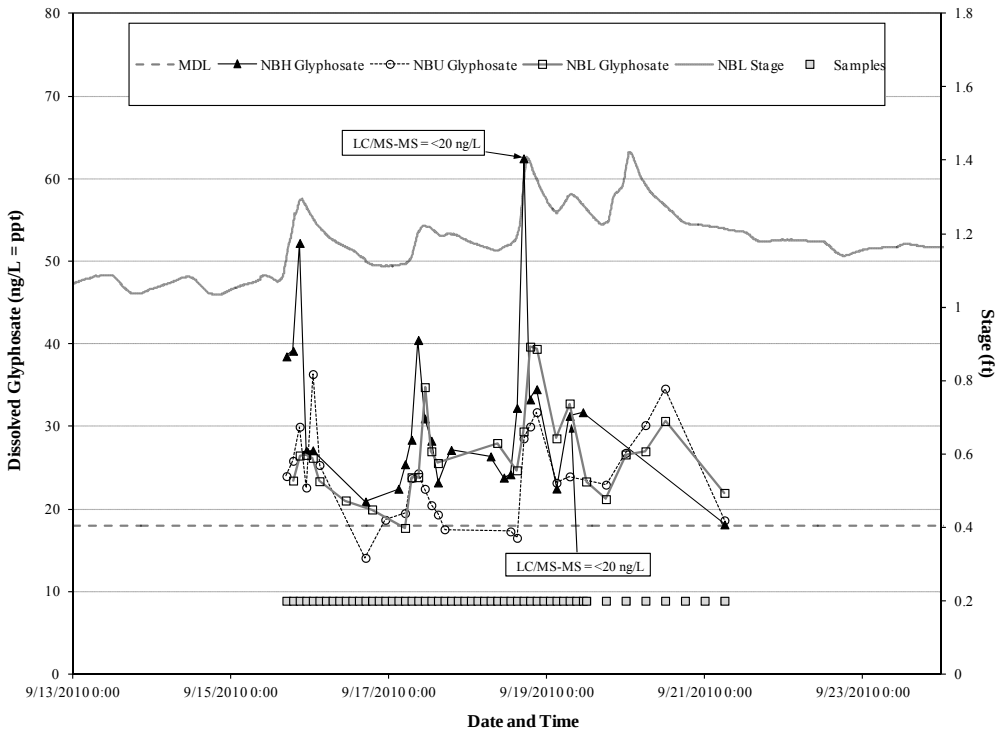

Figure 4.7 Dissolved Glyphosate at NBH, NBU, and NBL during Third Storm Event after Application of Herbicides with Results from LC/MS-MS Confirmations [all concentrations plotted regardless of MDL]

Concentrations of dissolved AMPA measured by NCASI in these samples ranged from <4 to 9 ng/L. As with the baseflow samples (Section 4.4) and samples collected during the first and second storm events (Section 4.5), all these concentrations are low enough to be impacted by the background interferent known to be present in samples and are less than the ICAL LCL (15 ng/L). In addition, as observed in the results from the first and second storm events, there was no pulse in dissolved AMPA at any site during the third storm event.

Overall, it is highly probable that there was no measurable AMPA in any of the samples collected during the third storm event, and that what was being measured was the background interferent. Again, however, without additional data, this is only speculation. Thus, the most defensible statement regarding dissolved AMPA is that concentrations in streamwater collected at all three sites during the third storm were <15 ng/L.

As noted in Section 4.1.2, sulfometuron methyl and metsulfuron methyl were not detected in any sample analyzed as part of this study. In addition, dissolved imazapyr was not measured at concentrations greater than 0.4 µg/L in any sample collected during the first two storm events (Section 4.5), and there was no evidence of an imazapyr pulse during either storm event. Based on

these results, no samples from the third storm event (or any subsequent storm event) were analyzed for determination of these herbicides.

4.7 Dissolved AMPA and Glyphosate in Streamwater during Fifth Storm Event after Application

Results from analysis of samples collected during the first three storm events showed that dissolved glyphosate was <20 ng/L at all three sampling sites by the third storm event, and that dissolved AMPA was, effectively, indistinguishable from background in all three storm events. Likewise, even though some samples collected during the first two storm events returned imazapyr detects from NCASI's analysis, none of these detects were at concentrations high enough to be free of high bias attributable to background interference and none exceeded the ICAL LCL. In addition, on a storm- and site-specific basis, none of these data showed any evidence for a pulse of dissolved imazapyr. Thus, overall, results from the first three storm events indicate that glyphosate, AMPA, and imazapyr were at background levels by the third storm event at the latest. This, coupled with the fact that sulfometuron methyl and metsulfuron methyl were not detected above background in any sample, suggested that analysis of samples collected from subsequent storm events would serve no purpose. However, based on the NBL stage data shown in Figure 4.1, the fifth storm event following application was the largest event to manifest during this study, so some of these samples were analyzed for AMPA and glyphosate only. Dissolved glyphosate results from these analyses are shown in Figure 4.8.

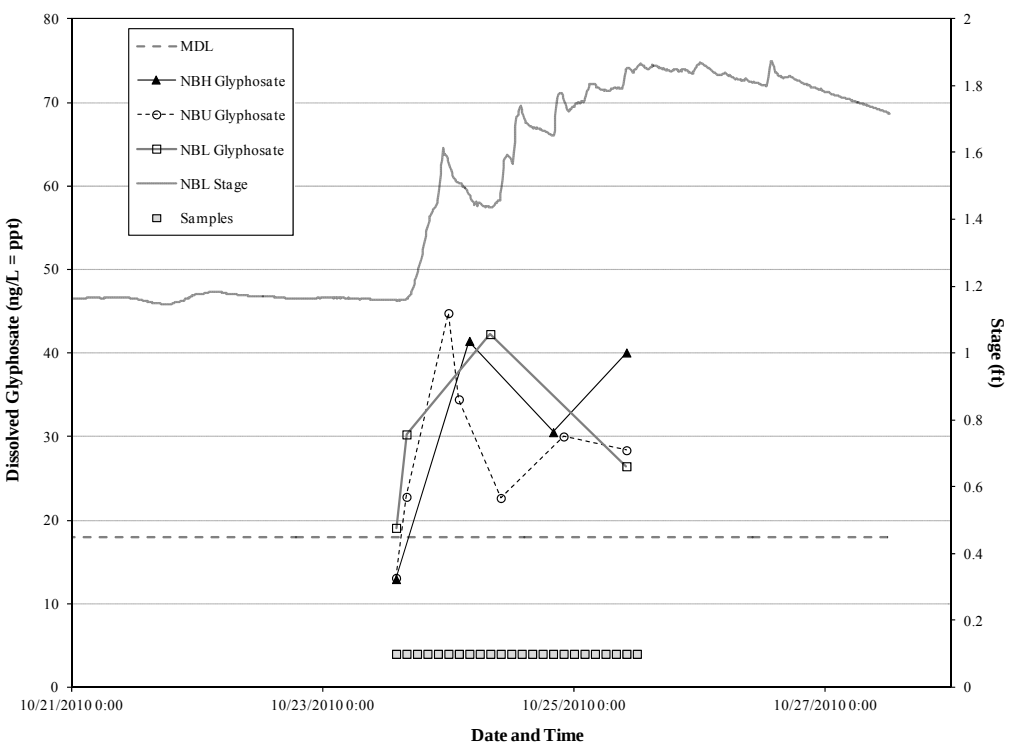

Figure 4.8 Dissolved Glyphosate at NBH, NBU, and NBL during Fifth Storm Event after Application of Herbicides [all concentrations plotted regardless of MDL]

The dissolved glyphosate results shown in Figure 4.8 are similar to those from the second (Figure 4.5; NBU and NBL only) and third (Figure 4.7; all three sites) storm events in that there was no clear pulse in dissolved glyphosate at any site and all concentrations were low enough to be impacted (i.e.,

biased) by background. Thus, these concentrations probably reflect background interference more than the presence of dissolved glyphosate. This is supported by the result from the single LC/MS-MS analysis performed on any of these samples, which returned a non-detect (<20 ng/L) for the sample collected two hours after the sample showing the highest concentration (41 ng/L) at NBH in Figure 4.8. Altogether, these results support concluding that dissolved glyphosate was <20 ng/L in all these samples.

Results for dissolved AMPA in these samples were also similar to those from analysis of samples collected during the earlier storm events. Measured dissolved AMPA concentrations ranged from <4 to 6 ng/L and showed no evidence of a pulse. Thus, the most defensible interpretation of these results is that dissolved AMPA was <15 ng/L in all samples collected during this storm event.

4.8 AMPA and Glyphosate on Suspended Sediments

The LCL of the calibration used to quantify AMPA and glyphosate on SS was 1.2 ng/mL (a.e.) in an extract regardless of the mass of SS actually extracted. That is, the calibration LCL corresponds to different concentrations on solids but is constant when expressed in terms of sample volume as long as 80 mL of sample was filtered. The only caveat to this is the requirement that the total mass of SS extracted not exceed 10 mg/L; thus, SS in an 80 mL sample should be ≤125 mg/L (Appendix C, Section 1.3). As noted, SS concentrations were not measured in samples. However, it was the assessment of all involved that SS was generally low (<125 mg/L). As a consequence, only a handful of SS analyses were performed, and in all cases a full 80 mL of sample was filtered.

The results of these analyses (Appendix C, Table C1.11) showed that regardless of the true SS concentration in each sample, the mass of both AMPA and glyphosate on SS contributed to the total mass found in samples was *de minimis*. As an example, the single highest dissolved glyphosate concentration found in any sample by NCASI's analysis was 149 ng/L (the corresponding LC/MS-MS result was 115 ng/L). When the unfiltered (whole) split of this sample was filtered, the filtrate and SS fractions analyzed separately, and the results summed the total glyphosate concentration was 155 ng/L (NCASI's analysis). Thus, results suggest that glyphosate on SS was equivalent to ≈6 ng/L (or ≈4% of the total mass of glyphosate in this sample). However, this almost certainly overstates the contribution of SS to total glyphosate because the background interference impacting glyphosate in extracts obtained from filtrates also manifested in SS extracts (Appendix B, Section 3.2). This means that even when there is no glyphosate on sample SS, an associated measured total concentration is expected to be ≈13 ng/L higher than a dissolved concentration solely as a consequence of the background interference.

As discussed in detail in Appendix C, Section 1.3.5, in no case was the mass of glyphosate found on SS >13 ng/L. Thus, regardless of the apparent relative (percent) increase in glyphosate resulting from adding glyphosate on SS to dissolved glyphosate (Appendix C, Table C1.11), the increase can be attributed to background interference in the SS measurement.

The ultimate interpretation of these results is that there was no measurable glyphosate on sample SS. Obviously, the low level of SS in these samples may have been the primary factor contributing to this outcome.

Analysis of AMPA results follows the discussion of glyphosate; that is, any AMPA on sample SS can be attributed to background interference acting on the SS measurement. Thus, the final interpretation is that there was no measurable AMPA on sample SS (see Appendix C, Section 1.3.5 for additional discussion).

5.0 SUMMARY

5.1 Dissolved AMPA and Glyphosate Concentrations in Streamwater

5.1.1 *Streamwater Collected during Application of Herbicides*

As shown in Figure 4.2, there was a clear pulse of dissolved glyphosate at NBH during application of the herbicides. This pulse showed the highest concentration in the first sample collected after application was initiated, and then tailed off over approximately six hours; that is, the pulse persisted for no more than six hours, and the "peak" persisted for only two to three hours. Glyphosate in this pulse "peaked" at 62 ng/L without background subtraction, or 42 ng/L after subtracting the event- and site-specific background signal. No glyphosate pulse was detected in samples collected at NBL, and no samples were collected at NBU during the application (autosampler malfunction).

As might be expected, AMPA was not detected in any sample collected during the application.

5.1.2 *Streamwater Collected during Storm Events*

Results support the following statements regarding dissolved glyphosate in streamwater collected during storm events (Sections 4.5, 4.6, and 4.7):

1. During the first two storm events after application (8 and 10 DAT) dissolved glyphosate manifested in streamwater as discrete pulses with a duration of 8 to 10 hours.
2. No pulses in dissolved glyphosate were observed in later storm events.
3. The maximum concentration observed during storm events decreased from NBH to NBU to NBL (i.e., decreased going downstream from the application site).
4. The maximum concentration observed at each site decreased with each storm event.
5. Dissolved glyphosate in streamwater collected at NBL during the first storm event (8 DAT) was <20 ng/L[3] (i.e., no pulse of dissolved glyphosate was observed at NBL during any storm event).
6. Dissolved glyphosate was <20 ng/L at NBU by the second storm event (10 DAT).
7. Dissolved glyphosate was <20 ng/L at NBH by the third storm event (24 DAT).
8. The highest dissolved glyphosate concentration found in any sample was 115 ng/L at NBU during the first storm (8 DAT); this concentration persisted for no more than two to three hours.

The last of these statements should be qualified by noting that no pulse in dissolved glyphosate was observed at NBH during the first storm event. Based on the totality of the results, a pulse at NBH is to be expected, and it would also be expected that the maximum concentration in this pulse would be higher than that seen at NBU during the same storm event. Thus, the 115 ng/L observed at NBU during the first storm event following application of herbicide may not have been as high as would have been found at NBH during the same storm event.

Measured dissolved AMPA was <12 ng/L in all baseflow and storm event samples, and dissolved AMPA in streamwater collected during storm events was generally at concentrations equivalent to those found in baseflow. In no case was a clear pulse of dissolved AMPA observed during a storm event. Taken together, these factors suggest that all measurements reflect variability in the background interferent known to be present in all samples rather than the actual presence of AMPA. The measured concentrations certainly carry high bias due to this background, and all were less than the 15 ng/L ICAL LCL for AMPA. Thus, the most defensible conclusion that can be drawn from

[3] Based on results obtained from LC/MS-MS confirmation analysis (Section 4.5).

these results is that dissolved AMPA was <15 ng/L in all streamwater samples collected during storm events.

5.1.3 *Streamwater Collected during Post-Application Baseflow Conditions*

Results from streamwater collected during baseflow conditions showed dissolved glyphosate at ≈25 ng/L (based on results from the LC/MS-MS confirmation analysis) at all three sites 3 DAT. The next baseflow sample, collected 19 DAT, showed <20 ng/L dissolved glyphosate at all three sites, and all subsequent baseflow samples also showed <20 ng/L. Thus, results show that baseflow immediately following the application contained ≈25 ng/L of dissolved glyphosate for a short period (days to perhaps two weeks) and that concentrations dropped to <20 ng/L by 19 DAT.

Results suggested that there was 6 to 7 ng/L dissolved AMPA in baseflow at all three sites 3 DAT, and that concentrations dropped to <4 ng/L in baseflow at NBH and NBU by the next baseflow sampling (19 DAT) but remained at these approximate levels throughout the study period at NBL (the last baseflow sample analyzed for determination of AMPA and glyphosate was collected 103 DAT). However, these measured concentrations are all in the range of concentrations measured in various pre-application (background) samples, which gave concentrations as high as 7 ng/L (as AMPA), and all are below the ICAL LCL for AMPA (15 ng/L). Thus, the most defensible conclusion is that dissolved AMPA was <15 ng/L in all baseflow samples.

5.2 AMPA and Glyphosate on Suspended Sediments

Glyphosate and AMPA were not found on sample SS at concentrations greater than the background interferent known to be present in SS extracts (Section 4.8). Combining this with the observation that all samples contained little to no SS indicates that export of glyphosate and AMPA on SS was truly *de minimis*.

5.3 Dissolved Imazapyr, Sulfometuron Methyl, and Metsulfuron Methyl Concentrations in Streamwater

5.3.1 *Streamwater Collected during Application of Herbicides*

Due to a malfunction of the autosampler, no samples were collected at NBH during application of herbicides, and because of the low concentrations found in samples collected at NBU none of the samples collected at NBL were analyzed.

Samples collected at NBU during the application gave imazapyr measurements ranging from <0.2 (i.e., non-detect) to 0.3 µg/L (Appendix F). However, no clear pulse of imazapyr was observed. As discussed in Section 4.3, concentrations at these levels are subject to bias due to background interference and are below the ICAL LCL for imazapyr (0.6 µg/L). Although it cannot be proven, this suggests that what was being measured in these samples was background, not imazapyr. Because of the uncertainties concerning sample-to-sample variability in the background signal and the resulting uncertainties regarding detection, the most defensible conclusion to be drawn from these results is that dissolved imazapyr was <0.6 µg/L in NBU baseflow samples collected during the application of herbicides.

Sulfometuron methyl and metsulfuron methyl were not detected in any sample collected during this study, including those collected at NBU during application of herbicides. Thus, all that can be said about these herbicides in samples collected during the application is that concentrations never exceeded MDLs, which were 0.5 µg/L and 1.0 µg/L for sulfometuron methyl and metsulfuron methyl, respectively.

5.3.2 *Streamwater Collected during Storm Events*

Although measured imazapyr exceeded the MDL (0.2 µg/L) in a handful of storm event samples, the highest concentration detected was 0.4 µg/L, indicating that all measured imazapyr concentrations were low enough to be biased high due to the impact of background interference and were less than the associated ICAL LCL, which was 0.6 µg/L. In addition, in no case was there any evidence for a pulse of imazapyr during a storm event. Taken together, these factors suggest that all results for imazapyr in samples collected during storm events reflect variability in the background interferent rather than the presence of imazapyr. However, this cannot be proven. Thus, the most defensible conclusion that can be drawn from these results is that dissolved imazapyr was <0.6 µg/L in all samples collected during post-application storm events.

Sulfometuron methyl and metsulfuron methyl were not detected in any sample collected during this study. Thus, all that can be said about these herbicides in samples collected during storm events is that concentrations never exceeded MDLs, which were 0.5 µg/L and 1.0 µg/L for sulfometuron methyl and metsulfuron methyl, respectively.

5.3.3 *Streamwater Collected during Post-Application Baseflow Conditions*

Imazapyr was not detected (i.e., <0.2 µg/L) in the vast majority of baseflow samples collected after application of herbicides, and exceeded the MDL only in one baseflow sample collected at NBH and one collected at NBU. As discussed in Section 4.4, concentrations at these levels are subject to bias due to background interference and are below the ICAL LCL for imazapyr (0.6 µg/L). Although it cannot be proven, this suggests that what was being measured in these samples was background, not imazapyr. Ultimately, because of the uncertainties concerning sample-to-sample variability in the background signal and the resulting uncertainties regarding detection, the most defensible conclusion to be drawn from these results is that dissolved imazapyr was <0.6 µg/L in all post-application baseflow samples.

Neither sulfometuron methyl nor metsulfuron methyl were detected in any sample collected during this study. Thus, all that can be said about these herbicides in post-application baseflow samples is that concentrations never exceeded MDLs, which were 0.5 µg/L and 1.0 µg/L for sulfometuron methyl and metsulfuron methyl, respectively.

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APPENDIX A

NOTES ON PROCEDURES FOR DETERMINING AMPA AND GLYPHOSATE IN SAMPLE FILTRATES AND ON SAMPLE SUSPENDED SEDIMENTS

This appendix provides details on the materials and methods used for determination of aminomethylphosphonic acid (AMPA) and glyphosate in both sample filtrates and sample suspended sediments (SS).

1.0 MATERIALS AND METHODS

1.1 Analytical Standards

AMPA (#MET-1051A) and glyphosate (#PS-1051) were purchased as solids from Chem Service, Inc. (West Chester, Pennsylvania). Individual primary standards were prepared by diluting exact masses into 0.1N hydrochloric acid (HCl; ACS reagent grade, EMD #HX0603-75). All secondary (i.e., spiking) solutions were also prepared in 0.1N HCl. Fluorenylmethylchloroformate (FMOC-Cl) derivatives of AMPA (#10205800) and glyphosate (#04151000) were purchased as solids from Crescent Chemical Co. (Islandia, New York), and individual primary standards were prepared by diluting exact masses into high performance liquid chromatography (HPLC) grade methanol (Honeywell Burdick and Jackson #230-4).

1.2 Sample Filtrations

Samples for determination of dissolved AMPA and glyphosate were filtered upon receipt at nominally 0.7 µm using 47 mm glass fiber filters (GFF) (Pall Life Sciences #66258) and 300 mL magnetic polysulfone filter funnels (VWR #28143-550). Filtration was performed on a solid phase extraction (SPE) vacuum manifold (Sigma Aldrich #57044) equipped with a vacuum pump (Gast #DOA P161-AA), allowing ≈160 mL of filtrate to be collected in a 250 mL high density polyethylene (HDPE) bottle with a polypropylene cap (Nalgene #042744).

1.3 Derivatization of Sample Filtrates

The derivatization regent was 6.5mM 9-fluorenylmethylchloroformate (HPLC grade FMOC-Cl; Sigma Aldrich #23184) prepared in HPLC grade acetonitrile (Macron #2856-10). This reagent was prepared on the day of use.

An 80 mL volume of sample filtrate in a 125 mL HDPE bottle (Thermo Scientific #332189-004) was adjusted to pH 1 using 6N HCl (ACS reagent grade, EMD #HX063-75) and held for one hour. Subsequently, 6M potassium hydroxide (KOH) (semiconductor grade pellets, Sigma Aldrich #306568) was added to obtain a pH between 2.5 and 9, followed rapidly by addition of 10 mL of 40mM pH 9 borate buffer (sodium tetraborate decahydrate; Alfa Aesar #40114) and then 10 mL 6.5mM FMOC-Cl. Bottles were then capped, shaken, and placed in a 35°C water bath for a minimum of two hours.

After removal from the water bath, 1 mL of formic acid (ACS reagent grade; Sigma Aldrich #33015) was added and the sample was filtered through a 47 mm 0.45 µm nylon membrane (Whatman #7404-004) into a 250 mL HPDE bottle holding 4 mL of 1M ethylenediaminetetraacetic acid (EDTA; EMD #EX0550). The bottle used in the derivatization was then rinsed three times using ≈33 mL reagent water (Sybron/Barnstad #D2798) for each rinse (for a total of 100 mL), and each rinse was passed through the nylon membrane and collected in the same bottle holding the derivatized sample. The sample was then passed to post-derivatization cleanup.

1.4 Post-Derivatization Solid-Phase Extraction Cleanup

Post-derivatization cleanup utilized an SPE cartridge and was performed on the same vacuum manifold used in sample filtration (Section 1.2 herein). The flow rate was controlled by adjusting the bleed on the vacuum applied to the manifold, and a small polypropylene/Teflon™ stopcock (Phenomenex #AH0-6049) between the SPE manifold liner (Sigma Aldrich #57059) and the SPE cartridge (Phenomenex Strata-X #8BS100-FCH) was used to isolate individual SPE cartridges. A 60 mL polypropylene reservoir (Varian #12121012) was stacked on top of the SPE cartridge using an adaptor cap (Sigma Aldrich #57020-U).

To condition an SPE cartridge, 5 mL methanol was added and allowed to soak into the packing for about one minute with the stopcock closed and the vacuum pump off. The stopcock was then opened until the methanol meniscus was just above the packing frit, when it was closed again. Then, 10 mL of 0.1% formic acid was added to the SPE cartridge and the stopcock was immediately opened. The stopcock was closed when the meniscus was ≈7 mm from the top of the SPE packing frit.

With the stopcock still closed, the vacuum pump was turned on, the vacuum was adjusted to ≈7″ mercury, and the 60 mL sample reservoir was filled with sample. The stopcock was opened and the vacuum was adjusted to ≈3″ mercury immediately after elution from the SPE cartridge was observed. This process resulted in a sample loading rate of ≈10 mL/min. After elution of all sample, the SPE cartridge was dried by pulling air through it for 30 minutes at maximum vacuum. The stopcock was closed, the vacuum pump was adjusted to ≈3″ mercury, and 3.5 mL HPLC grade dichloromethane (DCM; Honeywell Burdick and Jackson #300-4) was added to the SPE cartridge. The DCM was pulled through the cartridge and then dried for 15 minutes at maximum vacuum, after which the vacuum pump was shut off and the stopcock was closed.

A 15 mL graduated conic polypropylene tube (Nalge Nuc #36060) was placed below the SPE tube and 4 mL of methanol was added to the SPE cartridge. This methanol was allowed to soak for about one minute, after which the stopcock was opened and the methanol eluted by gravity. The vacuum pump was then turned on and the last bit of methanol was collected by incrementally increasing the vacuum.

1.5 Final Extract Concentration and Filtration

The methanol in the 15 mL conic tube was concentrated to 200 µL using nitrogen blowdown and a 50°C waterbath. The final volume was made up to 1 mL by adding 800 µL of reagent water. The conic tube was capped and shaken vigorously. A disposable glass pipette was used to transfer the contents of the tube to a 3 mL polypropylene syringe (Becton Dickinson #309585) fitted with a 0.45 µm nylon syringe filter (Phenomenex #AF3-3107-52), and the final extract was filtered into an HPLC autosampler vial.

1.6 Extraction of Suspended Sediment

Samples were filtered using 0.7 µm GFF filters (Section 1.2 herein) and the filter holding the SS was placed in a 125 mL HDPE bottle containing 80 mL 0.5M KOH (Sigma Aldrich #306568) and a 1″ Teflon™-coated stir bar. The bottle was tightly capped and placed in a sonic bath (Buehler Ultramet® II; #75-1970-115) for one hour. After sonication, the capped bottle was shaken to disintegrate the GFF. Working with eight or fewer bottles at a time, pH was adjusted to <9 (2.5 to 9) by addition of 6N HCl, and derivatization was initiated by addition of 10 mL of 40mM pH 9 borate buffer and then 10 mL 6.5mM FMOC-Cl. From this point forward, SS extracts were handed exactly as sample filtrates.

1.7 Instrumental Analysis

1.7.1 *Preparation of Calibration Standards*

Instrumental calibrations (ICALs) were generated using pre-derivatized chemicals purchased from Crescent Chemicals (Section 1.1 herein). Thus, primary standards containing AMPA-FMOC and glyphosate-FMOC were prepared by diluting an exact mass of purchased solid into 100% methanol. These single component primary solutions were prepared at 0.3 to 0.4 mg/mL, and were stable for at least 12 months when stored in amber glass vials in a freezer.

A series of intermediate spiking solutions (AMPA-FMOC plus glyphosate-FMOC in 100% methanol) were prepared from these primary standards at different concentrations and used in preparing all ICALs. These intermediate spike mixes were prepared at concentrations such that adding exactly 200 µL (using an auto pipette) of different intermediates to exactly 800 µL (auto pipette) of blank water gave a series of calibration solutions spanning a range from nominally 1 to 1000 ng/mL in the final 1 mL calibration standard. Given an initial sample volume of 80 mL, this calibration range corresponds to 12.5 to 12500 ng/L (ppt) in samples[4]. (Note that this calibration range reflects the sensitivity of a Waters 474 fluorescence detector and could have been pushed lower using a Waters 2475 detector.)

Once diluted to 80:20 water:methanol, standards were kept in a refrigerator. Although a formal stability study was not performed, these injection standards were observed to be stable for nominally 72 days. All instrumental sets, including analysis of sample extracts, included analysis of at least one freshly prepared calibration verification (CALVER) standard prepared from a methanol primary.

1.7.2 *Instrumental Analysis*

Final extracts were analyzed by HPLC (Waters Alliance 2695) with fluorescence detection (Waters 474 and Waters 2475). All chromatographic separations were performed on a Phenomenex Luna NH_2 column (Phenomenex #00G-4378-E0) in combination with a guard column configuration consisting of two 4x3 mm cartridges packed with the same material (Phenomenex #AJO-4302). The elution conditions are given in Table A1.

[4] Depending on the primary standard used in preparation, the lowest calibration level (LCL) of some ICALs was ≈1.2 ng/mL (corresponding to 15 ng/L in an 80 mL sample).

Table A1 HPLC Conditions

Column	Phenomenex Luna NHs, 5 µm 100 Å, 250x4.60 mm, reversed phase mode			
Mobile phase component A	pH 5.50 0.04 M phosphate buffer			
Mobile phase component B	Acetonitrile			
Column temperature	30.0°C			
Sample temperature	20.0°C			
Injection volume	25 µL			
Gradient	Min	%A	%B	Curve
	0	70	30	
	35	70	30	6 (linear)
	39	40	60	3 (convex)
	71	40	60	6
Fluorescence detector events	Min	Event	Action	
	0	λ_{Em}	875 nm	
	0	λ_{Ex}	800 nm	
	12	PMT gain	10	
	12	λ_{Ex}	264 nm	
	12	λ_{Em}	315 nm	
	30	PMT gain	1	
	53	PMT gain	20	

APPENDIX B

EXPERIMENTS TO CHARACTERIZE THE PERFORMANCE OF THE ANALYTICAL METHOD FOR DETERMINATION OF GLYPHOSATE AND AMPA

NCASI performed a number of stand-alone experiments to characterize various aspects of the analytical workflow for determining aminomethylphosphonic acid (AMPA) and glyphosate. The results are presented here.

1.0 INSTRUMENTAL CALIBRATIONS, CALIBRATION BIAS, AND SAMPLE BACKGROUND

1.1 Instrumental Calibrations

Over the course of this work, multiple instrument calibrations (ICALs) were generated using two different lots of pre-derivatized standards. In all cases, the factor triggering recalibration was the need to replace the high performance liquid chromatography (HPLC) column or perform instrumental maintenance. These calibrations were essentially indistinguishable (suggesting that recalibration was, in fact, not necessary).

Table B1 summarizes pooled response factor (RF) data from six ICALs generated using a Waters 474 fluorescence detector, which was the primary detector used in this work. Figure B1 is a plot showing the results for both AMPA and glyphosate from one of these ICALs, and Figure B2 gives example chromatograms showing the chromatographic peaks for AMPA-FMOC and glyphosate-FMOC from analysis of the lowest concentration standard used in this ICAL (≈1 ng/mL as extract concentration, equivalent to 12.5 ng/L in an 80 mL sample).

Table B1 Summary of Different Response Factor (RF) Data

Calibration Type[a]	Matrix	Description		AMPA	Glyph
ICAL	Injection solvent	RF statistics from pooling six ICALs developed using two separate lots of purchased pre-derivatized standards (extract concentrations ≈1-1000 ng/mL)	Mean RF[b]	36137	21932
			Std Dev[b]	892	938
			RSD[b]	2.5	4.3
DICAL	Laboratory blank water	Mean RF (5-1000 ng/L in extracts)	RF	28085	19552[c]
		DICAL vs. ICAL RF	(%)	78	89
		background concentration vs. ICAL (extract concentration)	(ng/mL)	0.12	0.51
DICAL	Needle Branch filtrate[d]	Mean RF (5-1000 ng/L in extracts)	RF	25495	19935
		DICAL vs. ICAL RF	(%)	71	91
		background concentration vs. ICAL (extract concentration)	(ng/mL)	0.60	0.50
DICAL	0.5M KOH	Mean RF (5-1000 ng/L in extracts)	RF	28009	17978
		DICAL vs. ICAL RF	(%)	78	82
		background concentration vs. ICAL (extract concentration)	(ng/mL)	0.51	0.84
DICAL	Needle Branch SS extract[e]	Mean RF (5-1000 ng/L in extracts)	RF	21896	17553
		DICAL vs. ICAL RF	(%)	61	80
		background concentration vs. ICAL (extract concentration)	(ng/mL)	3.6	1.5

[a] ICAL generated via dilution of pre-derivatized solids; DICAL generated by spiking sample matrix with underivatized analytes then performing derivatization.
[b] Mean calculated using individual mean RFs from each of six separate ICALs (n = 6).
Std Dev = standard deviation; RSD = relative standard deviation.
[c] Mean RF calculated using results spanning range from 10 to 1000 ng/mL in extracts (see text).
[d] From filtration of Needle Branch sample containing ≈155 mg/L (ppm) SS; herbicide spikes added to sample filtrate immediately prior to derivatization.
[e] rom filtration of Needle Branch sample containing ≈155 mg/L (ppm) SS; herbicide spikes added to SS extract immediately prior to derivatization.

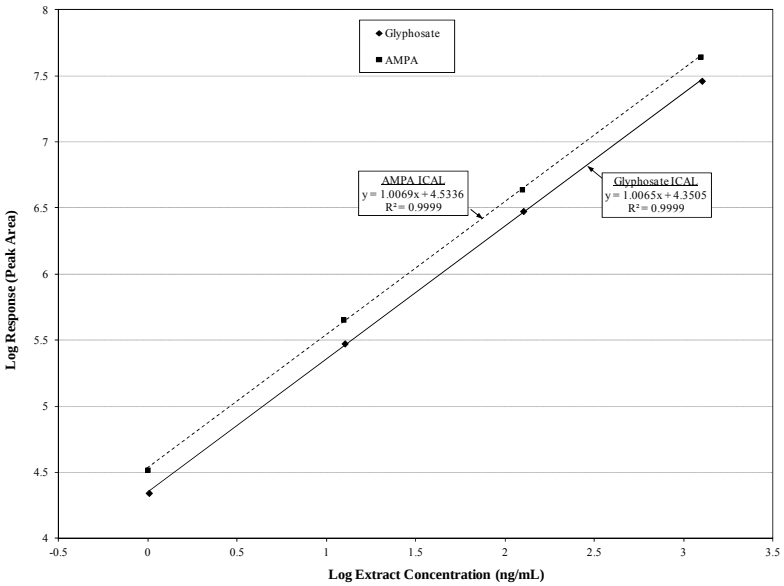

Figure B1 Example Instrumental Calibrations for AMPA and Glyphosate

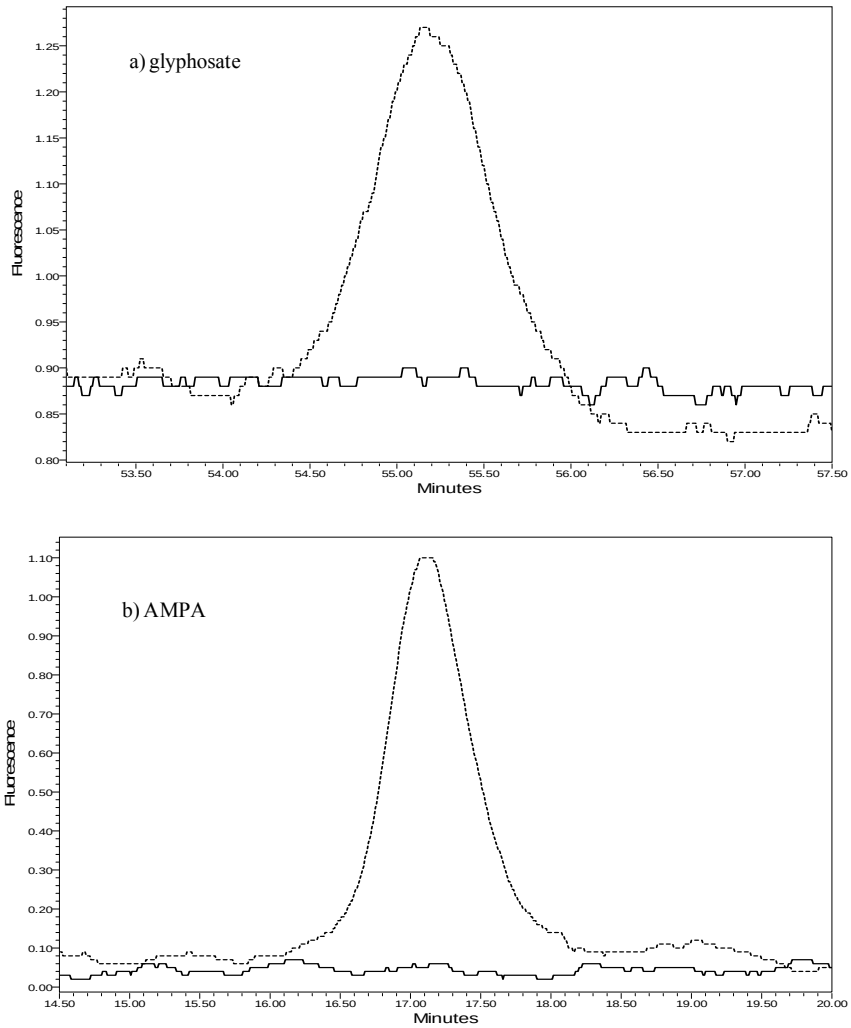

Figure B2 Chromatographic Traces Showing Peaks from Injection of ≈1 ng/mL (extract concentration) of a) Glyphosate-FMOC and b) AMPA-FMOC (chromatographic background from injection of ICAL blank included)

1.3 Derivatizations in Laboratory Blank Water and Sample Filtrates

ICALs were generated using purchased pre-derivatized solids; thus all quantifications in derivatized sample extracts assume 100% recovery from extract preparation (derivatization and cleanup). This assumption was checked by spiking laboratory water with underivatized AMPA and glyphosate and then carrying through the full analysis to obtain final extracts containing the FMOC derivatives. This experiment was also performed in a filtrate generated from a Needle Branch sample containing ≈155 mg/L suspended sediments (SS)[1]. Thus, calibrations ranging from ≈5 to ≈1000 ng/mL (concentration in final extracts) were generated in both matrices. This type of calibration will be referred to as a derivatized ICAL (or DICAL) to differentiate it from ICALs developed using purchased pre-derivatized solids (Section 1.1 herein). Results from both DICALs are summarized in Table B1, and are shown in Figures B3 (glyphosate) and B4 (AMPA).

[1] Section 2.1 herein describes the procedure used to generate this sample.

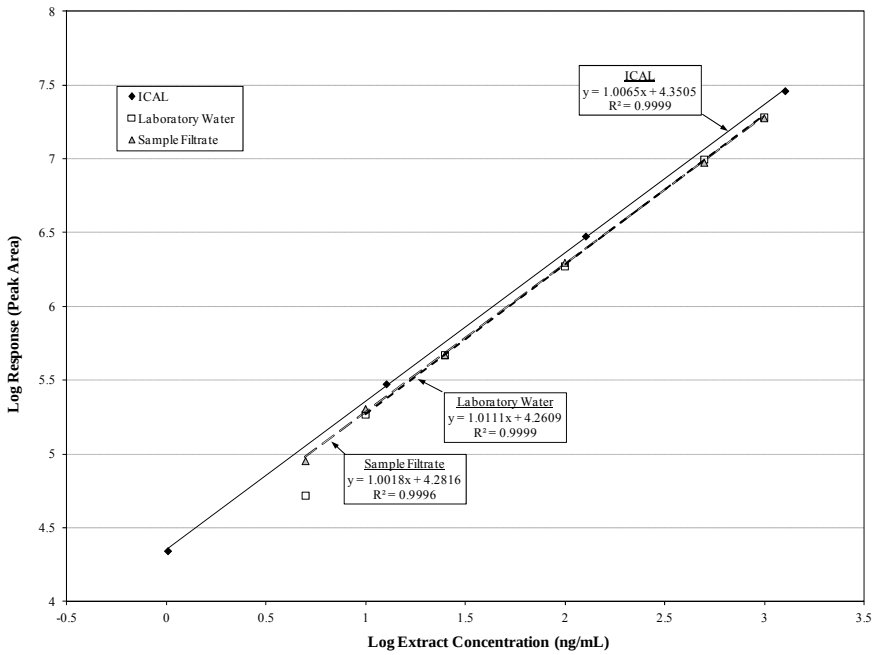

Figure B3 Example Glyphosate ICAL and DICALs Generated in Laboratory Water and a Needle Branch Filtrate

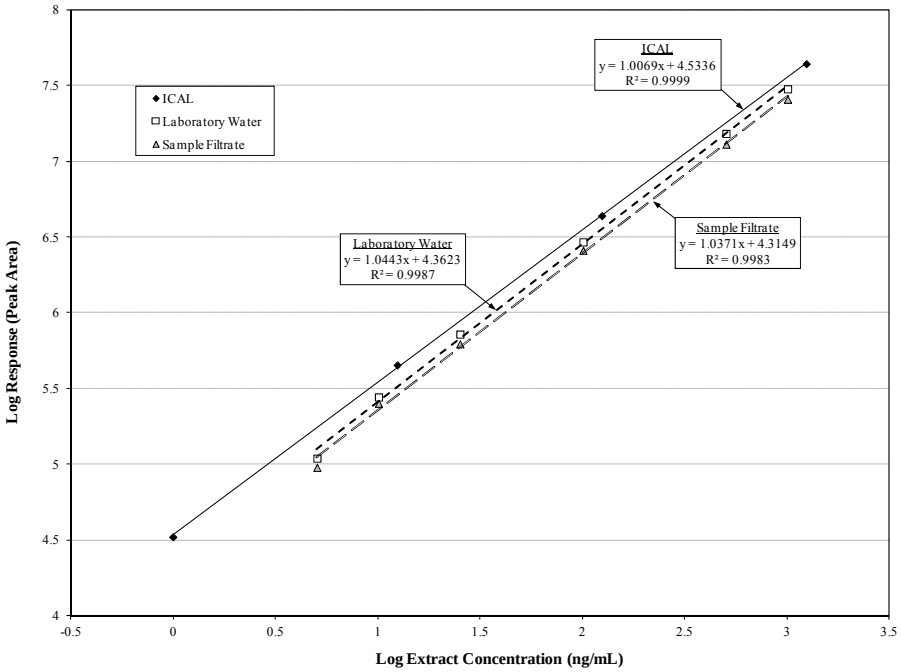

Figure B4 Example AMPA ICAL and DICALs Generated in Laboratory Water and a Needle Branch Filtrate

The sample preparation in laboratory blank water manifested background peaks interfering (i.e., co-eluting) with both AMPA and glyphosate. This background (Table B1) was equivalent to 0.12 ng/mL AMPA and 0.51 ng/mL glyphosate (extract concentrations calculated using the mean RF from the ICAL). In each case, the background chromatographic peaks were shifted relative to the associated

analyte, indicating that the peaks were not due to the presence of either AMPA or glyphosate. However, they co-eluted to the degree that only a single peak (misshapen at low analyte concentrations) was observed when analytes were present.

The blank control for the Needle Branch filtrate DICAL returned 0.60 ng/L as AMPA, while the interference impacting glyphosate was essentially no different than that found in the laboratory water derivatizations (≈0.50 ng/L as glyphosate). These results indicate that both the laboratory and the sample matrix contributed background affecting AMPA, but that all the background at the nominal retention time of glyphosate was from the laboratory only (note that this statement is only relevant to the specific sample used in this experiment). In any case, even though the background in these DICALs was equivalent to ≤10% of the lowest spike level, all results were background subtracted. Thus, the results given in Table B1 and shown in Figures B3 and B4 are all corrected for background interference.

The mean RFs for glyphosate (Table B1) in the two DICALs were effectively the same, and both were ≈10% lower than the mean RF from the multiple ICALs. Given that the stated purity for the underivatized glyphosate standard was 99.5%, this outcome cannot be attributed to standard purity. These results show that the sample matrix had no effect on the recovery of glyphosate from the Needle Branch filtrate beyond that manifesting in laboratory water.

The underivatized AMPA solid used in these experiments had a stated purity of 99%, so the low AMPA RF from the derivatizations also cannot be attributed to low purity. However, in this case, the RF from the Needle Branch filtrate DICAL was 7% lower than that from the laboratory water DICAL, suggesting some matrix effect on recovery of AMPA.

As noted, standard purity cannot explain the low RFs from the DICALs. The fact that both AMPA and glyphosate gave stable RFs at all spike levels (Figures B3 and B4) suggests that, with one exception, adsorption is also not a viable explanation because adsorption would be expected to manifest as a fixed bias affecting low concentrations to a greater extent than high concentrations. Thus, incomplete derivatization (i.e., <100% yield) and/or low recovery from the post-derivatization cleanup are the most likely explanations for these depressed RFs.

Although adsorption cannot explain the low glyphosate RFs from the two DICALs, it may be the reason for the low glyphosate response at 5 ng/mL in the laboratory water DICAL (this point was excluded from the mean listed in Table B1 and the linear regression shown in Figure B3). The strongest evidence for this is the good response (after subtracting background) at 5 ng/mL in the Needle Branch filtrate DICAL. Although it cannot be proven, this outcome is attributed to the ameliorative effect of dissolved organic carbon (DOC) on adsorption.

Overall, results indicate that use of the ICAL to quantify glyphosate in Needle Branch filtrates will return concentrations biased low by approximately 10%. The data also demonstrate that this level of bias is constant for concentrations ≥5 ng/mL (extract concentration), and that background interference manifested as ≈0.5 ng/mL in both laboratory water and Needle Brach filtrate (extract concentration as glyphosate). This level of background is high enough to bias results at the lowest calibration level (LCL) in the ICAL (≈1 ng/mL in extracts); thus the low end of the analytical working range in samples is limited by background, not by analytical sensitivity. Regardless, if an accurate measure of this background is available the use of background subtraction should allow quantifications to the ICAL LCL (1 ng/mL in extracts), although the resulting concentrations would be 10% low biased.

Results for AMPA indicate that use of the ICAL will give concentrations in Needle Branch filtrates biased low by approximately 30%, indicating a matrix effect on AMPA recovery. In addition, as with glyphosate, the data demonstrate that this level of bias is nominally constant for concentrations

≥5 ng/mL (extract concentration), and that the factor limiting the low end of the analytical working range is background interference.

1.4 Derivatizations in 0.5M Potassium Hydroxide and Suspended Sediment Extracts

The matrix for derivatizations performed as part of analyzing sample SS is 0.5M potassium hydroxide (KOH), so experiments analogous to those summarized in Section 1.3 herein were performed in 0.5M KOH and in an SS extract prepared from the same Needle Branch sample (≈155 mg/L SS) used to generate the filtrates used in the experiments summarized in Section 1.3. As part of these experiments, only 50 mL of this Needle Branch sample was used in order to limit the mass of SS present during the derivatization to <10 mg (see Section 2.4 herein). Briefly, the 47 mm 0.7 µm glass fiber filter (GFF) with SS was sonicated in 80 mL 0.5M KOH for one hour prior to spiking and derivatization; that is, spikes were added after extraction just prior to derivatization. Results from both these DICALs are summarized in Table B1 and are shown in Figures B5 (glyphosate) and B6 (AMPA).

The mean RF for AMPA from the 0.5M KOH DICAL (Table B1) was the same as that from the laboratory blank water DICAL, again suggesting either depressed yield from the derivatization, low recovery from the post-derivatization SPE cleanup, or a combination of the two. On the other hand, the AMPA RF from the Needle Branch SS DICAL was depressed by almost 20% relative to the RFs from the laboratory water and 0.5M KOH DICALs, indicating a matrix effect on overall recovery. In addition, the background interference impacting AMPA was notably higher in the SS extracts than in any other matrix, indicating that the sample matrix contributed the majority of this background.

The mean glyphosate RFs from the 0.5M KOH and Needle Branch SS DICALs were nominally the same, and were ≈7% lower than those from the laboratory water DICAL. This outcome indicates that the sample matrix did not have an observable impact on the recovery of glyphosate beyond that attributable to the 0.5M KOH. The results also show an increase in the background affecting glyphosate, and the data indicate that the sample matrix contributed approximately half of this background.

The background interfering with both AMPA and glyphosate in the Needle Branch SS extract was high relative to the background found in the associated filtrate sample (Table B1). The background impacting AMPA was equivalent to 3.6 ng/mL AMPA (or 72 ng/L in a 50 mL sample), while the background impacting glyphosate was equivalent to 1.5 ng/mL glyphosate (or 30 ng/L in a 50 mL sample). Given that the ICAL is good to 1 ng/L (extract concentration), these results show that the low end of the analytical working range in SS samples is limited by background, not by analytical sensitivity. If an accurate measure of this background is available, the use of background subtraction should allow quantifications to the ICAL LCL (1 ng/mL in extracts), although the resulting AMPA concentrations would be ≈40% low biased and the glyphosate concentrations would be ≈20% low biased.

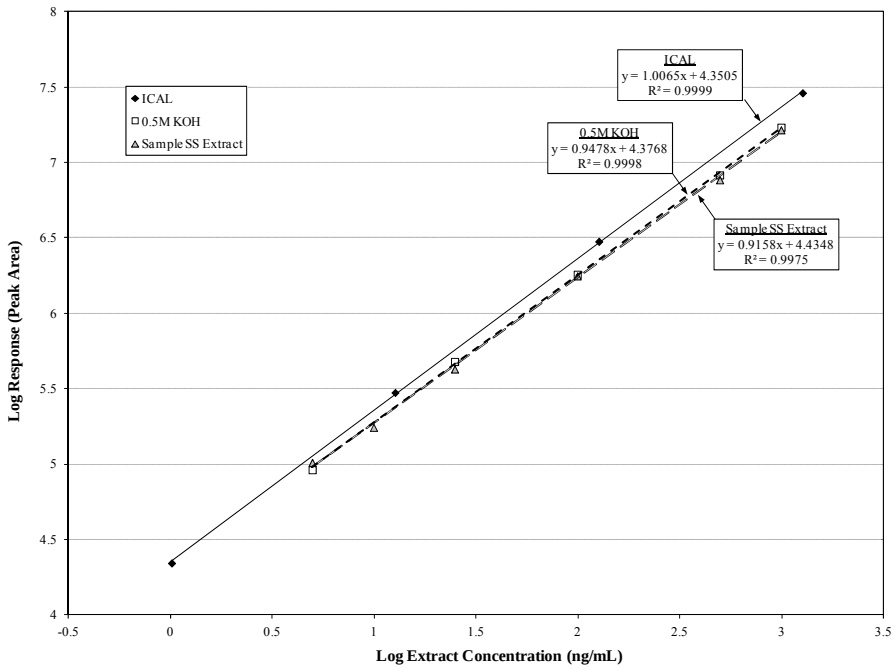

Figure B5 Example Glyphosate ICAL and DICALs Generated in 0.5M KOH and an Extract of Needle Branch Suspended Sediment

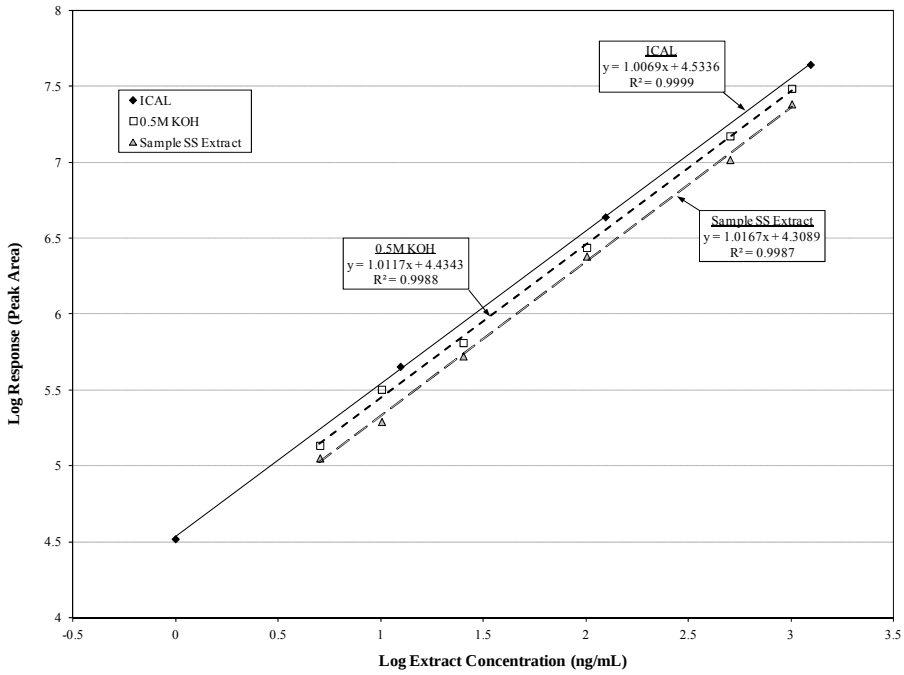

Figure B6 Example AMPA ICAL and DICALs Generated in 0.5M KOH and an Extract of Needle Branch Suspended Sediment

1.5 ICALs vs. DICALs

Although the reasons why remain unknown, the results presented in Table B1 show that use of the ICAL to quantify AMPA and glyphosate in sample extracts will return low-biased concentrations. In the case of sample filtrates, results indicate that quantifications versus the ICAL will return AMPA concentrations carrying an ≈30% low bias and glyphosate concentrations carrying an ≈10% low bias. SS extract results indicate that AMPA concentrations from quantifications versus the ICAL will be ≈40% low biased and glyphosate concentrations will be ≈20% low biased.

This might suggest that all quantifications should be generated versus the appropriate DICALs. However, this would require the assumption that these biases are fixed and are not subject to variability due to laboratory technique and/or sample-to-sample differences in matrix effects. If the analysis was being performed using liquid chromatography coupled with mass-spectrometry (LC/MS-MS), the use of labeled internal standards would mitigate these concerns about variability and matrix effects, but this approach will not work when using a fluorescence detector. Because of the uncertainty regarding causal factors leading to these apparent biases, ICALs were used in all quantifications and this study relied on results from ongoing quality assurance (e.g., control spikes, matrix spikes) to characterize bias in sample results (Appendix C, Section 1.2.3).

2.0 RECOVERY OF GLYPHOSATE AND AMPA FROM SUSPENDED SEDIMENTS

Because of the difficulties associated with separating SS from the extraction solution (0.5M KOH) after extraction, derivatization of the whole extract was attempted. Thus, the final 80 mL of extraction solution was derivatized while still containing all sample solids and the disintegrated GFF. A number of experiments were performed to characterize the efficacy of this procedure prior to initiating sample analysis.

2.1 Preparation of Streamwater Containing Known Amount of Suspended Sediment

Wet sediment was collected from the pool at NBL prior to application of herbicides. Once in the laboratory, the bulk sediment was sieved at 1 mm to remove non-settleable material. The wet material collected from sieving was allowed to settle for about two minutes, and the supernatant was then decanted into a 1 L glass bottle. This process was repeated until ≈800 mL of supernatant had been collected (Bottle A). Bottle A was shaken and allowed to settle for ≈15 minutes, after which ≈400 mL of the supernatant was decanted into a 500 mL glass bottle (Bottle B, 6190 mg/L solids). Approximately 400 mL of clear streamwater (collected at the same time as the sediment sample) was added to Bottle A, which was then shaken and allowed to settle for 45 minutes, after which ≈400 mL was decanted into another 500 mL glass bottle (Bottle C, 4870 mg/L solids).

When experimentation called for use of air-dried solids, either Bottle B or Bottle C was shaken and an aliquot was immediately withdrawn using a wide-mouth glass pipette. This aliquot was added to a tared glass petri dish. The petri dish was loosely covered with foil and placed on top of a laboratory oven (outside the oven) set at 105°C until all water had evaporated. The dried solids were scraped up and placed in a small glass vial. An exact mass of these solids was added to the relevant experimental sample (either laboratory water or a Needle Branch baseflow sample) to obtain a known SS concentration.

Whenever experimentation called for use of wet (or “never-dried”) solids, either Bottle B or Bottle C was shaken and an aliquot was immediately withdrawn using a wide-mouth glass pipette. This aliquot was added to the relevant experimental sample to obtain a final sample with a known SS concentration.

2.2 Impact of Air-Dried Suspended Sediment on Derivatization Yield

Initial experiments using fairly high levels (up to 1000 mg/L) of air-dried solids showed low and variable recoveries of both glyphosate and AMPA in the summed results from analyses of filtrate and solids fractions. In these experiments, AMPA and glyphosate were spiked into laboratory water containing air-dried solids and the mixture was equilibrated on a room temperature shaker table for ≈60 hours (sample bottles wrapped in foil during this period) prior to analysis. Subsequent spiking experiments indicated that poor recovery from the derivatization of the SS extract was driving these results, so experiments were performed to characterize the impact of the air-dried solids on derivatization yield.

Unspiked aliquots of laboratory water fortified with different masses of air-dried solids were equilibrated on a shaker table at room temperature for 60 hours (bottles wrapped in foil). After equilibration the solution was filtered (nominal 0.7 µm GFF). The resulting solids fractions (including the GFF) were extracted using 80 mL 0.5M KOH (sonicated for one hour at room temperature). The resulting SS extracts, including associated GFFs, were spiked with AMPA and glyphosate and then derivatized. The results of these experiments are given in Table B2. Note that all recoveries listed in the table were calculated versus paired controls generated by spiking laboratory water immediately prior to derivatization.

Table B2 Effect of Air-Dried Solids on Derivatization Yield in Solids Extracts[a]

Sample SS (mg/L)	Nominal Mass of Solids (mg)	Percent Recoveries[b]	
		AMPA	Glyphosate
12.5	1	95	99
62.5	5	89	96
125	10	92	104
250	20	79	84
500	40	70	77

[a] Air-dried solids added to 80 mL blank water and equilibrated on shaker table for ≈60 h at room temperature (bottles wrapped in foil) prior to spiking and derivatization; spike nominally 1250 ng/L (sample concentration), added to solids extracts immediately prior to derivatization.

[b] Recoveries calculated vs. experiment-specific controls in which spikes were added to laboratory blank water (without solids) immediately prior to derivatization.

The results show that the amount of air-dried solids affected recovery of both AMPA and glyphosate from derivatizations performed in solids extracts. Recoveries of both AMPA and glyphosate were generally good when the mass of air-dried solids in the original sample was ≤10 mg, but were clearly depressed when 20 mg SS was present. Although it is understood that air-dried solids may not have the same impact as native SS (i.e., never-dried SS), these results were taken to indicate that the original analytical sample should not contain more than ≈10 mg air-dried solids in order to obtain nominally quantitative recovery from derivatizations performed in the solids extract.

2.3 Spiking Experiments using Air-Dried Solids

Results presented in Section 2.2 herein showed that the presence of >10 mg air-dried Needle Branch solids (>125 mg/L in an 80 mL sample) depressed yield from the FMOC derivatization. The other factor potentially affecting overall recovery is extraction efficiency, and the ability of the KOH sonication to extract AMPA and glyphosate from air-dried Needle Branch solids was evaluated in a separate set of spiking experiments. In these experiments, 10 mg air-dried solids were added to 80 mL blank water in foil-wrapped HDPE bottles and the mixture was vigorously shaken. The analytes were

spiked just prior to the 60 hour equilibration period. Following equilibration, samples were filtered and the filtrate and solids fractions were analyzed separately. Results from multiple spikes are summarized in Table B3. All percent recoveries were calculated versus paired controls in which analytes were spiked into 80 mL of laboratory water prior to the 60 hour equilibration period.

Table B3 Recovery of AMPA and Glyphosate Spikes Added to Laboratory Blank Water Fortified with 125 mg/L (ppm) Air-Dried Solids[a]

Nominal Spike (ng/L)[b]	%Rec from Filtrate[c]		%Rec from Solids[c]		Overall %Rec[c]	
	AMPA	Glyph	AMPA	Glyph	AMPA	Glyph
1250	27	35	62	60	89	95
1250	22	28	69	61	91	89
3125	25	37	68	66	93	103
3125	23	34	64	60	87	94
6250	33	46	53	46	85	92
Mean	26	36	63	59	89	95
Std Dev	4.2	6.5	6.6	7.3	3.0	5.1
RSD	16	18	10	12	3	5

[a] 80 mL volumes of laboratory blank water with air-dried solids spiked with analytes and equilibrated on shaker table for 60 h at room temperature (bottles wrapped in foil) prior to filtration and separate analysis of filtrate and solids fractions.
[b] Spike level as sample concentration (ng/L).
[c] Recoveries calculated vs. experiment-specific controls in which spikes were added to laboratory blank water immediately prior to derivatization.

The results show that AMPA was recovered at nominally 90% (on average) and glyphosate was recovered at nominally 95% (on average) regardless of spike level. Although these results are specific to the air-dried Needle Branch solids used in the experiments, they show that the extraction can provide good recoveries of both analytes from SS as long as the mass of SS present during the derivatization is ≤10 mg.

2.4 Spiking Experiments Using Wet Solids

Following the experiments performed using air-dried solids, Needle Branch baseflow fortified with 155 mg/L native (never-dried) SS was spiked, equilibrated at room temperature in a foil-wrapped bottle for 60 hours, and then derivatized and analyzed. In order to limit the mass of solids present during derivatization of the solids fraction, a sample volume of 50 mL was used[2] (7.75 mg solids). Both spiked and unspiked samples were analyzed in these experiments, and spike recoveries were calculated after subtracting the background results (again, all recoveries were calculated vs. paired controls generated, in this case, by spiking Needle Branch baseflow water without any added SS immediately prior to derivatization). The results from these experiments are given in Table B4.

The overall (i.e., summed) recoveries of both AMPA and glyphosate were nominally 95%, indicating effective extraction of both analytes from native Needle Branch SS.

[2] The volume of the sample filtrate was made up to 80 mL with blank water prior to derivatization.

Table B4 Recovery of AMPA and Glyphosate Spikes Added to Pre-Application Needle Branch Water Containing Nominally 155 mg/L (ppm) Suspended Sediments[a]

Sample[b]	Nominal Spike (ng/L)[c]	%Rec from Filtrate[d]		%Rec from Solids[d]		Overall %Rec[d]	
		AMPA	Glyph	AMPA	Glyph	AMPA	Glyph
A	1000	43	33	58	54	101	87
B	2000	66	76	34	19	100	95
B	5000	58	65	40	31	97	96
B	5000	56	71	38	33	94	104
A	5000	14	8	81	83	95	91
A	5000	12	9	76	89	88	97
	Mean	42	44	54	52	96	95
	Std Dev	23.1	31.0	20.3	28.9	4.7	5.8
	RSD	56	71	37	56	5	6

[a] Pre-application baseflow samples fortified with SS harvested from pool above NBL (see text); AMPA and glyphosate spikes added to 50 mL sample and mixture equilibrated on shaker table for ≈60 h at room temperature (bottles wrapped in foil) prior to filtration and separate analysis of filtrate and solids fractions.
[b] Sample A generated using sediment and streamwater collected June 2009; sample B generated using sediment and streamwater collected August 2010 immediately before application of herbicides.
[c] Sample concentration (ng/L = ppt).
[d] Recoveries calculated vs. experiment-specific controls in which spikes were added to laboratory blank water immediately prior to derivatization.

2.5 Partitioning of Spiked Herbicides onto Needle Branch Suspended Sediments

The experiments were performed without any knowledge of whether or not a 60 hour equilibration period was sufficient to establish true steady-state partitioning between the dissolved and particulate phases. In addition, the ultimate capacity of the different solids for AMPA or glyphosate was not studied, so it is possible that the adsorptive capacity of the solids was exceeded in these experiments. In any case, in multiple experiments using air-dried or never-dried SS 50% or more of the spikes were found in the solids fraction, indicating that the 60 hour equilibration generated SS presenting a real challenge to the SS extraction. Thus, overall recoveries in Table B4 are taken as realistic measures of recoveries obtainable from samples containing up to 155 mg/L SS.

However, comparing the results presented in Table B4 for SS A and SS B suggests that the two never-dried SS samples had different affinities for AMPA and glyphosate. On average, ≈72% of the spiked AMPA was found on SS A after the 60 hour equilibration, but only ≈37% was found on SS B after the same equilibration period. For glyphosate, ≈75% of the spike mass was found on SS A, while ≈28% was found on SS B. These results indicate that the two SS samples did in fact have different affinities (or adsorptive capacities) for AMPA and glyphosate. Thus the analyte distributions between the dissolved and particulate phases observed in samples collected during the course of the study are expected to vary with both the concentration and the nature of the SS in samples. Therefore, the distributions in Table B4 cannot be assumed to be relevant to all samples.

3.0 STABILITY OF ANALYTES IN SAMPLES PRIOR TO FREEZING

It was essentially unavoidable that samples would sit in the field after collection and prior to freezing as final preservation. A stability study was performed to characterize the potential for changes in glyphosate or AMPA concentrations during this time.

3.1 Generation and Treatment of Stability Study Composite Samples

A series of 17 1 L grab samples of clear Needle Branch water was collected from the pool at NBL. Immediately after collection of these clear samples, the sediment in the pool was stirred and a series of five 1 L grabs of turbid water was collected. On return to the laboratory, SS concentrations in one of the clear grabs (the full 1 L was filtered) and in all five of the turbid grabs (100 mL of each) were measured. The concentration in the clear grab was 2.2 mg/L, and the concentrations in the turbid grabs ranged from 228 to 418 mg/L. All samples were stored in a refrigerator during SS determinations, which were performed overnight.

The next morning, a set of 34 composites was generated in pre-tared 500 mL HDPE bottles. The first of the 16 remaining clear grab samples was shaken and 25 mL decanted into a glass scintillation vial to a positive meniscus. This was added to composite Bottle #1. A second 25 mL aliquot was measured (after shaking the original grab sample) and added to composite Bottle #2. This was repeated until all 34 composites contained 25 mL from the first clear grab sample. The process was repeated using the second clear grab, this time starting with composite Bottle #34 and ending with composite Bottle #1.

The process was followed until all 34 composite bottles contained 350 mL of clear sample (using clear Grabs 1 to 14). After this, 25 mL from each of two of the turbid grabs were added to each composite to achieve a higher SS concentration. SS concentrations in Composites #1, #11, #23, and #34 were determined using the full volume of each bottle. The resulting SS concentration was 36 ±0.8 mg/L (n = 4). The 30 remaining composite bottles were assigned to different treatments according to Table B5 and then placed in a refrigerator.

Table B5 Bottle Assignments for Storage Stability Study[a]

Day	Blank Bottles	Spiked Bottles
0	5, 17, 30	4, 18, 31
1	8, 12, 24	13, 22, 26
2	3, 20, 33	2, 15, 32
4	9, 14, 29	10, 21, 25
7	7, 9, 27	6, 16, 28

[a] Bottles 1, 11, 23, and 34 consumed in determination of SS.

The next morning (two days after collection of the initial grab samples) all composites/samples were removed from the refrigerator, allowed to come to room temperature, and then spiked as indicated in Table B5 with nominally 200 ng AMPA and glyphosate to give final concentrations of ≈500 ng/mL (as sample concentration). The analysis of Day 0 samples was initiated immediately after spiking. All other samples were placed with their caps ajar in a ventilated closet maintained at room temperature (≈22°C), where they were kept in the dark for the periods noted in the table. On Days 1, 2, and 4, the appropriate bottles were capped and shaken, and then 160 mL was poured to waste. Each bottle was then capped and placed in a freezer. Analysis of the Day 7 samples, however, was initiated on Day 7 without freezing the samples.

Immediately after spiking, two 80 mL volumes were taken from each of the Day 0 bottles (blanks and spikes) after shaking, and the remaining volume (nominally 240 mL) was frozen. For two of the three Day 0 samples and blanks, one 80 mL volume was analyzed whole (i.e., without any filtration) as if it were a sample filtrate, and the second was filtered and separate analyses were performed on the filtrate and solids fractions. For the remaining sample (one spike and one blank), one of the 80 mL volumes was discarded, and the other was filtered and separate analyses performed on the filtrate and solids fractions. The Day 7 samples were treated exactly as described for the Day 0 samples.

3.2 Results

As discussed, Day 0 and Day 7 samples were analyzed without freezing, and each data set included two analyses of whole (unfiltered) sample (both spiked and unspiked) and three analyses of the associated filtrate and solids fractions. In all cases, quantifications were made versus the ICAL developed using pre-derivatized chemicals (Section 1 herein). The results from these analyses are summarized in Table B6.

Table B6 Analyte Stability in Room Temperature Needle Branch Water Containing 36 mg/L Suspended Sediments[a]

		Percent Recovery[b]			
		AMPA		Glyphosate	
		Day 0	Day 7	Day 0	Day 7
Samples Filtered Prior to Analysis[c]					
REP 1	filtrate	73.87	68.54	85.54	74.41
	solids	2.4	11.96	1.63	7.68
	sum	76.27	80.5	87.17	82.09
REP 2	filtrate	73.9	67.58	87.25	80.59
	solids	2.26	12.32	1.62	6.88
	sum	76.16	79.9	88.87	87.47
REP 3	filtrate	78.82	67.79	91.41	77.23
	solids	1.5	13.43	1.63	7.61
	sum	80.32	81.22	93.04	84.84
	Mean	77.6	80.5	89.7	84.8
	Std Dev	2.37	0.66	3.02	2.69
	RSD	3.1	0.8	3.4	3.2
Whole Sample Analyzed (n = 2)[d]					
	Mean	75.8	78.3	85.1	82.0
	RPD	1.99	4.58	5.36	0.63

[a] All quantification vs. ICAL developed using pre-derivatized AMPA and glyphosate.
[b] Recovery of nominal 500 ng/L (sample concentration) analyte spike to whole sample; each quantification blank subtracted using mean result from associated "fraction-specific" blank prior to calculating percent recovery.
[c] Filtrate and solids analyzed separately.
[d] Whole sample analyzed without filtration (i.e., unfiltered sample analyzed as filtrate).

The Day 0 results in Table B6 show AMPA recoveries on the order of 75 to 80% and glyphosate recoveries on the order of 85 to 90%. These are generally consistent with the RFs from derivatizations in sample matrix shown in Table B1. Thus, regardless of the reasons for the apparent low recovery from the Needle Branch samples (Section 1.5 herein), the results in Table B6 represent good overall recovery of both analyte spikes from the Day 0 replicates.

With respect to analyte stability, the results in Table B6 are generally consistent in that they suggest that on the order of 3 to 5% of the spiked glyphosate was lost over seven days at room temperature. Although the data clearly show that the fraction of glyphosate adsorbed to particulates increased over this period, the fact that AMPA recovery increased by nominally 3% during this time suggests that

some glyphosate was degraded to AMPA. In any case, the results do not show any loss of AMPA during storage, and the mass of glyphosate apparently lost over seven days at room temperature was considered trivial considering that field samples were always frozen within three days of collection and that temperatures were well below 22°C during the time samples were left in the autosamplers. Thus, the data presented in Table B6 were considered sufficient to document stability in field samples, so none of the intermediate stability samples (Days 1, 2, and 4) were analyzed.

As noted, unspiked samples were also analyzed as part of this experiment, and the mean backgrounds from these analyses were used in calculating background-corrected recoveries from the spiked samples listed in Table B6. Results of analyses of these multiple unspiked samples are summarized in Table B7.

Table B7 Analytical Background in Unspiked Storage Stability Study Samples

	AMPA[a]			Glyphosate[a]		
	Filtrate	SS	Sum	Filtrate	SS	Sum
Day 0 (n = 3)						
Mean	8.0	3.2	11.1	13.3	9.1	22.3
Std Dev	10.22	1.66	9.59	1.36	0.69	0.96
RSD	128	53	86	10	8	4
Day 7 (n = 3)						
Mean	6.0	4.2	10.2	13.1	25.7	38.8
Std Dev	3.03	0.24	2.87	0.96	0.61	0.46
RSD	51	6	28	7	2	1
Pooled (n = 6)						
Mean	7.0	3.7	10.7	13.2	NC[b]	NC[b]
Std Dev	6.83	1.22	6.35	1.06		
RSD	98	33	59	8		

[a] All results are ng/L sample concentration; all quantifications vs. ICAL developed using pre-derivatized AMPA and glyphosate.
[b] NC = not calculated.

The results in Table B7 show that the background interferent affecting AMPA was relatively stable over seven days of storage. On the other hand, the interferent affecting glyphosate increased in the SS extract over the seven-day storage period but was stable in the filtrate fraction. Although no explanation for this can be offered, this outcome does suggest that glyphosate in SS extracts will be more susceptible to bias due to background interference than AMPA.

APPENDIX C

DISCUSSION OF ANALYTICAL RESULTS

1.0 ANALYTICAL RESULTS FOR AMPA AND GLYPHOSATE

NCASI performed a number of stand-alone studies to characterize various aspects of the analytical workflow for determining AMPA and glyphosate. The details of these studies are given in Appendix B, which includes detailed summaries of the associated results. When appropriate, these data are referred to in this appendix, which gives a detailed discussion of the analytical results obtained from analysis of field samples for all analytes.

1.1 Stability of AMPA and Glyphosate in Samples Prior to Freezing

A stand-alone study was performed to characterize the stability of AMPA and glyphosate in field samples over the period between collection by the ISCO sampler and freezing. The details of this study and the associated results are presented in Appendix B, Section 3. Briefly, Needle Branch water holding ≈36 mg/L of suspended sediment (SS) was spiked with ≈500 ng/L AMPA and glyphosate and then held in open containers, in the dark, at room temperature. At prescribed times, spiked and unspiked samples were filtered and the resulting filtrate and solids fractions were analyzed. Results from these two analyses were summed to obtain total background-corrected recoveries for each point in time.

The results (Appendix B, Table B4) showed 3 to 5% losses of glyphosate over seven days of storage, while AMPA concentrations increased by nominally the same percentage over this period. This suggests loss of a small amount (≤5%) of glyphosate via degradation to AMPA and effectively no loss of AMPA. Given that field samples were always frozen within four days of collection, that sample temperatures ranged from 9 to 13°C at the time of collection, and that ambient temperature never exceeded 22°C, these results are considered to be high-biased estimates of the losses suffered in true field samples prior to freezing as final preservation.

1.2 Results for Dissolved AMPA and Glyphosate

1.2.1 *Laboratory QA/QC*

In addition to calibration verification, batch-specific (ongoing) laboratory quality assurance/quality control (QA/QC) included analysis of a blank control and a spiked blank control. A large volume grab sample of baseflow collected at NBL prior to application of herbicides served as this control sample. Splits of the control were frozen at the time of collection, but the largest volume was kept in a refrigerator and analytical volumes were taken as required. The SS concentration in this sample was not measured, but similar samples contained on the order of 2 mg/L SS (i.e., SS was barely measurable). Most analytical batches also included a matrix spike/matrix spike duplicate (MS/MSD) experiment performed on a randomly selected field sample. Results from these laboratory QA/QC analyses (except calibration verification) are summarized in Table C1.1.

Regardless of the matrix (control or field sample), mean spike recoveries of both AMPA and glyphosate were good (>80%) and there were effectively no differences between recoveries from spiked control and spiked field samples. This agreement in recoveries from the spiked control and matrix spikes reflects the fact that the control was in fact a field sample, and that SS was low in all samples (Section 1.3 herein).

The matrix spike recoveries in Table C1.1 show that the analysis gave quantitative recovery of dissolved AMPA and glyphosate from sample filtrates at the associated spike levels, which spanned the nominal range of 25 to 20,000 ng/L (sample concentrations). As shown by the plots in

Figure C1.1, although variability increased as spike level decreased, spike recovery was essentially constant across the entire range. For glyphosate specifically, this outcome is noteworthy because spikes were often less than the associated sample results, and the majority (60%) of glyphosate matrix spikes were at levels less than three times the associated sample results. Still, the mean recovery from this subset (i.e., where spikes were less than three times sample results) of matrix spikes was 98 ±9% (n = 15). Overall, these results show that the laboratory analysis provided good accuracy (good precision with low bias) at concentrations down to nominally 25 ng/L after background subtraction.

Results for the blank control (Table C1.1) show that there was low-level background impacting both AMPA and glyphosate. In the NBL baseflow sample used as the blank control, the glyphosate background consisted of a single chromatographic peak reflecting primarily laboratory background (Appendix B, Section 1.3). A single peak also interfered with AMPA, and in that case the sample matrix was the primary contributor (Appendix B, Section 1.3). In both instances, the retention times of the interfering peaks were slightly shifted relative to the associated analyte, indicating that the background was not due to contamination with actual analytes (Section 1.2.4 herein).

The average background concentrations in Table C1.1 reflect measurements made in refrigerated and frozen blank control (spiking was performed after thawing when frozen control was used), with either refrigerated or frozen control used on a batch-specific basis. As part of evaluating batch-specific results, recoveries of AMPA and glyphosate from batch-specific spiked controls were calculated after subtracting results from associated batch-specific blank control samples. Thus, spike recoveries from refrigerated and frozen controls were calculated by subtracting the result from a paired analysis of an unspiked aliquot of refrigerated or frozen control (as appropriate). However, some analytical batches included a spiked control but no unspiked control. In those cases, result from the most recent analysis of the appropriate refrigerated or frozen unspiked blank control was used to calculate spike recovery. These calculations led to the spiked blank control recoveries in Table C1.1.

Table C1.1 Summary of Laboratory QA/QC Results for Dissolved AMPA and Glyphosate

	Blank Control (ng/L)[a]		Spiked Blank Control[b] (%Rec)[d]		Sample (Matrix) Spikes[c]			
					AMPA		Glyphosate	
	AMPA	Glyphosate	AMPA	Glyphosate	%Rec[d]	%RPD[e]	%Rec[d]	%RPD[e]
Mean	3.2	12.9	87	101	93	3	98	4
Std Dev	1.6	2.6	5.9	7.0	4.8	2.6	7.1	4.4
RSD (%)	49	20	7	7	5	94	7	102
N	41	41	53	53	25	24	25	24

[a] Sample concentration reported without censoring (i.e., regardless of MDL).
[b] Spike levels 15 to 5000 ng/L (sample concentration).
[c] Spike levels 24 to 20,000 ng/L (sample concentration).
[d] %Rec = percent recovery.
[e] %RPD = relative percent difference.

Mean results from frozen and refrigerated controls were also compared to see if there were differences between the two data sets. As summarized in Table C1.2, freezing had the effect of decreasing variability in the background signal interfering with both AMPA and glyphosate. In addition, for both AMPA and glyphosate, the mean background from refrigerated controls was higher than the mean from frozen controls. However, in neither case were the means substantially different from each other or from the grand mean given in Table C1.1. Overall, these results indicate that even though freezing had a small effect on magnitude and variability, the background interference impacting each analyte was stable over the period during which samples were analyzed. This shows

that laboratory contamination was stable over this period, but says nothing about how the background contributed by the sample matrix may have varied from sample to sample (Section 1.2.4 herein).

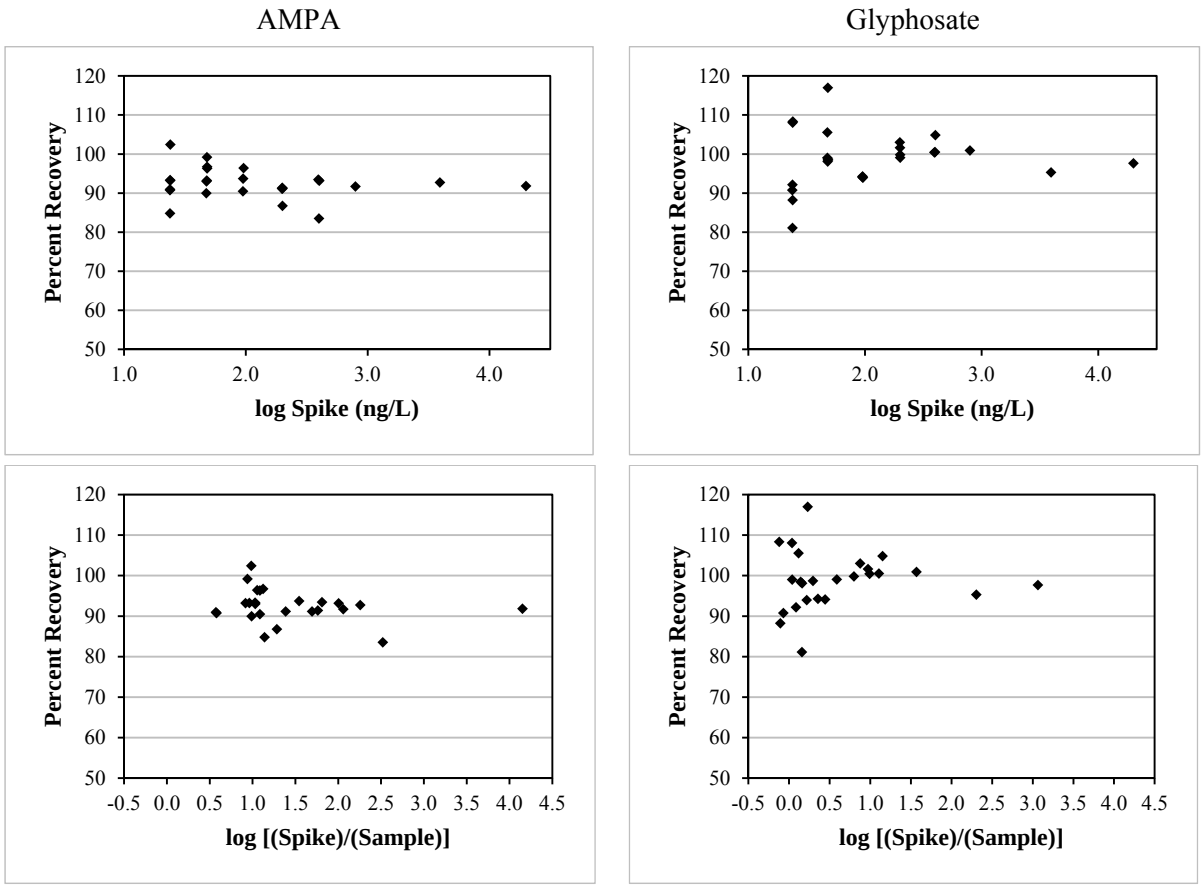

Figure C1.1 Matrix Spike Recoveries as a Function of Absolute Spike Level [top] and Relative Spike Level (ng/L)/(ng/L) [bottom]

Table C1.2 Results from Analysis of Frozen and Refrigerated Blank Controls[a]

	AMPA (ng/L)[b]		Glyphosate (ng/L)[b]	
	Frozen	Refrigerated	Frozen	Refrigerated
Mean	2.4	3.8	12.8	13.0
Std Dev	0.55	1.80	1.99	2.90
RSD (%)	23	48	16	22
n	16	25	16	25

[a] Grand mean of all blank controls is given in Table C1.1.
[b] Sample concentrations reported without censoring (i.e., regardless of MDL).

1.2.2 *Method Detection Limits, Minimum Levels, and Reporting*

Study-specific method detection limits (MDLs) and minimum levels (MLs) were obtained via replicate analyses of the blank control spiked with ≈15 ng/L AMPA and glyphosate (USEPA 1984; Muir and Sverko 2006). This experiment was performed in refrigerated blank control, and the replicate analyses (n = 7) were carried out in a single analytical batch that did not include any analyses of unspiked control. Thus, the means from the unspiked refrigerated blank controls (Table C1.2) were used to calculate recoveries from this MDL experiment.

Calculation of an MDL in accordance with EPA procedures (USEPA 1984) is based on data developed in a single analytical batch, so the resulting values reflect short-term intra-batch variability only. Because there were background peaks in the blank control co-eluting (i.e., interfering) with both AMPA and glyphosate, MDLs and MLs can also be calculated based on the variability in this background, and pooling the results from batch-specific analyses of this control allows calculation of MDLs and MLs reflecting longer-term batch-to-batch variability. These calculations were performed using the pooled results from both frozen and refrigerated control samples (Table C1.2), and the results are given in Table C1.3 (all quantifications were vs. the instrumental calibration, or ICAL; Appendix B, Section 1.5).

Table C1.3 Summary of Study-Specific MDLs and MLs for Dissolved AMPA and Glyphosate

	Spiked Refrigerated Blank Control[a]				Unspiked Refrigerated Blank Control[b]		Unspiked Frozen Blank Control[b]	
	AMPA		Glyphosate		AMPA	Glyphosate	AMPA	Glyphosate
	ng/L[c]	%Rec[d]	ng/L[c]	%Rec[d]	ng/L[c]	ng/L[c]	ng/L[c]	ng/L[c]
Mean	16.3	84	30.0	113	3.8	13.0	2.4	12.8
Std Dev	0.55	3.70	0.79	5.06	1.80	2.90	0.55	1.99
RSD (%)	3	4	3	4	48	22	23	16
n	7	7	7	7	25	25	16	16
MDLs and MLs[e]								
MDL	1.7		2.5		4.5	7.2	1.4	5.2
ML	5.5		7.9		18.0	29.0	5.5	19.9
MDLs and MLs accounting for sample background[f]								
MDL	5.5		15.5		8.3	20.3	3.8	18.0
ML	9.3		20.9		21.8	42.1	7.9	32.7

[a] Blank control sample spiked with ≈15 ng/L AMPA and glyphosate (a.e.) prior to initial filtration; all analyses in one analytical batch.
[b] Mean results from analyses of refrigerated and frozen controls performed over multiple analytical batches (from Table C1.2).
[c] Sample concentrations reported without censoring (i.e., regardless of MDL).
[d] Individual quantifications from spiked blank control background subtracted using mean result from refrigerated (unspiked) blank control prior to calculation of spike recovery.
[e] Analyte-specific MDL calculated as (SD*t) where t=3.143 (n=7), 2.492 (n=25), or 2.602 (n=16); analyte-specific ML calculated as (SD*10).
[f] Analyte-specific MDL calculated as [(mean blank)+(SD*t)]; analyte-specific ML calculated as [(mean blank)+(SD*10)].

Absent any accounting for background, the MDL and ML calculated for AMPA based on the long-term mean from analysis of the frozen blank control sample are essentially indistinguishable from those calculated using results from the spiking experiment, while values calculated using results from the refrigerated blank control are higher than those from the spiking experiment. This probably reflects the impact of freezing on the variability of the AMPA background (Section 1.2.1 herein),

which may have masked some of the batch-to-batch variability manifesting in the results from the refrigerated blank control. On the other hand, freezing had only a small effect on variability in the background peak interfering with glyphosate (Tables C1.2 and C1.3). Thus, the MDLs/MLs calculated using results from the refrigerated and frozen control samples are in nominal agreement, and both are higher than the metrics from the MDL experiment. Regardless of all this, because all samples were subjected to a freeze-thaw cycle and were analyzed over many analytical batches, MDLs and MLs based on results from analyses of the frozen control are the most relevant to results from analyses of samples.

MDLs and MLs calculated without regard to background represent the smallest concentration increment above the analyte-specific background that can be detected, and would be the appropriate metrics defining detection whenever this analyte-specific background was truly zero. However, the blank control gave chromatographic peaks interfering with both AMPA and glyphosate, so the final study-specific MDLs are calculated as the mean background plus the MDL calculated using the appropriate standard deviation (Muir and Sverko 2006). Thus, from Table C1.3 the final study-specific MDLs (as sample concentrations) are 3.8 ng/L for AMPA and 18.0 ng/L for glyphosate; the corresponding MLs are 7.9 ng/L for AMPA and 32.7 ng/L for glyphosate.

As noted, MDLs are driven by variability and carry no information relevant to bias. However, the results presented in Table C1.3 show good (>80%) recoveries of the nominal 15 ng/L spikes of both analytes. This outcome is most notable for glyphosate because the signal-to-background ratio was essentially 1 (i.e., the spike was nominally equal to the background). These recoveries show that the laboratory analysis can provide accurate background-subtracted (or background-corrected) quantifications of AMPA and glyphosate in sample matrix (NBL baseflow) to15 ng/L, which is the lower calibration level (LCL) of the ICAL. This outcome is consistent with the matrix spike results, which showed good background-subtracted recoveries of both AMPA and glyphosate to ≈25 ng/L (Section 1.2.1 herein).

1.2.3 *Field QA and Overall Recovery*

On delivery to the laboratory, all samples for determination of dissolved AMPA and glyphosate were filtered prior to freezing for long-term storage. For a limited number of samples (one or two per site per sampling episode), an additional volume was spiked with AMPA and glyphosate, filtered, and then frozen. Results from these spikes provide the most authoritative measure of overall recovery in that they reflect all aspects of the analysis other than the period between sample collection and freezing. As noted in Section 1.1 herein, <5% of the glyphosate present in samples was lost during this period, while effectively no AMPA was lost.

Figure C1.2 gives plots showing recovery of the pre-filtration, pre-freeze spikes as a function of spike level (sample concentrations as ng/L) and time spent frozen. Overall, these plots show that recovery of both analytes was effectively independent of spike level (all spikes greater than ≈400 ng/L), an outcome generally consistent with matrix spike results (Section 1.2.1 herein) and results from the MDL experiments (Section 1.2.2 herein). However, the plots also suggest losses of both AMPA and glyphosate after 450 days in the freezer, and that AMPA recovery was a bit lower than that from MS/MSD and MDL experiments. The summaries presented in Table C1.4 substantiate these observations.

As seen in Table C1.4, all spikes analyzed within 300 days of freezing gave nominally uniform recoveries regardless of spike level. Mean recovery was about 80% for AMPA. This is slightly lower than the means for AMPA in the MDL/ML experiment (84%, Table C1.3) and the MS/MSD experiments (87%, Table C1.1). More significantly, recovery of AMPA from samples held in a freezer for >450 days was clearly depressed, with a mean of 59 ±8.9% (n = 4). This clearly indicates

losses of AMPA and/or the constituents giving rise to the background signal when samples are kept in the freezer for extended times (>450 d).

Overall, these results indicate that, absent any recovery correction, AMPA quantifications in samples analyzed within 300 days of freezing should be considered 20% low biased (i.e., 80% of true value) as a result of losses incurred during initial filtration, the freeze-thaw cycle, and analysis of the thawed sample. Because no measurable losses of AMPA were observed in the room temperature storage stability study (Section 1.1 herein and Appendix B, Section 3.2), overall recovery of dissolved AMPA from samples analyzed within 300 days of freezing is estimated to be ≈80% (0.80*1). Similarly, overall recovery of dissolved AMPA from samples analyzed after being in a freezer for >450 days is estimated to be ≈60%.

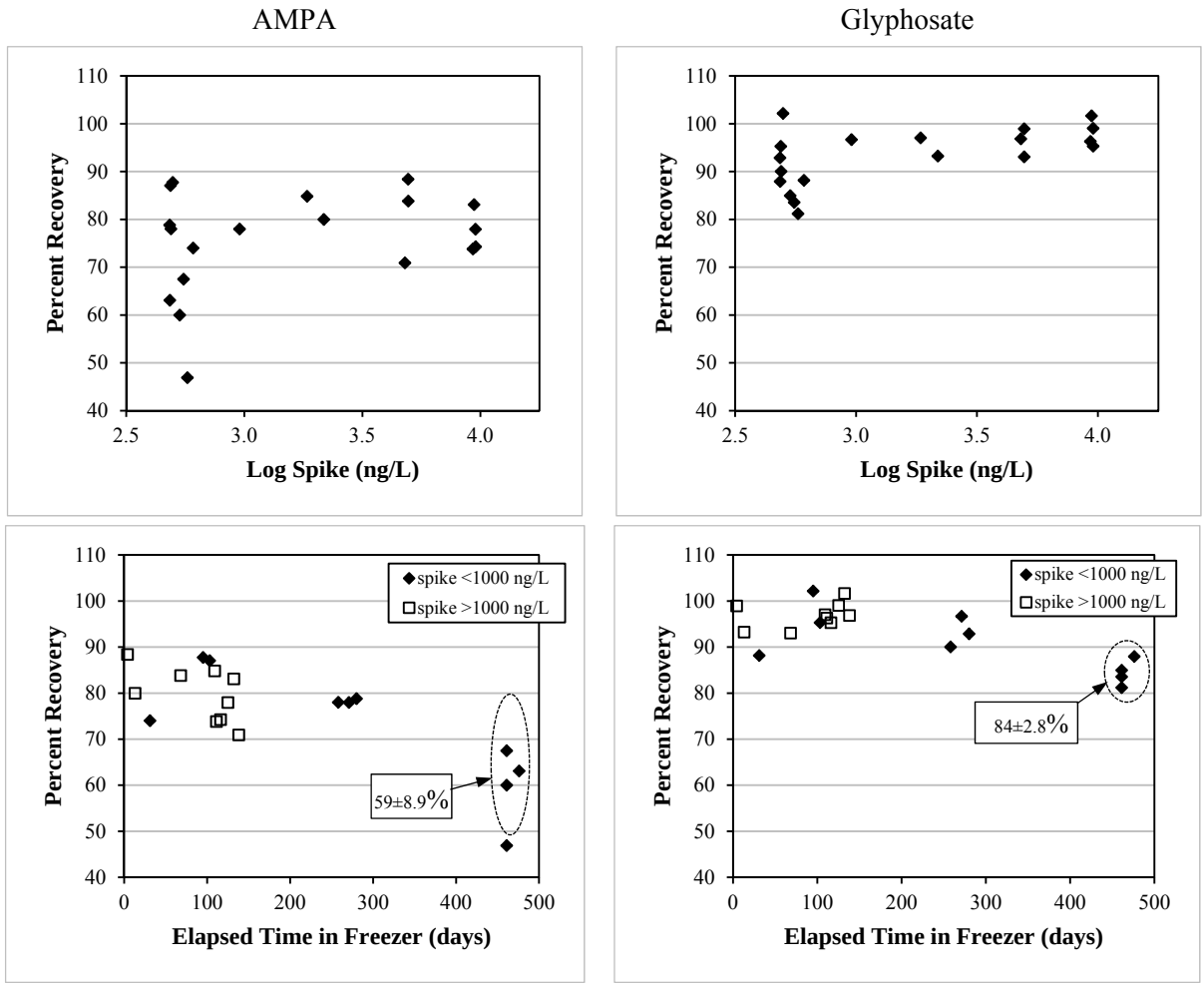

Figure C1.2 Recoveries of Pre-Filtration, Pre-Freezing Spikes as a Function of Absolute Spike Level (top) and Time Spent in Freezer Prior to Thawing and Analysis (bottom)

Results for glyphosate were more consistent; the mean from samples analyzed within 300 days of freezing was nominally 95% and the mean from samples analyzed after >450 days in a freezer was 84%. The mean for samples analyzed within 300 days of freezing is ≈5% lower than that from the MS/MSD experiments (95%, Table C1.1), but almost 20% lower than that from the MDL experiment (113%, Table C1.3). However, as noted, the MDL experiment was performed at a spike level

equivalent to the background, so a small amount of bias in background subtraction due to variability in the background would explain this high-biased measure of recovery from the MDL experiment.

In any case, the results presented in Table C1.4 give the best measure of bias in sample results and indicate that glyphosate quantifications in samples analyzed within 300 days of freezing should be considered ≈5% low biased (i.e., 95% of true value) as a result of losses incurred during initial filtration, the freeze-thaw cycle, and analysis of thawed sample. Assuming that 5% of the dissolved glyphosate in a sample is lost during the period of time between collection and freezing (Appendix B, Section 3.2), overall recovery of dissolved glyphosate in samples analyzed within nominally 300 days of freezing is thus estimated to be ≈90% (0.95*0.95). Similarly, overall recovery of dissolved glyphosate from samples analyzed after being in a freezer for >450 days is estimated to be ≈80% (0.84*0.95).

Table C1.4 Recovery of AMPA and Glyphosate Spikes Added to Samples Immediately Prior to Initial Filtration and Freezing

	AMPA		Glyphosate	
	%Rec	%RPD[a]	%Rec	%RPD[a]
All Spikes				
Mean	76	3.0	93	1.5
Std Dev	10.6	5.6	6.0	2.0
RSD	14	187	6	136
n	19	13	19	13
All Spikes >1000 ng/L[b]; all analyzed within 300 days of freezing				
Mean	80	3.7	97	1.5
Std Dev	5.9	7.2	2.8	2.2
RSD	7	196	3	145
n	9	8	9	8
Spikes <1000 ng/L[c]; analyzed within 300 days of freezing				
Mean	81	2.0	94	0.8
Std Dev	5.5	2.0	5.0	0.7
RSD	7	101	5	85
n	6	3	6	3
Spikes <1000 ng/L[d]; analyzed after being frozen >450 days				
Mean	59	2.0	84	2.4
Std Dev	8.9		2.8	
RSD	15		3	
n	4	2	4	2

[a] Relative percent difference between duplicate analyses (not performed on all samples).
[b] Spike levels nominally 2000 to 10,000 ng/L (sample concentration).
[c] Spike levels nominally 500 to 900 ng/L (sample concentration).
[d] All spikes nominally 500 ng/L (sample concentration).

1.2.4 *Dissolved Glyphosate Concentrations and the Impact of Background Interference*

Appendix D gives results for dissolved glyphosate (acid equivalent or a.e.) in all analyzed sample filtrates. As discussed in greater detail here, the analytical background noted in Sections 1.2.1 and 1.2.2 herein was not subtracted prior to reporting these concentration results, nor have they been corrected to account for losses incurred during analysis (Section 1.2.3 herein).

As noted in Sections 1.2.1 and 1.2.2 herein, both AMPA and glyphosate were impacted by background interference. The observation that this background was relatively stable in the blank

control (Section 1.2.1 herein) suggests that the results could be background corrected (background subtracted) to remove this source of high bias. However, this would require an assumption that the background measured in the single blank control sample (NBL baseflow collected before application of herbicides) represents the background in all samples collected during this study. This would require that the background at NBL did not change over time (e.g., diurnally or with the seasons), which seems improbable. Moreover, assuming that the result from this (or any) NBL baseflow reflects background in samples of NBL during storm events is even more tenuous, to say nothing of the assumption that it would apply to baseflow or storm event samples collected at NBU or NBH. Ultimately, due to an absolute inability to characterize the potentially dynamic contribution of site-specific background to total analytical signal (chromatographic peak area), results in Appendix D are reported without background subtraction. This precludes reporting of low-biased results due to overcorrection in the event that background in any given sample was actually less than the mean found in the blank control. Thus, all concentrations in Appendix D are understood to carry some high bias. As discussed in greater detail here, the magnitude of this bias did in fact vary from sample to sample, but was effectively unknowable for any specific sample.

The background peaks co-eluting (i.e., interfering) with both AMPA and glyphosate eluted at slightly different retention times than the analytes (Section 1.2.1 herein), but were generally inseparable from them. However, after installation, one specific HPLC column showed improved resolving power for a very small number of injections (nominally one analytical batch of samples). The analyses performed during this brief period provided additional chromatographic evidence that the background peak interfering with glyphosate was not glyphosate and, more importantly, that samples collected throughout the course of the study added variable increments to the background contributed by the laboratory. Unfortunately, this improved separation did not also manifest for AMPA, which elutes much earlier than glyphosate.

Most analytical batches included analyses of both a blank control (NBL baseflow) and a spiked blank control. Figure C1.3 shows chromatograms (glyphosate peak only) from these analyses performed as part of two analytical batches, one of which was performed using the noted (“best”) column when it was new and the other during the next analytical batch. These chromatographic traces clearly show the glyphosate peak (30 ng/L spike) partially resolved from the background peak during the brief period during which this column showed improved resolution (“best” resolution in Figure C1.3), and the merging of these two peaks after column performance had degraded to a level equivalent to all the other columns (“normal” resolution in Figure C1.3) used in this work. Thus, these chromatograms show that the background peak found in the blank control was not due to glyphosate.

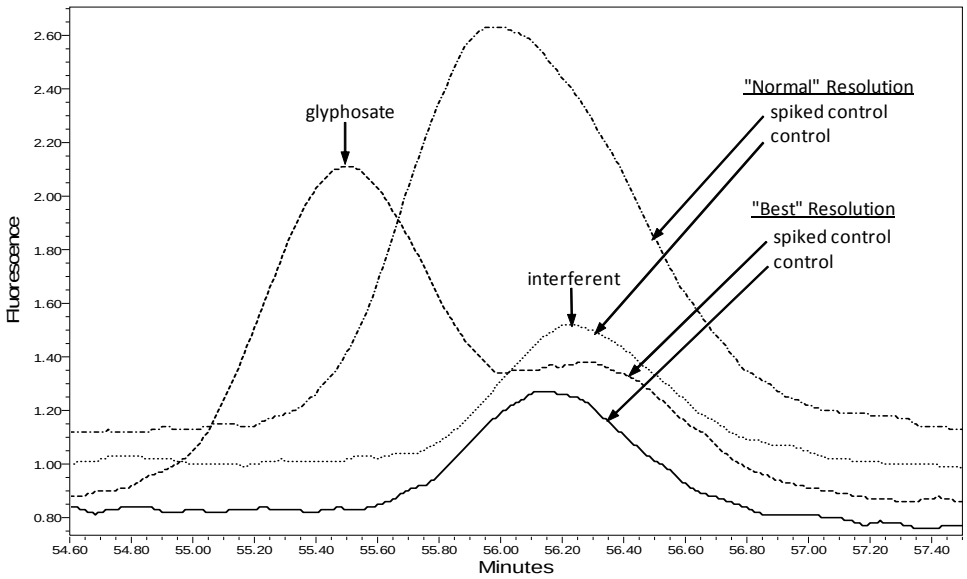

Figure C1.3 Chromatographic Traces Showing Effect of HPLC Column Resolving Power on Separation of Glyphosate from Background Peak in Blank Control Sample

Figure C1.4 shows the chromatogram from analysis of a sample collected from NBL during a storm event along with the chromatogram from analysis of a matrix spike (95 ng/L glyphosate) performed on the same sample. These chromatograms were generated as part of the one analytical batch in which the "best" column provided some separation between glyphosate and the background peak, and taken together show separation of spiked glyphosate from the background peak present in the unspiked sample. In this case, the unspiked sample clearly contains no detectable glyphosate even though the reported result (by the convention outlined herein) was 42 ng/L. Thus, these chromatograms provide additional evidence that the background peak is not due to glyphosate, while also showing that the concentration of whatever gives rise to this peak can increase during storm events, in this case from ≈13 ng/L (as glyphosate) in the blank control to 42 ng/L (as glyphosate) in this storm event sample.

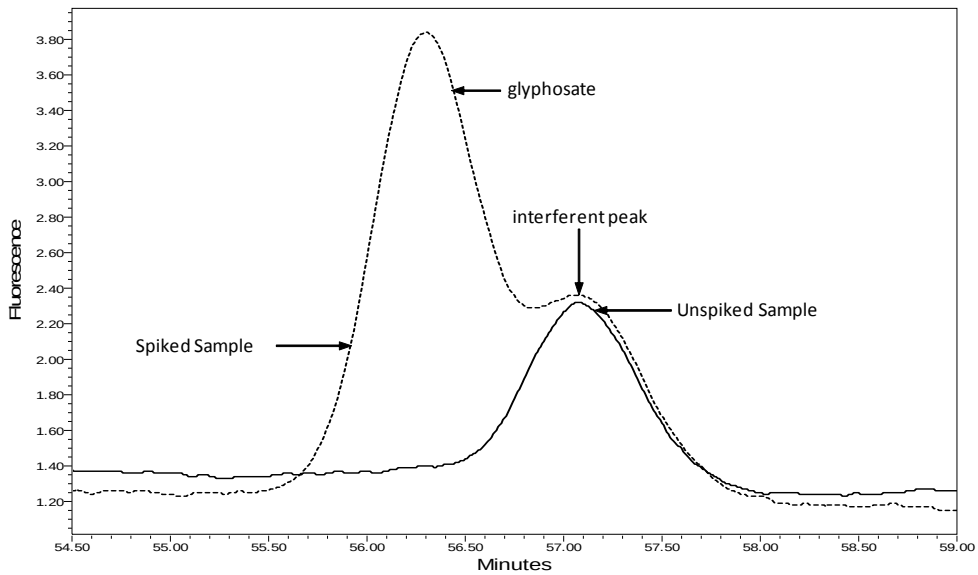

Figure C1.4 Chromatographic Traces Showing Partial Resolution of Spiked Glyphosate from Sample Background in Sample #14 Collected at NBL on 09/01/2010 (storm event sample)

Figure C1.5 shows the chromatograms from two separate samples collected from NBH during a storm event. These samples were collected one hour apart (i.e., consecutive samples), and so might be expected to have nominally the same concentrations of glyphosate and whatever molecule gives the interfering peak. One of these samples was analyzed as part of the analytical batch using the "best" column, while the other was analyzed as part of a different batch using a normal column. The sample analyzed using the "best" column clearly shows a doublet at the retention time of glyphosate, with the glyphosate peak (leading peak of doublet) giving a quantification of 29 ng/L and the background peak giving a quantification of 39 ng/L (note that this value is in general agreement with the 42 ng/L from Figure C1.4). Again, however, the result reported for glyphosate reflected the total area of this split peak, or 68 ng/L. Analysis of the second sample performed on the normal column gave a single peak only, and the reported glyphosate result was 72 ng/L. This is in good agreement with the 68 ng/L reported for the first sample, and almost certainly carries ≈40 ng/L of bias due to co-elution with the background peak (i.e., the true concentration is almost certainly closer to 30 ng/L than to 72 ng/L).

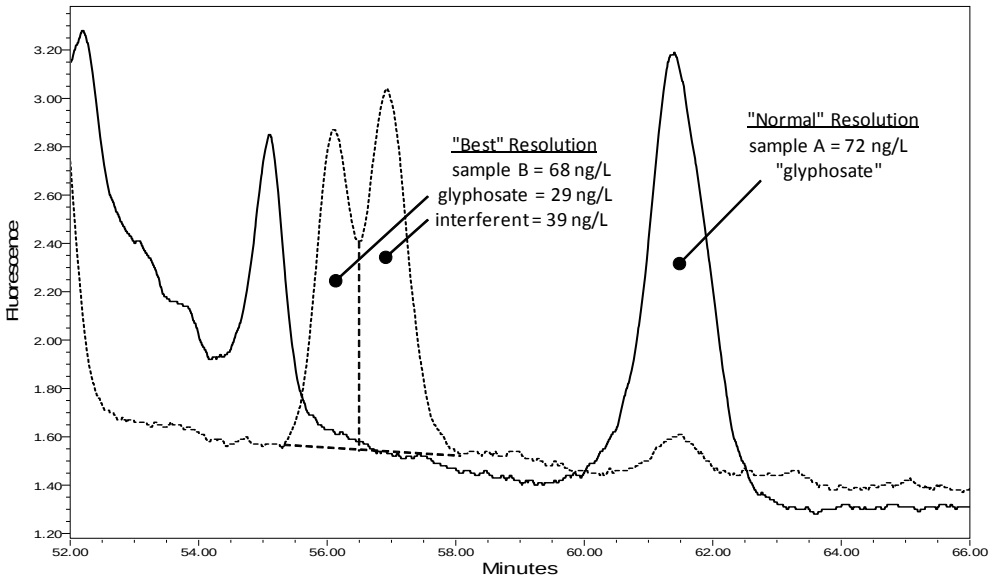

Figure C1.5 Chromatographic Traces Comparing Separation of Glyphosate from Sample Background in Samples #3 and #4 Collected at NBH on 09/01/2010 (storm event samples)

Clearly, Appendix D results for dissolved glyphosate carry a variable amount of high bias resulting from the variable background present in samples. As illustrated by the examples discussed here, this bias can be as high as 100% in samples collected during storm events.

Results from the pre-freeze spikes (Table C1.4) indicate that recovery of glyphosate from the overall analytical process (sample collection through analysis) was on the order of 95% when samples were analyzed within 300 days of being frozen and ≈84% when kept frozen for >450 days. Thus, the analysis provides glyphosate quantifications that are low biased by 5 to 15% depending on the time a sample was kept frozen prior to thawing and analysis. As noted, the results presented in Appendix D were not recovery corrected to account for this low bias, and one of the primary reasons for this was the observation that a reported concentration can in fact be 100% high biased due to the presence of background interference.

Ultimately, the absolute bias in the dissolved glyphosate concentrations provided in Appendix D cannot be characterized. However, all evidence supports high bias outweighing low bias by a considerable margin; thus Appendix D results are considered maximum possible concentrations. This issue is addressed again in Section 1.2.6 herein.

1.2.5 *Dissolved AMPA Concentrations*

Appendix E gives results for dissolved AMPA in all analyzed sample filtrates. The concentrations in Appendix E have not been recovery corrected (Section 1.2.3 herein), and are reported without background subtraction.

As with glyphosate, the AMPA quantifications in Appendix E are impacted by background interference from constituents native to samples. Unfortunately, none of the HPLC columns were able to effectively separate the AMPA peak from this background peak, so there are limited results showing how this background might have varied from sample to sample. The few results relevant to this question are given in Appendix E. Specifically, dissolved AMPA measured in baseflow samples collected at NBU immediately prior to application of herbicides was 7.2 ±0.04 ng/L (n = 3). This concentration is notably higher than the background concentrations found at NBH or NBL during this

same period, which were nominally equivalent to the means from the blank control (3.2 ng/L; Table C1.1), which was a pre-application baseflow collected at NBL. These results show that the background interference impacting dissolved AMPA can vary from sample to sample, and that concentrations as high as ≈7 ng/L can be attributed solely to the presence of non-AMPA background.

AMPA recoveries from the pre-freeze spikes (Table C1.4) show that recovery of AMPA from the overall analytical process (sample collection through analysis) was on the order of 80% when samples were analyzed within 300 days of being frozen and ≈60% when kept frozen for >450 days. Thus, the analysis provides AMPA quantifications that are low biased by 20 to 40% depending on the time a sample was kept frozen prior to thawing and analysis. As noted, the results presented in Appendix E were not recovery corrected to account for this low bias. The reason for this is that all measured dissolved AMPA concentrations were <12 ng/L (most were <8 ng/L), so they are well within the range of the background signal and almost certainly carry 3 to 7 ng/L of high bias, which means that background interference almost certainly contributed >40% high bias to all measured AMPA concentrations. Thus, Appendix E results almost certainly carry a net high bias.

Besides the issues noted, all measured AMPA concentrations were less than the ICAL LCL (15 ng/L), and so must be considered estimates. Combining this with the uncertainties regarding background interference means that, overall, the absolute bias in dissolved AMPA concentrations provided in Appendix E cannot be characterized, and it is well within the realm of possibility that all reported concentrations are true false positives. For all these reasons, the concentrations in Appendix E are not considered reliable measures of AMPA in samples. Ultimately, the most defensible statement that can be made is that AMPA was not present in any sample at concentrations >15 ng/L (the ICAL LCL for AMPA).

1.2.6 *LC/MS-MS Confirmation of Dissolved Glyphosate and AMPA Concentrations*

A small number of samples were submitted to AXYS Analytical Services (Sidney, British Columbia) for confirmatory analysis. AXYS applies nominally the same sample preparation procedure used by NCASI, but uses LC/MS-MS as the instrumental finish where NCASI used LC/FLUOR. Use of LC/MS-MS provides additional specificity, so results should be less susceptible to background interference. Table C1.5 compares results from the two analyses on a sample-specific basis, including analysis of one of NCASI's ICAL standards containing FMOC derivatives of both glyphosate and AMPA.

The analysis of NCASI's ICAL standard returned 110% recovery of AMPA and 115% recovery of glyphosate, indicating that AMPA quantifications reported by AXYS are ≈10% high biased relative to quantifications reported by NCASI and glyphosate quantifications reported by AXYS are ≈15% high biased relative to NCASI's quantifications[1]. Despite this inter-laboratory bias, in no case did AXYS report a higher sample-specific concentration than NCASI. In the case of AMPA, this comparison is obviously limited by the nominal 20 ng/L reporting limit used by AXYS to censor its results. Because NCASI's quantifications ranged from 2 to 12 ng/L, the analysis performed by AXYS cannot confirm NCASI's results for dissolved AMPA.

AXYS reported glyphosate above its reporting limit (≈20 ng/L based on the AXYS ICAL LCL) in 5 of 17 samples analyzed. Results for the remaining 12 samples were nominally <20 ng/L. NCASI's results for two of these samples were also <20 ng/L, and one sample was not analyzed by NCASI. However, for the remaining nine samples reported as <20 ng/L by AXYS, NCASI's reported concentrations ranged from 28 to 62 ng/L. As an example, Sample #15 collected at NBH on

[1] One factor understood to contribute to this inter-laboratory bias is the fact that AXYS generated its calibration via derivatization of native analytes as opposed to simple dilution of pre-derivatized solids (see Appendix B, Section 1).

09/18/2010 returned a result of 62 ng/L from NCASI's analysis but was reported as <20 ng/L by AXYS. Overall, this outcome supports the conclusion that NCASI's results for dissolved glyphosate are high biased.

Considering the five samples for which AXYS reported measured concentrations, the relative bias ranged from 25 to 100%; that is, NCASI's results were 25 to 100% higher than results reported by AXYS. On an absolute basis, the differences in reported concentrations ranged from 6.6 to 42 ng/L, amounts that are in line with background levels measured in the blank control sample (Section 1.2.1 herein) and noted in Section 1.2.4 herein.

Table C1.5 Comparison of Dissolved Concentrations from HPLC/FLUOR and HPLC/MS-MS Analyses of Selected Samples[a]

				AMPA (ng/L)		Glyphosate (ng/L)	
Site	Date	Time	#[b]	NCASI[c]	AXYS	NCASI[c]	AXYS
Field Samples							
NBL	08/25/10	14:45	BF	6	<18.7	33	26.4
NBL	08/30/10	09:00	11	9	<24.0	51	<19.1
NBL	09/01/10	03:00	5	6	<18.9	48	<18.9
NBL	09/14/10	14:00	BF	8	<18.8	34	<18.8
NBL	09/18/10	21:00	18	3	<19.4	39	<19.4
NBL	10/08/10	14:00	BF	6	<18.5	28	<18.5
NBL	12/11/10	(d)	3	4[e]	<19.2	18[e]	<19.2
NBH	08/25/10	10:30	BF	7	<18.4	30	23.4
NBH	08/29/10	23:00	1	7	<18.2	45	24.6
NBH	08/30/10	07:00	9	5	<19.3	31	<19.3
NBH	09/01/10	04:00	6	10	<20.1	84	42
NBH	09/18/10	17:00	15	4	<19.8	62	<19.8
NBH	09/19/10	07:00	22	3	<20.0	31	<20.0
NBH	10/24/10	06:00	9	NA[f]	<19.9	NA[f]	<19.9
NBU	08/30/10	07:00	9	6	<27.5	149	115
NBU	09/01/10	06:00	8	7	<18.2	47	<18.2
NBU	09/14/10	14:45	BF	2	<20.1	17	<20.1
NCASI Standards							
NCASI calibration solution[g]					110%		115%

[a] NCASI analysis of pre-freeze filtrates by HPLC/FLUOR; AXYS analysis of split samples by LC/MS-MS; all results are sample concentrations.
[b] Numerical sequence of sample collection during date-specific storm event (one event could include multiple triggering of ISCO sampler; BF = baseflow grab sample.
[c] NCASI results from Appendix D (glyphosate) and Appendix E (AMPA) reported without censoring (i.e., regardless of MDL).
[d] Time unknown.
[e] NCASI result is dissolved concentration from analysis of whole sample (Table C1.10).
[f] NA = not analyzed.
[g] FMOC derivatives diluted and analyzed; result is percent recovery vs. AXYS's calibration.

Overall, the results in Table C1.5 show that NCASI's analysis returned high-biased concentrations for glyphosate in the Needle Branch samples, and that this bias routinely exceeds 25%. This is attributed to the inability of the HPLC/FLUOR analysis to discriminate against the background interference known to be present at variable levels in samples; that is, the bias is attributed to interference acting on the HPLC/FLUOR analysis.

1.3 AMPA and Glyphosate on Suspended Sediment

Aliquots (≈180 mL) of a small number of whole samples were frozen to allow determination of total concentrations via separate analysis of sample filtrates and the associated solids collected by filtration. As noted in Section 3.1 of the main text, these samples were selected based on a visual determination that they contained relatively high levels of SS (no SS measurements were made). For a subset of these samples, an additional volume of whole sample was spiked with AMPA and glyphosate prior to freezing. On thawing, these samples were analyzed as outlined in Section 3.3 of the main text to give AMPA and glyphosate concentrations in filtrate (dissolved analyte according to Section 3.2 of the main text) and on SS.

Results presented in Appendix B, Section 2, showed that >10 mg of Needle Branch SS has the potential to depress yield from the FMOC derivatization. Thus, the SS concentration in samples should be ≤125 mg/L in order to allow analysis of an 80 mL sample. Based on professional judgment, all samples contained <125 mg/L SS, so all analyses of whole samples used an 80 mL sample volume. In addition, because of the general absence of SS in the field samples, a limited number (n = 12) of these samples were subjected to this analysis.

Appendix B (Section 2, Table B4) also gives results of experiments characterizing the performance of the extraction when applied to Needle Branch SS. These experiments were performed on volumes of pre-application Needle Branch baseflow fortified with SS also collected from Needle Branch. The results of these experiments show that spiking a 50 mL sample containing ≈155 mg/L SS and allowing the spike to equilibrate for ≈60 hours resulted in up to 81% of the spiked AMPA and 89% of the spiked glyphosate being found in the solids fraction. Subsequent analyses of the filtrate and solids fractions were able to recover 96 ±4.7% (n = 6) of the spiked AMPA and 95 ±5.8% (n = 6) of the spiked glyphosate when spikes ranged from 1000 to 5000 ng/L. In these experiments, all recoveries were calculated versus spiked controls with little to no SS, so they are relative recoveries characterizing the efficacy of SS extraction when isolated from the rest of the overall analysis (derivatization, etc.). The results in Appendix B, Table B4, show that the extraction can effectively recover both AMPA and glyphosate from Needle Branch SS.

1.3.1 *Laboratory QA/QC*

The results presented for filtrates in Tables C1.1 to C1.3 are considered relevant to filtrates obtained during analysis of whole samples. Because of the limited number of analyses ultimately performed, extensive batch-specific QA/QC for SS analyses was not performed. Instead, data developed as part of the experimentation discussed in Appendix B are considered to be relevant. Table C1.6 summarizes the levels of background interference found in some experimental SS extracts generated from extraction of a pre-application Needle Branch baseflow sample fortified to hold ≈36 mg/L Needle Branch SS (results from Appendix B, Table B7).

The background interfering with glyphosate in the SS extracts (sample with ≈36 mg/L SS) ranged from ≈9 to ≈26 ng/L as glyphosate (Table C1.6). The higher result was associated with samples that had been left at room temperature for seven days prior to filtration and analysis, and the lower result was from samples analyzed after only a few hours at room temperature. A single analysis performed on solids collected from a sample with ≈155 ng/L SS (Appendix B, Table B1) gave 1.5 ng/L as glyphosate. Overall, these results suggest that the amount of solids is not the primary factor

controlling the magnitude of interference, while also showing that this interference is variable. Regardless, these concentrations are all above the 0.8 ng/L glyphosate background found in a single method blank analyzed as part of testing derivatization yield in 0.5M potassium hydroxide (KOH) (Appendix B, Table B1), indicating that the sample matrix contributes the bulk of the background impacting determination of glyphosate in SS extracts.

Table C1.6 Summary of Background Concentrations Relevant to Analysis of Suspended Sediments[a]

	Background Concentrations (ng/L)[b]		
	AMPA	Glyphosate[c]	Glyphosate[d]
Mean	3.7	9.1	25.7
Std Dev	1.2	0.7	0.6
RSD (%)	33	8	2
n	6	3	3

[a] From stability study, Appendix B, Table B7; results reflect Needle Branch water containing ≈36 mg/L (ppm) SS.
[b] Sample concentrations reported without censoring (i.e., regardless of MDL).
[c] Results from Day 0 samples (refrigerated samples brought to room temperature immediately prior to analysis).
[d] Results from Day 7 samples (samples held at room temperature for seven days prior to analysis).

The background interfering with AMPA in the same experimental SS extracts was not dependent on time spent at room temperature, and the overall mean was equivalent to 3.7 ±1.2 ng/L (n = 6) AMPA. This result is nominally the same as that from pre-application Needle Branch baseflow filtrates (3.2 ng/L; Table C1.1), while a single analysis performed on a sample with ≈155 mg/L SS (Appendix B, Table B1) gave 3.6 ng/L. Overall, these results suggest that the amount of solids did not impact the level of interference. In any case, these concentrations are well above the 0.5 ng/L AMPA background found in a single method blank analyzed as part of testing derivatization yield in 0.5M KOH (Appendix B, Table B1), indicating that the sample matrix contributed the bulk of this background.

Although limited, the results presented in Table C1.6 show that AMPA and glyphosate concentrations measured in SS extracts from Needle Branch samples are subject to background interference from sample constituents. Despite the observation that the background interfering with AMPA was relatively constant in the experimental extracts, analogous to the situation with sample filtrates (Section 1.2.5 herein), it cannot be assumed that it will be constant in all samples. The same holds true for the background interference acting on glyphosate, which was more variable than the AMPA background. Thus, results from SS analyses were not background subtracted, so all reported concentrations carry some (unknown) high bias resulting from background interference.

Table C1.7 summarizes results from analyses of both filtrate and solids fractions from matrix spike experiments performed on whole samples. As noted in the table, in one experiment, spikes were added to a thawed whole sample just prior to initial filtration. Thus, calculating spike recovery requires summing concentrations found in the resulting filtrate and solids fractions, and results reflect overall method performance. In another experiment, the thawed sample was filtered and the two fractions were spiked prior to acidification of the filtrate and extraction of the solids. In that case, reported spike recoveries reflect post-filtration losses in the two fractions separately. In a final experiment spikes were added to each fraction immediately prior to derivatization (i.e., after

extraction); thus, recoveries reflect derivatization and post-derivatization cleanup only (in each fraction).

Table C1.7 Recovery of Laboratory Matrix Spikes Added to Whole (Unfiltered) Samples at Different Points in the Analytical Procedure[a]

	Whole Sample		Filtrate (Dissolved)		Solids		
	AMPA	Glyph	AMPA	Glyph	AMPA	Glyph	Spiking[b]
% Rec	87	98					spike whole sample prior to initial filtration
% Native on solids[c]	7.2	18					
% Spike on solids[d]	0.6	1					
% Rec			87	99	90	94	separate spikes to filtrate and solids fractions after filtration and before solids extraction
% Native on solids[c]	11	27					
% Spike on solids[d]							
% Rec			85	96	86	76	separate spikes to filtrate and solids fractions immediately before derivatization (after solids extraction)
% Native on solids[c]	41	17					
% Spike on solids[d]							

[a] Mean percent recoveries from duplicate spikes performed on one sample only.
[b] All spikes were in the range from 300 to 400 ng/L (sample concentration).
[c] Percent of measured analyte (native chemical) found in solids fraction of unspiked sample.
[d] Percent of measured analyte found in solids fraction of spiked sample; only relevant when spike was added to whole sample prior to filtration.

As seen in Table C1.7, good (>80%) recoveries of both AMPA and glyphosate were obtained when spikes were added prior to sample filtration, and effectively the same levels of recovery were obtained in both filtrate and solids fractions when spikes were added after filtration. Recovery of glyphosate was very good (94 to 99%), and was effectively the same as that obtained when a sample containing 155 mg/L SS was spiked at much higher glyphosate concentrations as part of method development (mean recovery = 95%; Appendix B, Table B4). However, AMPA recovery (87 to 90%) was a little lower than that obtained during method development (mean recovery = 96%; Appendix B, Table B4).

Recovery of both analytes in both fractions decreased when spikes were added after extraction and immediately before derivatization. The greatest decrease was observed for glyphosate in the solids fraction, which decreased from 94% in the post-filtration pre-extraction spike to 76% in the post-extraction spike (Table C1.7). This result is lower than any single recovery obtained during method development (Appendix B, Section 2), which included analyses of samples containing 155 mg/L SS. Overall, this outcome is counterintuitive in that spiking just prior to derivatization would be expected to give recoveries no worse than those obtained when spikes are added prior to extraction and/or filtration.

Table C1.7 also shows the relative amount of each analyte found in the solids fraction from analyses of unspiked samples. These limited results show that this amount was variable, ranging from 7 to 41% for AMPA and from 17 to 27% for glyphosate. These mass fractions are generally lower than those measured when samples with 155 mg/L SS were spiked and equilibrated for ≈60 hours prior to extraction as part of method development (Appendix B, Table B4). Two unique samples of Needle

Branch SS were used in the Appendix B experiments; 58 to 81% of spiked AMPA was found on the solids when SS A was used, while 34 to 40% was found on the solids when SS B was used (Appendix B, Table B4). With SS A, 54 to 89% of spiked glyphosate was found on the solids, and for SS B 19 to 33% was found on the solids.

Overall, these results suggest that the SS in Needle Branch samples has variable affinity for AMPA and glyphosate. That is, the relative mass of each analyte found on Sample SS will depend on both the "nature" and the concentration of the sample-specific SS. As discussed (Section 1.3 herein), the results presented in Appendix B, Section 2 support good recovery of both analytes from Sample SS as long as the absolute mass of SS included in the derivatization is ≤10 mg (≤125 mg/L SS in an 80 mL sample).

Although SS was not measured in the samples included in Table C1.7, visual inspection indicated that SS varied from sample to sample, and this may have contributed to the variability seen in distributions of both AMPA and glyphosate between the dissolved (filtrate) and particulate (solids) compartments in unspiked samples. However, based on the totality of the data, the low recovery of glyphosate from the pre-derivatization spike (Table C1.7) cannot be attributed to abnormally high solids in that sample. Thus, this result remains an anomaly.

1.3.2 *Method Detection Limits*

The MDLs and MLs in Table C1.3 are relevant to the filtrates obtained during analysis of previously unfiltered (whole) samples. As noted in Section 3.3 of the main text, no MDLs or MLs were determined for the solids analysis.

1.3.3 *Field QA*

A number of whole (i.e., unfiltered) samples were spiked with AMPA and glyphosate prior to freezing, and results from these spikes provide the most authoritative measure of overall recovery in that they reflect all aspects of the analysis other than the period between collection by the ISCO samplers and freezing. As noted in Section 1.1 herein, <5% of glyphosate in the samples was lost during this period, while effectively no AMPA was lost.

Because of very low SS concentrations (≤2 mg/L), a number of frozen unfiltered pre-application baseflow samples and their associated spikes were analyzed without filtration; that is, the thawed sample was acidified and then analyzed as if it was a filtrate (neither filtration nor solids extraction was performed). In addition, one unfiltered frozen field sample with unknown SS was analyzed this way, as was the associated pre-freeze spike. Results from these analyses are summarized in Table C1.8.

Table C1.8 Recovery of AMPA and Glyphosate Added to Whole (Unfiltered) Samples Prior to Freezing when Thawed Samples were Analyzed Whole[a]

Sample			Sample Result (ng/L)[b]		Spike Concentration (ng/L)		Recovery (%)	
Site	Date	#[c]	AMPA	Glyph	AMPA	Glyph	AMPA	Glyph
NBL	07/07/10	PA	3	12	2489	2496	86.4	97.1
NBL	12/11/10	3	4	18	1201	1205	65.3	83.1
NBL	07/07/10	PA	3	12	50	50	89.9	96.8
NBL	07/07/10	PA	3	14	50	50	92.2	101.8
						Mean	83	95
						Std Dev	12.3	8.1
						RSD (%)	15	9
						N	4	4

[a] Unfiltered sample and unfiltered sample spike frozen and both analyzed as a filtrate upon thawing (i.e., no filtration, and derivatization performed in sample matrix).
[b] Sample concentrations reported without censoring (i.e., without regard to MDL).
[c] Sample number for date-specific storm event sampling; PA = pre-application baseflow grab sample.

Results for glyphosate (Table C1.8) are generally consistent with matrix spike results (Table C1.7) and results obtained during method development (Appendix B, Table B4) in that they show, with one exception, recoveries >90%. AMPA recoveries in Table C1.8 are also generally consistent with matrix spike results (Table C1.7), again with one exception, but are about 10% lower, on average, than those obtained during method development (Appendix B, Table B4).

Table C1.9 shows results from analyses of pre-freeze sample spikes obtained when thawed samples were filtered and the filtrate and solids fractions were analyzed separately. With the exception of one baseflow grab, these samples were collected during storm events.

Table C1.9 Recovery of AMPA and Glyphosate Added to Whole (Unfiltered) Samples Prior to Freezing when Thawed Samples were Filtered and Filtrate and Solids Fractions were Analyzed Separately

Sample			Sample Result (ng/L)[a]		Spike Concentration (ng/L)		Recovery[b] (%)	
Site	Date	#[c]	AMPA	Glyph	AMPA	Glyph	AMPA	Glyph
NBL	12/11/10	6	15	33	1338	1342	71.8	83.6
NBL	12/11/10	3	7	36	1201	1205	67.3	87.8
NBH	12/10/10	17	18	17	724	726	75.1	92.8
NBU	12/10/10	10	3	16	669	671	74.2	88.8
NBU	12/03/10	BF	11	14	651	653	76.0	89.3
						Mean	72	88
						Std Dev	3.5	3.8
						RSD (%)	5	4
						N	5	5

[a] Sample concentrations reported without censoring (i.e., regardless of MDL).
[b] Recovery based on summed results from analyses of filtrate and solids fractions.
[c] Sample number for date-specific storm event sampling; PA = pre-application baseflow grab sample, BF = post-application baseflow grab sample.

As the table shows, the mean recovery for glyphosate was 88 ±3.8% (n = 5). This is lower than the mean from analyses of unfiltered samples (95 ±8.1%; Table C1.8) or spiked samples filtered after a 60 hour equilibration (95 ±6%; Appendix B, Table B4). The results in Table C1.9 provide the best measure of overall recovery for glyphosate. Assuming that up to 5% of glyphosate is lost during the period of time between collection and freezing (Section 1.1 herein), overall recovery of total glyphosate is estimated to be ≈84% (0.88*0.95) from samples frozen whole (unfiltered).

From Table C1.9, the mean recovery for AMPA was 72 ±3.5% (n = 5). Again, this is lower than the means from analyses of unfiltered samples (83 ±12.3%; Table C1.8) or spiked samples filtered after a 60 hour equilibration (96 ±5%; Appendix B, Table B4). Again, however, these results (Table C1.9) provide the best measure of overall recovery for AMPA. Thus, because no measurable losses of AMPA were observed in the room temperature storage stability study (Section 1.1 herein), overall recovery of total AMPA is estimated to be ≈72% (0.72*1) from samples frozen whole.

1.3.4 *The Impact of Freezing on Measured Concentrations of Dissolved AMPA and Glyphosate*

The purpose of examining the impact of freezing on measured dissolved concentrations in frozen whole samples is to characterize the potential for dissolved AMPA and glyphosate to irreversibly bind to sample solids during the freeze-thaw cycle. If this was to occur, subsequent measurements of AMPA and glyphosate in thawed whole samples would return low-biased measurements of dissolved chemicals and high-biased measures of the amount of chemicals adsorbed to solids. Although the data are insufficient to determine whether the observed change was due to loss of the background interferent or dissolved AMPA, this phenomenon manifested in the results from analysis of frozen and refrigerated blank control, which showed that the background interfering with AMPA decreased by ≈37% on freezing (Table C1.2) even though sample SS was very low (≤2 mg/L).

Table C1.10 compares concentrations of dissolved AMPA and glyphosate measured in sample filtrates generated prior to freezing to dissolved concentrations measured in filtrates generated from filtering whole samples after thawing (i.e., unfiltered samples frozen, thawed, and then filtered).

Table C1.10 Comparison of Dissolved Concentrations from Analyses of Filtrates Generated Prior to Freezing and Filtrates Generated after Thawing Frozen Whole Samples

Sample			Dissolved Sample Concentrations (ng/L)[a]				RPD[e] (%)	
			Whole Sample Filtered after Thawing[c]		Sample Filtered Prior to Freezing[d]			
Site	Date	#[b]	AMPA	Glyph	AMPA	Glyph	AMPA	Glyph
NBU	08/30/10	9	4	144	6	149	-33.0	-3.4
NBH	09/01/10	7	5	68	9	70	-43.4	-3.9
NBU	08/30/10	7	3	62	2	62	11.7	-0.7
NBH	09/18/10	15	9	48	4	62	99.8	-22.7
NBH	08/30/10	2	5	33	8	38	-42.7	-13.3
NBU	09/01/10	8	6	32	7	47	-5.8	-31.3
NBL	09/18/10	18	4	28	3	39	7.8	-27.7
NBL	08/30/10	15	2	26	10	32	-77.5	-18.7
NBL	09/15/10	2	3	24	4	23	-36.0	0.4
NBL	12/11/10	3	4	18	NA	NA		
NBU	08/30/10	24	7	17	19	17	-61.6	-1.2
NBL	12/11/10	6	6	14	NA	NA		
NBL	08/22/10	4	4	14	2	12	150.9	12.2

(Continued on next page. See notes at end of table.)

Table C1.10 Continued

Sample			Dissolved Sample Concentrations (ng/L)[a]				RPD[e] (%)	
			Whole Sample Filtered after Thawing[c]		Sample Filtered Prior to Freezing[d]			
Site	Date	#[b]	AMPA	Glyph	AMPA	Glyph	AMPA	Glyph
NBH	12/10/10	17	7	13	NA	NA		
NBU	12/10/10	10	2	12	NA	NA		
NBU	12/03/10	BF	10	10	3	11	240.1	-9.9
						Mean	18	-10
						Std Dev	96.7	13.0
						RSD (%)	552	-130
						n	12	12

[a] Sample concentrations reported without censoring (i.e., regardless of MDL).
[b] Sample number for date-specific storm event sampling; BF = baseflow grab sample.
[c] Unfiltered samples thawed and filtered, then filtrate and solids fractions analyzed separately (results for filtrates are dissolved herbicide); results sorted highest to lowest glyphosate concentration in analysis of post-freeze filtrate samples.
[d] Samples filtered immediately on return to laboratory and resulting filtrate frozen; NA indicates that pre-freeze filtrate was not analyzed.
[e] Relative percent difference in dissolved herbicide calculated as [((post-freeze–pre-freeze)/pre-freeze)*100].

These results show that the impact of freezing on dissolved AMPA was variable, with measured dissolved concentrations decreasing in seven samples and increasing in five samples. However, only three of the 28 measured dissolved AMPA concentrations were >10 ng/L, and the apparent difference between measured concentrations was ≤8 ng/L in all but one sample. In multiple samples these differences were on the order of the dissolved AMPA MDL, which was 3.8 ng/L (Table C1.3). At these concentrations, individual measurements are impacted by variability in the background signal, which was 2.4 ±0.55 ng/L (n = 16; Table C1.2) in frozen blank control. Considering that SS probably varied somewhat in these samples (SS was not measured), this background could well have been higher in any given field sample. Regardless, given the uniformly low concentrations of dissolved AMPA, variability in the background at 2 to 5 ng/L was probably a significant factor driving the relative percent differences (RPDs) given in Table C1.10.

On the other hand, dissolved glyphosate in multiple samples was well above the background signal found in frozen blank control (12.8 ±1.99 ng/L, n = 16; Table C1.2), and for samples with more than three times this background (>38.4 ng/L) in the pre-freeze filtrate, freezing unfiltered sample had the uniform effect of decreasing measured dissolved glyphosate. However, the general trend indicates smaller RPDs as concentrations increased, and differences in concentrations were always less than the dissolved glyphosate MDL, which was 18.0 ng/L (Table C1.3). This suggests that variability in the background signal probably impacted dissolved glyphosate RPDs.

Variability in analyte- and sample-specific background interference could be the primary factor driving the RPDs in Table C1.10. Although the limited data available suggest that the background interference that impacted both AMPA and glyphosate did not vary with the mass of SS present in any given sample (Section 1.3.1 herein), it is still possible that the RPDs for both AMPA and glyphosate given in Table C1.10 reflect differences in sample-specific SS concentrations, and that samples with more SS showed greater RPDs due to irreversible adsorption of AMPA and/or glyphosate to these solids over the freeze-thaw cycle. Unfortunately, without measured SS concentrations, this potential cannot be examined.

1.3.5 *Total Glyphosate and AMPA Concentrations*

Table C1.11 summarizes results from analyses of whole (unfiltered) samples that were filtered after thawing. The filtrate and solids fractions were analyzed separately as outlined in Section 3.3 of the main text.

These results indicate that total AMPA ranged from 44 to 375% of measured dissolved AMPA. Obviously, a true total concentration can never be less than a dissolved concentration, so these results suggest loss of either AMPA or the background interferent from some samples during the freeze-thaw cycle. Regardless, the differences between the measured total and pre-freeze dissolved AMPA concentrations were ≤8 ng/L for all samples, and for many samples the difference was ≤3 ng/L. Differences of these magnitudes are consistent with the apparent AMPA concentrations found in the blank control (Section 1.2.1 herein) and blanks generated during development of the SS methodology (Table C1.6). Thus, for all samples, the apparent contribution from the SS fraction can be attributed to the background known to impact the SS analysis (Table C1.6), showing that results are consistent with there being little to no AMPA on sample SS.

Although including SS in the analysis appears to have doubled the mass of glyphosate found in some samples, the relative contribution of the SS fraction to the measured total generally increased as the original dissolved concentration decreased, and for all but two samples, the concentration found in the SS fraction was ≤13 ng/L. This is consistent with the apparent glyphosate concentrations found in pre-application filtrate controls (Table C1.1) and blanks generated during development of the SS methodology (Table C1.6). Thus, for these samples, the apparent contribution from the SS fraction to total glyphosate can be attributed to background interference in the SS measurement, which is known to vary on a sample-specific basis (Section 1.3.1 herein).

The glyphosate concentrations found on SS from two samples (NBL #3 and NBL #6 from 12/11/2010) were ≈19 ng/L. In neither case was the pre-freeze filtrate analyzed, so the dissolved concentration reported in Table C1.10 and used to calculate the total/dissolved value in Table C1.11 was measured in filtrate generated after thawing unfiltered frozen sample. As discussed in Section 1.3.4, freezing unfiltered samples and then measuring dissolved glyphosate can result in low-biased measurements of dissolved glyphosate, and the results presented in Table C1.10 suggest this bias can approach 30% in some samples. This alone will add a high bias to a total/dissolved value even if none of this “lost” dissolved glyphosate is found on the solids. The bias in the total/dissolved value would be even greater if this lost glyphosate was shifted to sample SS and measured as part of the SS analysis. For these reasons, total/dissolved glyphosate for the four samples collected on 12/10/2010 and 12/11/2010 are considered biased, and so were excluded from calculation of the means given in Table C1.11.

Table C1.11 Contribution of AMPA and Glyphosate Adsorbed onto Suspended Sediments to Measured Total Concentrations

Sample			Total Concentrations (ng/L)[b,c]		Relative Percent (total/dissolved)[d]	
Site	Date	#[a]	AMPA	Glyph	AMPA	Glyph
NBU	08/30/10	9	9	155	148	104
NBH	09/01/10	7	6	76	71	108
NBU	08/30/10	7	4	68	174	108
NBH	09/18/10	15	11	64	264	102
NBH	08/30/10	2	5	40	62	105
NBU	09/01/10	8	7	44	105	94
NBL	09/18/10	18	5	41	147	104
NBL	08/30/10	15	5	35	44	110
NBL	09/15/10	2	4	35	95	148
NBL	12/11/10	3	7	36	197[e]	207[e]
NBU	08/30/10	24	12	20	65	118
NBL	12/11/10	6	15	33	254[e]	236[e]
NBL	08/22/10	4	5	17	314	142
NBH	12/10/10	17	18	17	271[e]	126[e]
NBU	12/10/10	10	3	16	114[e]	133[e]
NBU	12/03/10	BF	11	14	375	125
				Mean	155	114
				Std Dev	108.0	16.6
				RSD (%)	70	15
				n	12	12

[a] Sample number for date-specific storm event sampling; BF = baseflow grab sample.
[b] Unfiltered (i.e., whole) samples thawed and filtered, then filtrate and solids fractions analyzed separately and results summed to get total; results sorted highest to lowest glyphosate concentration in analysis of post-freeze filtrate samples (Table C1.10).
[c] Sample concentrations reported without censoring (i.e., regardless of MDL).
[d] Calculated using dissolved result from pre-freeze filtrate (Table C1.10).
[e] Sample-specific result calculated using dissolved result from analysis of post-freeze filtrate (Table C1.10) because pre-freeze filtrate was not analyzed; these specific ratios were excluded from calculation of the mean.

Ultimately, the results in Table C1.11 are consistent with there being little to no glyphosate on sample SS.

2.0 ANALYTICAL RESULTS FOR IMAZAPYR, SULFOMETURON METHYL, AND METSULFURON METHYL

2.1 Stability of Imazapyr, Sulfometuron Methyl, and Metsulfuron Methyl in pH 7 Preserved Samples

Previous work by NCASI (2007) showed that buffering at pH 7 was an effective means of preserving imazapyr and sulfometuron methyl in filtered (0.45 µm) streamwater for up to 30 hours at 50°F. However, filtration at 0.45 µm may have effectively sterilized samples, and no data on metsulfuron methyl were developed as part of that work.

More recently, Fischer, Michael, and Gibbs (2008) reported results from a study examining the stability of multiple herbicides in unfiltered (i.e., whole) streamwater held in 1 L ISCO HDPE sector bottles at “ambient temperatures” for up to 24 days. Both samples were collected from streams in

Alabama. Sample collected from Weogufka Creek had a pH of 6.5 and was characterized as being "clear," while sample collected from a stream in the Escambia Experimental Forest had a pH of 5.1 and was characterized as "visibly amber colored" and containing suspended sediment (SS was not measured). In that study, samples were spiked with pH 7 potassium phosphate buffer at a final concentration of 0.000125M as a preservative and all herbicides were spiked at 100 µg/L. Results for imazapyr, sulfometuron methyl, and metsulfuron methyl are summarized in Table C2.1, and show that over 6 days of storage at ambient temperature ≈5% of spiked sulfometuron methyl, ≈2% of spiked imazapyr, and effectively 0% of spiked metsulfuron methyl were lost from the pH 7 buffered, "amber colored" streamwater. These results are considered sufficient to document stability of these same herbicides in the samples collected as part of Needle Branch study, which were preserved using a final potassium phosphate buffer concentration of 0.01M, contained very low levels of SS, were at temperatures ranging from 9 to 13°C at the time of collection, and were frozen within nominally three days of collection during which time ambient temperature was no greater than ≈70°F.

Table C2.1 Recovery of Herbicides from Streamwater Samples after Various Periods Standing at Ambient Temperature in 1L ISCO HDPE Sector Bottles[a]

	Weogufka Creek Water		Escambia Streamwater	
Herbicide	Day 2	Day 24	Day 6	Day 24
Imazapyr	104.6 ±0.6*	93.4 ±2.0*	98.3 ±0.9*	99.3 ±0.5
Metsulfuron methyl	101.7 ±0.4*	100.0 ±0.8	100.0 ±1.1	97.1 ±0.4*
Sulfometuron methyl	97.7 ±0.8	91.1 ±0.9*	94.8 ±1.9*	84.3 ±3.9*

Source: excerpted from Fischer, Michael, and Gibbs 2008.

[a] Values are mean percent recovery relative to day 0 with standard deviations, n = 4; * = significant difference from day 0 mean (P <0.05).

Fischer, Michael, and Gibbs (2008) also examined the efficacy of freezing as a means of long-term preservation. Relevant results are summarized in Table C2.2, and show that over 12 months storage at ≤-15°C ≈15% of spiked sulfometuron methyl, ≈6% of spiked imazapyr, and ≈3% of spiked metsulfuron methyl were lost from the pH 7 buffered, "amber colored" streamwater.

Table C2.2 Herbicide Recoveries from Streamwater Samples after Freezer Storage at <-15°C[a]

	Weogufka Creek Water			Escambia Streamwater		
Herbicide	Month 3	Month 6	Month 12	Month 3	Month 6	Month 12
Imazapyr	97.8 ±0.7*	102.4 ±0.8*	94.9 ±1.8*	93.9 ±0.5*	95.8 ±0.8*	93.9 ±0.2*
Metsulfuron methyl	108.8 ±0.3*	131.0 ±0.8*	95.5 ±1.2*	99.2 ±0.2	99.5 ±0.6	97.0 ±0.4*
Sulfometuron methyl	105.9 ±0.5*	99.2 ±0.4*	103.4 ±0.1*	101.1 ±2.5	91.6 ±1.4*	84.5 ±2.8*

Source: excerpted from Fischer, Michael, and Gibbs 2008.

[a] Values are mean percent recovery relative to day 0 with standard deviations, n = 4; * = significant difference from day 0 mean (P <0.05).

2.2 Results for Dissolved Imazapyr, Sulfometuron Methyl, and Metsulfuron Methyl

2.2.1 *Laboratory QA/QC*

In addition to a calibration verification, batch-specific (ongoing) laboratory QA/QC included analysis of a blank control and a spiked blank control. A large volume grab sample collected at NBL on 07/06/2012 (approximately two years after application of herbicides) served as the blank control sample. Aliquots of this refrigerated sample were taken as required. The SS concentration in this sample was on the order of 2 mg/L. Most analytical batches also included an MS/MSD experiment

performed on a randomly selected field sample. These matrix spikes were added to the analytical volumes of thawed samples immediately prior to filtration. Results from laboratory QA/QC analyses (except calibration verification) are summarized in Table C2.3.

Table C2.3 Summary of Laboratory QA/QC Results for Dissolved Imazapyr, Sulfometuron Methyl, and Metsulfuron Methyl

	Blank Control (µg/L)[a]				Spiked Blank Control (%Rec)[b]			
Herbicide	Mean	SD	%RSD	n	Mean	SD	%RSD	n
Imazapyr	0.10	0.037	39	23	90	7.9	9	21
Sulfometuron methyl	0.23	0.102	44	23	91	4.3	5	21
Metsulfuron methyl	0.38	0.227	59	23	82	13.1	16	21

	Sample (Matrix) Spikes[c]							
	Percent Recovery				Relative Percent Difference			
	Mean	SD	%RSD	n	Mean	SD	%RSD	n
Imazapyr	94	6.6	7	6	3.3	1.7	51	5
Sulfometuron methyl	92	1.6	2	6	3.7	2.0	55	5
Metsulfuron methyl	88	7.6	9	6	4.9	3.7	75	5

[a] Sample concentrations reported without censoring (i.e., regardless of MDL).
[b] Blank control spiked with imazapyr at 0.625 to 6.25 µg/L (sample concentrations) and sulfometuron methyl and metsulfuron methyl at 1 to 6.25 µg/L (sample concentrations).
[c] Spike levels from 1 to 25 µg/L (sample concentrations) for imazapyr and 2 to 25 µg/L (sample concentrations) for sulfometuron methyl and metsulfuron methyl.

Regardless of the matrix (blank control or samples), mean spike recoveries for imazapyr, sulfometuron methyl, and metsulfuron methyl were >80%, and there were effectively no differences between recoveries from spiked blank control versus spiked samples. This agreement between the recoveries reflects the reality that the blank control was in fact a field sample and that SS was low in all samples. Figure C2.1 provides plots showing the recovery of each analyte from the control and matrix spikes as a function of spike level.

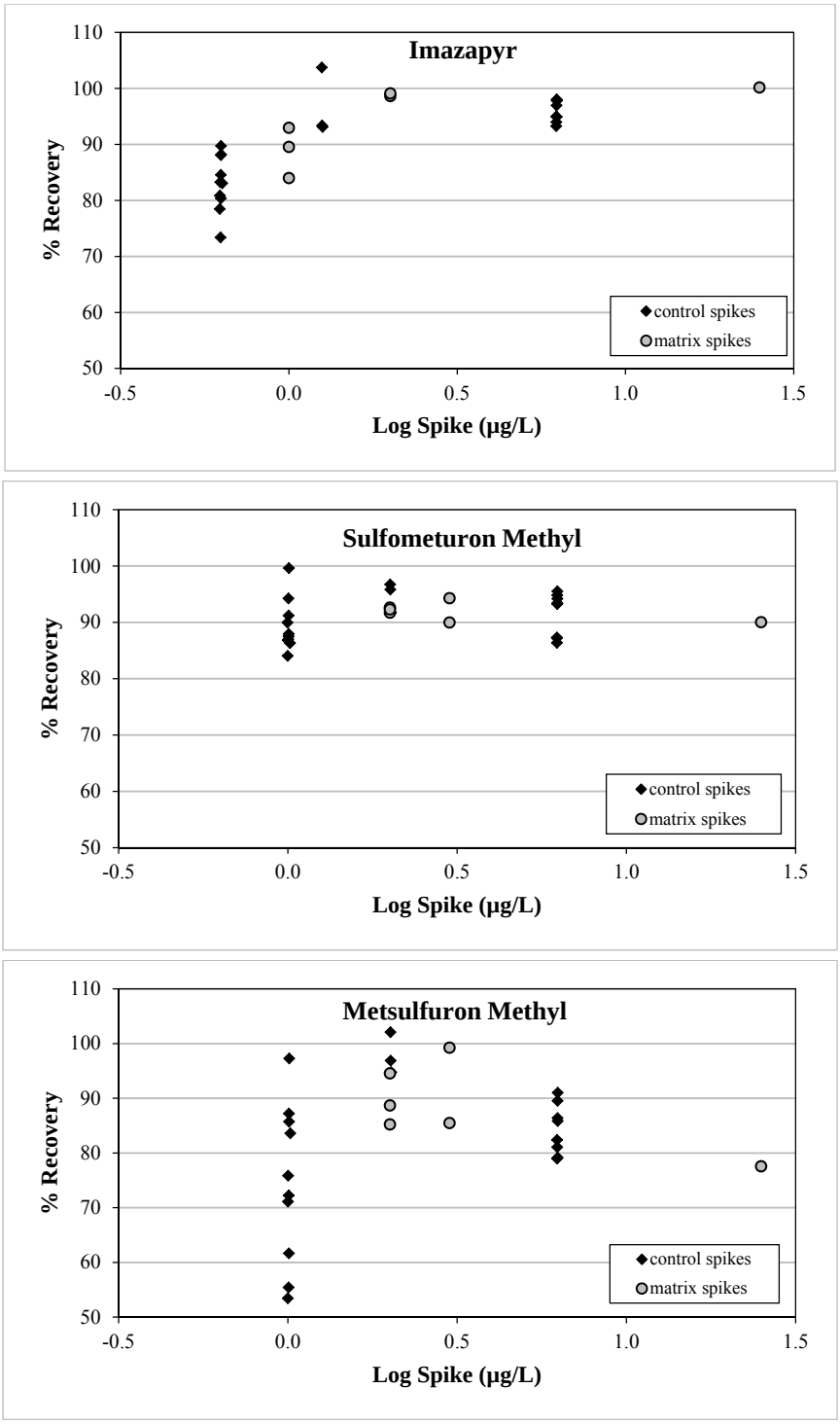

Figure C2.1 Analyte-Specific Recoveries from Control and Matrix Spikes as a Function of Absolute Spike Level

In the case of imazapyr, recovery at the lowest spike level (0.6 µg/L in control spikes) averaged 83 ±5.0% (n = 10) and increased to 95 ±4.5% (n = 17) at spike levels ≥1 µg/L, while recovery of sulfometuron methyl was essentially constant at ≈90% (91 ±3.9%, n = 27) across the entire spike range (all spikes ≥1 µg/L). Recovery of metsulfuron methyl at the lowest spike level (1 µg/L in control spikes) was low and quite variable, with a mean of 74 ±14.5% (n = 10); recovery increased to an average of 89 ±7.1% (n = 16) at spike levels between 2 and 6 µg/L, and then dropped to 78% (n = 1) at 25 µg/L. Additional results reported in Section 2.2.3 herein (Figure C2.2) support concluding that the 78% recovery at 25 µg/L was an anomaly.

2.2.2 *Study-Specific Method Detection Limits and Minimum Levels*

Study-specific MDLs and MLs were established via replicate analyses of a blank control sample (baseflow collected at NBL) and the same sample spiked at ≈1 µg/L (sample concentration) sulfometuron methyl and metsulfuron methyl and ≈0.6 µg/L (sample concentration) imazapyr. All these analyses were performed in a single analytical batch, so the results reflect intra-batch variability only. An analysis of the blank control was also performed as part of every sample preparation batch, and pooling the results allows calculation of an MDL reflecting long-term batch-to-batch variability. Results of all these MDL calculations are summarized in Table C2.4.

As discussed in Section 1.2.2 herein, because analysis of samples proceeded over an extended period (≈4 months), an MDL incorporating long-term inter-batch variability is more relevant to the resulting data set than an MDL developed from analyses performed in only one analytical batch. In addition, the blank control gave chromatographic peaks interfering with all three analytes, and this background must be accounted for in derivation of the MDLs. Thus, study-specific MDLs (as sample concentrations) are based on results from replicate analyses of the unspiked blank control. As shown in Table C2.4, the resulting MDLs are 0.2 µg/L for imazapyr, 0.5 µg/L for sulfometuron methyl, and 1.0 µg/L for metsulfuron methyl. Corresponding MLs are 0.5 µg/L for imazapyr, 1.3 µg/L for sulfometuron methyl, and 2.6 µg/L for metsulfuron methyl.

The results presented in Table C2.4 show good (>80%) recoveries of spiked imazapyr and spiked sulfometuron methyl from the MDL experiment, and these recoveries are nominally equivalent to mean recoveries from the control and matrix spikes made at comparable spike levels (Table C2.3). These results show that the laboratory analysis can provide accurate background-subtracted (or background-corrected) quantifications of imazapyr in sample matrix (NBL baseflow) to 0.6 µg/L, which is the ICAL LCL for imazapyr. These data also show that the laboratory analysis can provide accurate background-subtracted (or background-corrected) quantifications of sulfometuron methyl in sample matrix (NBL baseflow) to 1.0 µg/L.

Recovery of metsulfuron methyl from the MDL experiment was also consistent with recoveries from control and matrix spikes made at comparable concentrations (≈1 µg/L), although in all cases recovery was relatively low (71 to 74%).

Table C2.4 Summary of Study-Specific MDLs and MLs for Dissolved Imazapyr (IMAZ), Sulfometuron Methyl (SMM), and Metsulfuron Methyl (MSM)

	Spiked Blank Control[a] (µg/L)[b]			Unspiked Blank Control (µg/L)[b]			Blank-Subtracted Spike Recoveries (%Rec)		
	IMAZ	SMM	MSMs	IMAZ	SMM	MSMs	IMAZ	SMM	MSM
Method Detection Limit Experiment									
Mean	0.600	1.027	1.296	0.084	0.140	0.589	82.7	89.0	70.6
Std Dev	0.034	0.033	0.130	0.023	0.044	0.227	5.47	3.28	13.02
RSD (%)	6	3	10	28	31	39	7	4	18
N	8	8	8	8	8	8	8	8	8
MDLs and MLs[c]									
MDL	0.10	0.10	0.39	0.07	0.13	0.68			
ML	0.34	0.33	1.30	0.23	0.44	2.27			
MDLs and MLs accounting for sample background[d]									
MDL	0.19	0.24	0.98	0.15	0.27	1.27			
ML	0.43	0.47	1.89	0.32	0.58	2.86			
Replicate Analyses of Unspiked Blank Control[e]									
Mean				0.095	0.231	0.382			
Std Dev				0.037	0.102	0.227			
RSD (%)				39	44	59			
N				23	23	23			
MDLs and MLs[f]									
MDL				0.09	0.26	0.57			
ML				0.37	1.02	2.27			
MDLs and MLs accounting for sample background[g]									
MDL				0.19	0.49	0.95			
ML				0.46	1.26	2.65			

[a] Blank control sample spiked with ≈0.6 µg/L (a.e. sample concentration) imazapyr and ≈1 µg/L (a.i. sample concentration) sulfometuron methyl and metsulfuron methyl.
[b] Sample concentrations reported without censoring (i.e., regardless of MDL).
[c] Analyte-specific MDL calculated (SD*2.998); analyte-specific ML calculated (SD*10).
[d] Analyte-specific MDL calculated [(mean blank)+(SD*2.998)]; analyte-specific ML calculated [(mean blank)+(SD*10)].
[e] From Table C2.3.
[f] Analyte-specific MDL calculated (SD*2.508); analyte-specific ML calculated (SD*10).
[g] Analyte-specific MDL calculated [(mean blank)+(SD*2.508)]; analyte-specific ML calculated [(mean blank)+(SD*10)].

2.2.3 *Field QA*

Figure C2.2 and Table C2.5summarize recoveries of spikes added to unfiltered, pH-preserved samples prior to freezing (all spikes ≥4.8 µg/L). These spike recoveries provide the most authoritative measure of overall recovery in that they reflect all aspects of the analysis other than the period between collection by the ISCO samplers and freezing. As discussed in Section 2.1 herein, losses during this period are assumed to be negligible.

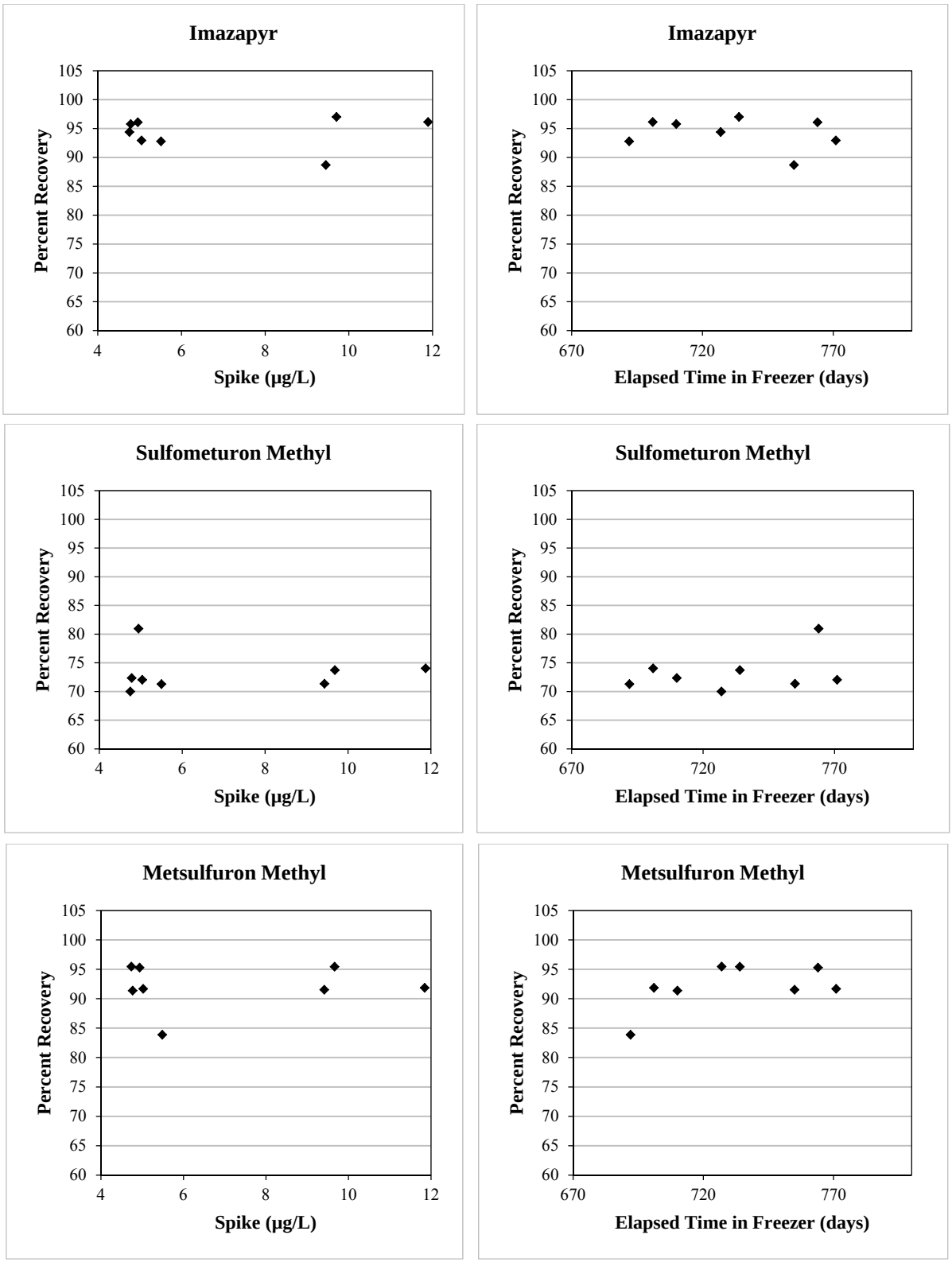

Figure C2.2 Recoveries of Spikes Added to Samples Prior to Freezing as a Function of Absolute Spike Level [left] and Elapsed Time Frozen Prior to Thawing and Analysis [right]

Table C2.5 Recovery of Herbicide Spikes Added to Samples Immediately Prior to Freezing[a]

	Percent Recovery			
	Mean	Std Dev	RSD (%)	n
Imazapyr	94	2.7	3	8
Sulfometuron methyl	73	3.4	5	8
Metsulfuron methyl	92	3.8	4	8

[a] Spike levels from 4.8 to 11.9 µg/L.

It is worth noting that these results (Table C2.5) differ somewhat from those summarized in Table C2.2 (Fischer, Michael, and Gibbs 2008). Most notably, NCASI's results show losses of 26% of sulfometuron methyl, while Table C2.2 shows a maximum loss of 15%. This difference could be due to the much lower spike levels used by NCASI (all spikes <11.9 µg/L vs. 100 µg/L), the increased time NCASI's samples were frozen (up to approximately two years vs. one year), and/or some unidentified matrix effect. However, even with these differences, NCASI's results for imazapyr and metsulfuron methyl are, on average, consistent with the results in Table C2.2. In any case, the Table C2.5 results are taken as the best measure of overall recovery from NCASI's analysis.

The plots in Figure C2.2 show recovery of the pre-freeze spikes as a function of spike level and time spent frozen. Overall, recoveries of all three analytes were effectively independent of spike level (all spikes ≥4.8 µg/L) and elapsed time in the freezer. Thus, the means in Table C2.5 represent recovery across the full spike range (≈5 to 12 µg/L).

Mean recoveries of imazapyr and metsulfuron methyl from the pre-freeze spikes were >90% (Table C2.5), consistent with control spike and matrix spike results (Figure C2.1) at comparable spike levels (≥4.8 µg/L). This shows that the freeze-thaw cycle did not affect recovery of these two analytes at concentrations >4.8 µg/L, suggesting that results from control and matrix spikes can be used to characterize recovery of dissolved imazapyr and metsulfuron methyl from frozen samples at concentrations <4.8 µ/L. These results (Section 2.2.1 herein) showed that imazapyr recovery was at 83 ±5.0% (n = 10 control spikes; Figure C2.1) at 0.6 µg/L, which is the ICAL LCL, and increased to 95 ±4.5% (n = 17 control and matrix spikes) at concentrations ≥1 µg/L. Thus, recovery of dissolved imazapyr from frozen samples is estimated to be ≈83% for concentrations <1 µg/L and ≈94% (Table C2.5) for concentrations ≥1 µg/L.

For metsulfuron methyl, results from the control and matrix spikes showed that recovery was 74 ±14.5% (n = 10) at 1 µg/L, and increased to 89 ±7.1% (n = 16) at concentrations ≥2 µg/L (Section 2.2.1 herein). Thus, recovery of dissolved metsulfuron methyl from frozen samples is estimated to be ≈74% for concentrations <2 µg/L and ≈94% (Table C2.5) for concentrations ≥2 µg/L.

The mean recovery of sulfometuron methyl from pre-freeze spikes (73 ±3.4%, Table C2.5) was almost 20% lower than the mean recovery when results from control spikes and matrix spikes were pooled (91 ±3.9%, n = 27). This indicates loss of dissolved sulfometuron methyl during the freeze-thaw cycle; thus recovery from the pre-freeze spikes is the only metric useful for characterizing recovery of dissolved sulfometuron methyl from frozen samples. Recovery of sulfometuron methyl at all concentrations is estimated to be 73%.

2.2.4 *Dissolved Imazapyr Concentrations*

Appendix F gives results for dissolved imazapyr (a.e.) in all samples analyzed. As with AMPA and glyphosate, none of these reported concentrations were recovery corrected, nor were any corrections for background interference made.

As with AMPA (Section 1.2.5 herein), all measured imazapyr concentrations were low enough to be biased by background interference, which averaged ≈0.1 µg/L (0.095 ±0.037 µg/L, n = 23; Table C2.4) in the blank control. Additional results from samples collected at NBU (Appendix F) immediately before the application of herbicides showed a background of ≈0.2 µg/L (0.175 ±0.051 µg/L, n = 3), demonstrating that background can vary from sample to sample.

As noted in Section 2.2.3, results from analysis of the pre-freeze spikes provide the best measure of overall analytical accuracy. As seen in Table C2.5, mean recovery of imazapyr from the pre-freeze spikes was 94 ±2.7% (n = 8). However, all these spikes were at concentrations ≥4.8 µg/L, which is a nominal order of magnitude higher than the highest concentration found in any sample (0.4 µg/L). Therefore, results from the control and matrix spikes made at 0.6 µg/L are more relevant, and these spikes were recovered at 83 ±5.0% (n = 10) (Section 2.2.1), indicating that the results in Appendix F are nominally 20% low biased. On the other hand, given that background interference from constituents native to samples can be as high as ≈0.2 µg/L (as imazapyr), sample concentrations ≤0.4 µg/L can carry up to 50% high bias in the absence of background subtraction.

Besides the issues noted, all measured imazapyr concentrations were less than the ICAL LCL (0.6 µg/L), and so must be considered estimates. Combining this with the uncertainties regarding background interference means that, overall, the absolute bias in the dissolved imazapyr concentrations provided in Appendix F cannot be characterized, and it is well within the realm of possibility that all reported concentrations are true false positives. For all these reasons, the concentrations given in Appendix F are not considered to be reliable measures of imazapyr in samples and, ultimately, the most defensible statement that can be made is that imazapyr was not present in any sample at concentrations >0.6 µg/L (the ICAL LCL for imazapyr).

5.2.5 *Dissolved Sulfometuron Methyl Concentrations*

Appendix G gives results for dissolved sulfometuron methyl (a.i.) in all samples analyzed. Sulfometuron methyl was not found in any sample at concentrations exceeding the MDL of 0.5 µg/L; that is, the reportable result from analysis of all samples was <0.5 µg/L.

5.2.6 *Dissolved Metsulfuron Methyl Concentrations*

Appendix H gives results for dissolved metsulfuron methyl (a.i.) in all samples analyzed. Metsulfuron methyl was not found in any sample at concentrations exceeding the MDL of 1.0 µg/L; that is, the reportable result from analysis of all samples was <1.0 µg/L.

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APPENDIX D

DISSOLVED GLYPHOSATE CONCENTRATIONS

The study-specific method detection limit (MDL) for dissolved glyphosate in Needle Branch samples was determined to be 18.0 ng/L (ppt; Appendix C, Section 1.2.2). This metric was developed via replicate analyses of a single pre-application baseflow sample collected at NBL (the blank control). This sample consistently gave a chromatographic peak co-eluting with glyphosate averaging 12.8 ng/L (as glyphosate) in the HPLC/FLUOR analysis, a value slightly lower than the lower calibration level (LCL) of the instrumental calibration (ICAL), which was 15 ng/L. The fact that the experimental MDL is higher than the ICAL LCL reflects the impact of this background interference; that is, the low end of the analytical working range for dissolved glyphosate was limited by background interference, not by instrumental sensitivity or analytical variability.

As noted, background interference was observed in the pre-application baseflow sample used as the blank control during sample analysis. As discussed in Appendix C, Sections 1.2.4 and 1.2.6, the magnitude of this background clearly varied from sample to sample and results showed it to be as high as ≈40 ng/L (as glyphosate) in samples collected during storm events. Because it is known that this background was not stable, none of the measured concentrations were back subtracted.

The fact that samples contributed variable background interference also means that the MDL will vary from sample to sample; that is, the MDL cited is not universally relevant. However, there are no data allowing calculation of unique MDLs reflecting different background levels. Thus, the MDL based on the blank control was used to censor all concentration results even though the true MDL for many samples would be higher. In the tabulation herein, concentrations less than this MDL are reported as "<18" (ng/L) and are flagged "U" to signify that the associated result is not statistically different than the mean background concentration (as glyphosate) found in the blank control. Because the MDL (18.0 ng/L) was greater than the ICAL LCL, all reported concentrations fall within the calibration range.

Sample concentrations less than three times the mean background signal from replicate analyses of frozen blank control (12.8 ±2.9 ng/L, n = 16; Appendix C, Table C1.2) are flagged with a "B" to signify that the associated result is high biased by a minimum of 50%. This level of high bias is considered a minimum because (1) concentrations less than this threshold (38.4 ng/L) will carry >50% bias assuming a stable background at the level found in the blank control (12.8 ng/L); and (2) the background varied from sample to sample and is known to have reached ≈40 ng/L in some samples (i.e., the 50% bias threshold would be as high as 120 ng/L in some samples).

Because of the variability in this background interference, the concentrations given in the tabulation should be considered high biased by some unknown amount, and thus should be considered maximum possible concentrations. Note that this leaves the potential that some of the reported concentrations are true false positives. Results discussed in Appendix C, Section 1.2.6, illustrate this potential.

The table herein lists all samples collected for determination of AMPA and glyphosate during the course of this study. However, not all samples were analyzed (Section 2.3 in main text), so there are many samples for which no concentration results are given. These samples are included here for completeness only.

All concentrations in the tabulation are acid equivalents (a.e.) of glyphosate.

DISSOLVED GLYPHOSATE CONCENTRATIONS

Sample Tracking						Sample Results							
									MS/MSD[e]			Field Spike[f]	
Site	Date	Time	#[a]	Days Frozen[b]		SA (ng/L)[c]	Data Flags[d]		%Rec	Spike (≈ng/L)		%Rec	Spike (≈ng/L)
NBH	8/22/10	9:00	1	231		19	B						
NBH	8/22/10	10:00	2	224		<18	U						
NBH	8/22/10	11:00	3	245		<18	U						
NBH	8/22/10	12:00	4	224		62							
NBH	8/22/10	13:00	5	224		54							
NBH	8/22/10	14:00	6	231		30	B						
NBH	8/22/10	15:00	7	231		30	B						
NBH	8/22/10	16:00	8	141		27	B						
NBH	8/22/10	17:00	9										
NBH	8/22/10	18:00	10										
NBH	8/22/10	19:00	11										
NBH	8/22/10	20:00	12	224		19	B						
NBH	8/22/10	21:00	13										
NBH	8/22/10	22:00	14										
NBH	8/22/10	23:00	15										
NBH	8/23/10	0:00	16	141		23	B						
NBH	8/23/10	1:00	17										
NBH	8/23/10	2:00	18										
NBH	8/23/10	3:00	19										
NBH	8/23/10	4:00	20										
NBH	8/23/10	5:00	21										
NBH	8/23/10	6:00	22										
NBH	8/23/10	7:00	23										
NBH	8/23/10	8:00	24	231		<18	U						
NBH	8/25/10	10:30	BF	153		30	B,C						
NBH	8/29/10	23:00	1	187		45	C						
NBH	8/30/10	0:00	2	215		38	B,1						
NBH	8/30/10	1:00	3	68		29	B						
NBH	8/30/10	2:00	4	215		39			99.0	50			
NBH	8/30/10	3:00	5	187		33	B						
NBH	8/30/10	4:00	6	166		31	B						
NBH	8/30/10	5:00	7	173		32	B						
NBH	8/30/10	6:00	8	132		31	B						
NBH	8/30/10	7:00	9	166		31	B,C						
NBH	8/30/10	8:00	10	132		24	B					101.6	9000
NBH	8/30/10	9:00	11	196		28	B						
NBH	8/30/10	10:00	12	132		25	B						
NBH	8/30/10	11:00	13										
NBH	8/30/10	12:00	14										
NBH	8/30/10	13:00	15	196		26	B						
NBH	8/30/10	14:00	16										
NBH	8/30/10	15:00	17										
NBH	8/30/10	16:00	18	196		25	B						
NBH	8/30/10	17:00	19										
NBH	8/30/10	18:00	20										
NBH	8/30/10	19:00	21										

(Continued on next page. See notes at end of table.)

DISSOLVED GLYPHOSATE CONCENTRATIONS

Sample Tracking						Sample Results							
									MS/MSD[e]			Field Spike[f]	
Site	Date	Time	#[a]	Days Frozen[b]		SA (ng/L)[c]	Data Flags[d]		%Rec	Spike (≈ng/L)		%Rec	Spike (≈ng/L)
NBH	8/30/10	20:00	22	173		22	B						
NBH	8/31/10	23:00	1	185		48							
NBH	9/1/10	0:00	2	171		56							
NBH	9/1/10	1:00	3	194		68							
NBH	9/1/10	2:00	4	185		72							
NBH	9/1/10	3:00	5	179		82							
NBH	9/1/10	4:00	6	164		84	C						
NBH	9/1/10	5:00	7	171		70	1						
NBH	9/1/10	6:00	8	130		69							
NBH	9/1/10	7:00	9	179		58							
NBH	9/1/10	8:00	10	171		54							
NBH	9/1/10	9:00	11										
NBH	9/1/10	10:00	12										
NBH	9/1/10	11:00	13	220		35	B						
NBH	9/1/10	12:00	14										
NBH	9/1/10	13:00	15										
NBH	9/1/10	14:00	16	130		34	B						
NBH	9/1/10	15:00	17										
NBH	9/1/10	16:00	18										
NBH	9/1/10	17:00	19										
NBH	9/1/10	18:00	20										
NBH	9/1/10	19:00	21										
NBH	9/1/10	20:00	22										
NBH	9/1/10	21:00	23										
NBH	9/1/10	22:00	24	171		33	B		98.1	50			
NBH	9/10/10	14:30	BF	137		<18	U						
NBH	9/14/10	15:15	BF	190		19	B						
NBH	9/15/10	17:00	1	241		38	B						
NBH	9/15/10	19:00	2	241		39							
NBH	9/15/10	21:00	3	241		52							
NBH	9/15/10	23:00	4	241		27	B						
NBH	9/16/10	1:00	5	241		27	B						
NBH	9/16/10	3:00	6										
NBH	9/16/10	5:00	7										
NBH	9/16/10	7:00	8										
NBH	9/16/10	9:00	9										
NBH	9/16/10	11:00	10										
NBH	9/16/10	13:00	11										
NBH	9/16/10	15:00	12										
NBH	9/16/10	17:00	13	241		21	B						
NBH	9/16/10	19:00	14										
NBH	9/16/10	21:00	15										
NBH	9/16/10	23:00	16										
NBH	9/17/10	1:00	17										
NBH	9/17/10	3:00	18	241		22	B						
NBH	9/17/10	5:00	19	241		25	B						

(Continued on next page. See notes at end of table.)

DISSOLVED GLYPHOSATE CONCENTRATIONS

Sample Tracking						Sample Results							
									MS/MSD[e]			Field Spike[f]	
Site	Date	Time	#[a]	Days Frozen[b]		SA (ng/L)[c]	Data Flags[d]		%Rec	Spike (≈ng/L)		%Rec	Spike (≈ng/L)
NBH	9/17/10	7:00	20	241		28	B						
NBH	9/17/10	9:00	21	241		40							
NBH	9/17/10	11:00	22	241		31	B						
NBH	9/17/10	13:00	1	239		28	B		117.0	48			
NBH	9/17/10	15:00	2	319		23	B						
NBH	9/17/10	17:00	3										
NBH	9/17/10	19:00	4	319		27	B						
NBH	9/17/10	21:00	5										
NBH	9/17/10	23:00	6										
NBH	9/18/10	1:00	7										
NBH	9/18/10	3:00	8										
NBH	9/18/10	5:00	9										
NBH	9/18/10	7:00	10	319		26	B						
NBH	9/18/10	9:00	11										
NBH	9/18/10	11:00	12	319		24	B						
NBH	9/18/10	13:00	13	319		24	B						
NBH	9/18/10	15:00	14	319		32	B						
NBH	9/18/10	17:00	15	319		62	C,1						
NBH	9/18/10	19:00	16	319		33	B						
NBH	9/18/10	21:00	17	319		34	B						
NBH	9/18/10	23:00	18										
NBH	9/19/10	1:00	19										
NBH	9/19/10	3:00	20	319		22	B						
NBH	9/19/10	5:00	21										
NBH	9/19/10	7:00	22	319		31	B,C						
NBH	9/19/10	9:00	23										
NBH	9/19/10	11:00	24	319		32	B		108.3	50			
NBH	9/19/10	12:00	1										
NBH	9/19/10	18:00	2										
NBH	9/20/10	0:00	3										
NBH	9/20/10	6:00	4										
NBH	9/20/10	12:00	5										
NBH	9/20/10	18:00	6										
NBH	9/21/10	0:00	7										
NBH	9/21/10	6:00	8	201		18	B						
NBH	9/24/10	15:30	BF	123		20	B						
NBH	10/1/10	12:30	BF	315		<18	U						
NBH	10/8/10	12:00	BF	308		<18	U						
NBH	10/8/10	18:00	1										
NBH	10/8/10	21:00	2										
NBH	10/9/10	0:00	3	476		21	B					87.9	485
NBH	10/9/10	3:00	4										
NBH	10/9/10	6:00	5										
NBH	10/9/10	9:00	6										
NBH	10/9/10	12:00	7										
NBH	10/9/10	15:00	8										

(Continued on next page. See notes at end of table.)

DISSOLVED GLYPHOSATE CONCENTRATIONS

Sample Tracking					Sample Results						
							MS/MSD[e]		Field Spike[f]		
Site	Date	Time	#[a]	Days Frozen[b]	SA (ng/L)[c]	Data Flags[d]	%Rec	Spike (≈ng/L)	%Rec	Spike (≈ng/L)
NBH	10/9/10	18:00	9							
NBH	10/9/10	21:00	10							
NBH	10/10/10	0:00	11							
NBH	10/10/10	3:00	12							
NBH	10/10/10	6:00	13							
NBH	10/10/10	9:00	14							
NBH	10/11/10		NS							
NBH	10/14/10	13:00	BF	103	24	B	98.7	50		
NBH	10/22/10	13:30	BF	294	<18	U				
NBH	10/23/10	14:00	1	461	<18	U			83.5	555
NBH	10/23/10	16:00	2							
NBH	10/23/10	18:00	3							
NBH	10/23/10	20:00	4							
NBH	10/23/10	22:00	5							
NBH	10/24/10	0:00	6							
NBH	10/24/10	2:00	7							
NBH	10/24/10	4:00	8	77	41					
NBH	10/24/10	6:00	9			C				
NBH	10/24/10	8:00	10							
NBH	10/24/10	10:00	11							
NBH	10/24/10	12:00	12							
NBH	10/24/10	14:00	13							
NBH	10/24/10	16:00	14							
NBH	10/24/10	18:00	15							
NBH	10/24/10	20:00	16	77	31	B				
NBH	10/24/10	22:00	17							
NBH	10/25/10	0:00	18							
NBH	10/25/10	2:00	19							
NBH	10/25/10	4:00	20							
NBH	10/25/10	6:00	21							
NBH	10/25/10	8:00	22							
NBH	10/25/10	10:00	23	77	40		100.4	400		
NBH	10/25/10	12:00	24							
NBH	11/5/10	12:30	BF	81	<18	U				
NBH	11/18/10	times unknown	1							
NBH			2							
NBH			3							
NBH			4							
NBH			5							
NBH			6							
NBH			7							
NBH			8							
NBH			9							
NBH			10							
NBH			11							
NBH			12							

(Continued on next page. See notes at end of table.)

DISSOLVED GLYPHOSATE CONCENTRATIONS

Sample Tracking						Sample Results							
									MS/MSD[e]			Field Spike[f]	
Site	Date	Time	#[a]	Days Frozen[b]		SA (ng/L)[c]	Data Flags[d]		%Rec	Spike (≈ng/L)		%Rec	Spike (≈ng/L)
NBH			13										
NBH			14										
NBH			15										
NBH	11/20/10	12:00	BF	271		<18	U						
NBH	12/3/10	14:00	BF	258		<18	U						
NBH	12/10/10	times unknown	1										
NBH			3										
NBH			5										
NBH			7										
NBH			9										
NBH			11										
NBH			13										
NBH			15										
NBH			17				1						
NBH	12/11/10		NS										
NBU	8/22/10	9:00	1	134		18	B						
NBU	8/22/10	10:00	2										
NBU	8/22/10	11:00	3	134		<18	U						
		ISCO malfunction											
NBU	8/23/10	10:15	24	224		<18	U						
NBU	8/25/10	12:35	BF	125		21	B		100.9	800			
NBU	8/29/10	23:00	1	215		<18	U						
NBU	8/30/10	0:00	2	68		19	B		95.3	4000			
NBU	8/30/10	1:00	3										
NBU	8/30/10	2:00	4	118		<18	U						
NBU	8/30/10	3:00	5										
NBU	8/30/10	4:00	6	111		24	B						
NBU	8/30/10	5:00	7	125		62	1					99.0	9600
NBU	8/30/10	6:00	8	125		103							
NBU	8/30/10	7:00	9	111		149	C					96.3	9000
NBU	8/30/10	8:00	10	125		99							
NBU	8/30/10	9:00	11	118		80							
NBU	8/30/10	10:00	12	125		56							
NBU	8/30/10	11:00	13	27		39							
NBU	8/30/10	12:00	14										
NBU	8/30/10	13:00	15	118		27	B						
NBU	8/30/10	14:00	16										
NBU	8/30/10	15:00	17	111		21	B		101.6	200			
NBU	8/30/10	16:00	18										
NBU	8/30/10	17:00	19										
NBU	8/30/10	18:00	20	111		22	B						
NBU	8/30/10	19:00	21										
NBU	8/30/10	20:00	22										
NBU	8/30/10	21:00	23										
NBU	8/30/10	22:00	24	68		<18	U,1					93.2	5000

(Continued on next page. See notes at end of table.)

DISSOLVED GLYPHOSATE CONCENTRATIONS

Sample Tracking					Sample Results						
							MS/MSD[e]		Field Spike[f]	
Site	Date	Time	#[a]	Days Frozen[b]	SA (ng/L)[c]	Data Flags[d]	%Rec	Spike (≈ng/L)	%Rec	Spike (≈ng/L)
NBU	8/31/10	23:00	1	179	24	B				
NBU	9/1/10	0:00	2							
NBU	9/1/10	1:00	3	59	31	B	100.5	400		
NBU	9/1/10	2:00	4							
NBU	9/1/10	3:00	5	116	34	B				
NBU	9/1/10	4:00	6	164	52		99.1	200		
NBU	9/1/10	5:00	7	116	37	B				
NBU	9/1/10	6:00	8	164	47	C,1				
NBU	9/1/10	7:00	9	109	26	B				
NBU	9/1/10	8:00	10							
NBU	9/1/10	9:00	11							
NBU	9/1/10	10:00	12							
NBU	9/1/10	11:00	13	116	27	B			95.3	10000
NBU	9/1/10	12:00	14							
NBU	9/1/10	13:00	15	109	23	B				
NBU	9/1/10	14:00	16							
NBU	9/1/10	15:00	17							
NBU	9/1/10	16:00	18							
NBU	9/1/10	17:00	19							
NBU	9/1/10	18:00	20							
NBU	9/1/10	19:00	21	109	19	B				
NBU	9/1/10	20:00	22							
NBU	9/1/10	21:00	23							
NBU	9/1/10	22:00	24							
NBU	9/10/10	13:45	BF	109	<18	U			97.0	2000
NBU	9/14/10	14:45	BF	190	<18	U,C				
NBU	9/15/10	17:00	1	164	24	B				
NBU	9/15/10	19:00	2	170	26	B				
NBU	9/15/10	21:00	3	170	30	B				
NBU	9/15/10	23:00	4	179	23	B				
NBU	9/16/10	1:00	5	164	36	B	93.0	50		
NBU	9/16/10	3:00	6	170	25	B				
NBU	9/16/10	5:00	7							
NBU	9/16/10	7:00	8							
NBU	9/16/10	9:00	9							
NBU	9/16/10	11:00	10							
NBU	9/16/10	13:00	11							
NBU	9/16/10	15:00	12							
NBU	9/16/10	17:00	13	10	<18	U				
NBU	9/16/10	19:00	14							
NBU	9/16/10	21:00	15							
NBU	9/16/10	23:00	16	186	19	B				
NBU	9/17/10	1:00	17							
NBU	9/17/10	3:00	18							
NBU	9/17/10	5:00	19	186	19	B				
NBU	9/17/10	7:00	20	186	24	B				

(Continued on next page. See notes at end of table.)

DISSOLVED GLYPHOSATE CONCENTRATIONS

Sample Tracking						Sample Results							
									MS/MSD[e]			Field Spike[f]	
Site	Date	Time	#[a]	Days Frozen[b]		SA (ng/L)[c]	Data Flags[d]		%Rec	Spike (≈ng/L)		%Rec	Spike (≈ng/L)
NBU	9/17/10	9:00	21	186		24	B						
NBU	9/17/10	11:00	22	186		22	B						
NBU	9/17/10	13:00	1	184		20	B						
NBU	9/17/10	15:00	2	184		19	B						
NBU	9/17/10	17:00	3	184		<18	U						
NBU	9/17/10	19:00	4										
NBU	9/17/10	21:00	5										
NBU	9/17/10	23:00	6										
NBU	9/18/10	1:00	7										
NBU	9/18/10	3:00	8										
NBU	9/18/10	5:00	9										
NBU	9/18/10	7:00	10										
NBU	9/18/10	9:00	11										
NBU	9/18/10	11:00	12										
NBU	9/18/10	13:00	13	8		<18	U		97.6	20000			
NBU	9/18/10	15:00	14	217		<18	U						
NBU	9/18/10	17:00	15	217		29	B						
NBU	9/18/10	19:00	16	217		30	B						
NBU	9/18/10	21:00	17	217		32	B						
NBU	9/18/10	23:00	18										
NBU	9/19/10	1:00	19										
NBU	9/19/10	3:00	20	217		23	B						
NBU	9/19/10	5:00	21										
NBU	9/19/10	7:00	22	217		24	B						
NBU	9/19/10	9:00	23										
NBU	9/19/10	11:00	24										
NBU	9/19/10	12:00	1										
NBU	9/19/10	18:00	2	215		23	B						
NBU	9/20/10	0:00	3	215		27	B						
NBU	9/20/10	6:00	4	215		30	B						
NBU	9/20/10	12:00	5	215		35	B		98.5	48			
NBU	9/20/10	18:00	6										
NBU	9/21/10	0:00	7										
NBU	9/21/10	6:00	8	201		19	B						
NBU	9/24/10	14:30	BF	4		<18	U					99.0	5000
NBU	10/1/10	13:30	BF	315		<18	U						
NBU	10/8/10	13:00	BF	308		<18	U						
NBU	10/8/10	18:00	1										
NBU	10/8/10	21:00	2										
NBU	10/9/10	0:00	3										
NBU	10/9/10	3:00	4										
NBU	10/9/10	6:00	5										
NBU	10/9/10	9:00	6										
NBU	10/9/10	12:00	7										
NBU	10/9/10	15:00	8										
NBU	10/9/10	18:00	9										

(Continued on next page. See notes at end of table.)

DISSOLVED GLYPHOSATE CONCENTRATIONS

Sample Tracking						Sample Results							
									MS/MSD[e]			Field Spike[f]	
Site	Date	Time	#[a]	Days Frozen[b]		SA (ng/L)[c]	Data Flags[d]		%Rec	Spike (≈ng/L)		%Rec	Spike (≈ng/L)
NBU	10/9/10	21:00	10										
NBU	10/10/10	0:00	11										
NBU	10/10/10	3:00	12										
NBU	10/10/10	6:00	13										
NBU	10/10/10	9:00	14										
NBU	10/11/10		NS										
NBU	10/14/10	13:00	BF	302		19	B						
NBU	10/22/10	12:30	BF	95		<18	U					102.2	500
NBU	10/23/10	14:00	1	461		<18	U					85.0	534
NBU	10/23/10	16:00	2	70		23	B						
NBU	10/23/10	18:00	3										
NBU	10/23/10	20:00	4										
NBU	10/23/10	22:00	5										
NBU	10/24/10	0:00	6	70		45							
NBU	10/24/10	2:00	7	13		34	B					93.2	2000
NBU	10/24/10	4:00	8										
NBU	10/24/10	6:00	9										
NBU	10/24/10	8:00	10										
NBU	10/24/10	10:00	11	13		23	B						
NBU	10/24/10	12:00	12										
NBU	10/24/10	14:00	13										
NBU	10/24/10	16:00	14										
NBU	10/24/10	18:00	15										
NBU	10/24/10	20:00	16										
NBU	10/24/10	22:00	17	70		30	B						
NBU	10/25/10	0:00	18										
NBU	10/25/10	2:00	19										
NBU	10/25/10	4:00	20										
NBU	10/25/10	6:00	21										
NBU	10/25/10	8:00	22										
NBU	10/25/10	10:00	23	70		28	B		104.8	400			
NBU	11/5/10	11:40	BF	286		<18	U						
NBU	11/18/10	times unknown	1										
NBU			2										
NBU			3										
NBU			4										
NBU			5	31		29	B					88.2	500
NBU			6										
NBU			7										
NBU			8										
NBU			9										
NBU			10										
NBU			11										
NBU			12										
NBU			13										
NBU			14										

(Continued on next page. See notes at end of table.)

DISSOLVED GLYPHOSATE CONCENTRATIONS

Sample Tracking						Sample Results							
									MS/MSD[e]			Field Spike[f]	
Site	Date	Time	#[a]	Days Frozen[b]		SA (ng/L)[c]	Data Flags[d]		%Rec	Spike (≈ng/L)		%Rec	Spike (≈ng/L)
NBU			15										
NBU	11/20/10	11:00	BF	271		<18	U					96.7	1000
NBU	12/3/10	13:00	BF	258		<18	U,1						
NBU	12/10/10	times unknown	2										
NBU			4										
NBU			6										
NBU			8										
NBU			10				1						
NBU			12										
NBU	12/11/10		NS										
NBL	8/22/10	9:00	1	231		<18	U						
NBL	8/22/10	10:00	2	224		<18	U						
NBL	8/22/10	11:00	3										
NBL	8/22/10	12:00	4	224		<18	U,1						
NBL	8/22/10	13:00	5	224		<18	U						
NBL	8/22/10	14:00	6	231		<18	U						
NBL	8/22/10	15:00	7	231		<18	U						
NBL	8/22/10	16:00	8	147		<18	U						
NBL	8/22/10	17:00	9										
NBL	8/22/10	18:00	10										
NBL	8/22/10	19:00	11										
NBL	8/22/10	20:00	12	224		<18	U						
NBL	8/22/10	21:00	13										
NBL	8/22/10	22:00	14										
NBL	8/22/10	23:00	15										
NBL	8/23/10	0:00	16	147		<18	U						
NBL	8/23/10	1:00	17										
NBL	8/23/10	2:00	18										
NBL	8/23/10	3:00	19										
NBL	8/23/10	4:00	20										
NBL	8/23/10	5:00	21										
NBL	8/23/10	6:00	22										
NBL	8/23/10	7:00	23										
NBL	8/23/10	8:00	24	231		<18	U						
NBL	8/25/10	14:45	BF	153		33	B,C						
NBL	8/29/10	23:00	1	181		37	B						
NBL	8/30/10	0:00	2										
NBL	8/30/10	1:00	3	196		43							
NBL	8/30/10	2:00	4										
NBL	8/30/10	3:00	5	196		36	B						
NBL	8/30/10	4:00	6	166		26	B						
NBL	8/30/10	5:00	7	173		42							
NBL	8/30/10	6:00	8	138		39							
NBL	8/30/10	7:00	9	166		44							
NBL	8/30/10	8:00	10	138		38	B						

(Continued on next page. See notes at end of table.)

DISSOLVED GLYPHOSATE CONCENTRATIONS

Sample Tracking					Sample Results						
							MS/MSD[e]		Field Spike[f]	
Site	Date	Time	#[a]	Days Frozen[b]	SA (ng/L)[c]	Data Flags[d]	%Rec	Spike (≈ng/L)	%Rec	Spike (≈ng/L)
NBL	8/30/10	9:00	11	173	51	C				
NBL	8/30/10	10:00	12	187	43					
NBL	8/30/10	11:00	13	187	58		94.0	96		
NBL	8/30/10	12:00	14	138	37	B			96.8	4800
NBL	8/30/10	13:00	15	516	32	B,1	99.8	200		
NBL	8/30/10	14:00	16							
NBL	8/30/10	15:00	17							
NBL	8/30/10	16:00	18							
NBL	8/30/10	17:00	19							
NBL	8/30/10	18:00	20							
NBL	8/30/10	19:00	21	181	29	B				
NBL	8/31/10	23:00	1	179	29	B				
NBL	9/1/10	0:00	2	164	27	B				
NBL	9/1/10	1:00	3							
NBL	9/1/10	2:00	4	185	41					
NBL	9/1/10	3:00	5	171	48	C				
NBL	9/1/10	4:00	6	179	37	B				
NBL	9/1/10	5:00	7	164	39					
NBL	9/1/10	6:00	8	136	42					
NBL	9/1/10	7:00	9	171	43					
NBL	9/1/10	8:00	10	194	36	B				
NBL	9/1/10	9:00	11							
NBL	9/1/10	10:00	12	164	34	B				
NBL	9/1/10	11:00	13							
NBL	9/1/10	12:00	14	194	42		94.3	95		
NBL	9/1/10	13:00	15							
NBL	9/1/10	14:00	16	136	39					
NBL	9/1/10	15:00	17							
NBL	9/1/10	16:00	18							
NBL	9/1/10	17:00	19							
NBL	9/1/10	18:00	20							
NBL	9/1/10	19:00	21							
NBL	9/1/10	20:00	22							
NBL	9/1/10	21:00	23							
NBL	9/1/10	22:00	24	179	24	B				
NBL	9/10/10	12:30	BF	137	<18	U				
NBL	9/14/10	14:00	BF	190	34	B,C	94.1	95		
NBL	9/15/10	17:00	1							
NBL	9/15/10	19:00	2	226	23	B,1				
NBL	9/15/10	21:00	3	226	26	B				
NBL	9/15/10	23:00	4	226	27	B				
NBL	9/16/10	1:00	5	226	26	B				
NBL	9/16/10	3:00	6	226	23	B				
NBL	9/16/10	5:00	7							
NBL	9/16/10	7:00	8							
NBL	9/16/10	9:00	9							

(Continued on next page. See notes at end of table.)

DISSOLVED GLYPHOSATE CONCENTRATIONS

Sample Tracking						Sample Results							
									MS/MSD[e]			Field Spike[f]	
Site	Date	Time	#[a]	Days Frozen[b]		SA (ng/L)[c]	Data Flags[d]		%Rec	Spike (≈ng/L)		%Rec	Spike (≈ng/L)
NBL	9/16/10	11:00	10	334		21	B						
NBL	9/16/10	13:00	11										
NBL	9/16/10	15:00	12										
NBL	9/16/10	17:00	13										
NBL	9/16/10	19:00	14	334		20	B						
NBL	9/16/10	21:00	15										
NBL	9/16/10	23:00	16										
NBL	9/17/10	1:00	17										
NBL	9/17/10	3:00	18										
NBL	9/17/10	5:00	19	226		<18	U						
NBL	9/17/10	7:00	20	226		24	B						
NBL	9/17/10	9:00	21	226		24	B						
NBL	9/17/10	11:00	1	224		35	B						
NBL	9/17/10	13:00	2	224		27	B						
NBL	9/17/10	15:00	3	224		26	B						
NBL	9/17/10	17:00	4										
NBL	9/17/10	19:00	5										
NBL	9/17/10	21:00	6										
NBL	9/17/10	23:00	7										
NBL	9/18/10	1:00	8										
NBL	9/18/10	3:00	9										
NBL	9/18/10	5:00	10										
NBL	9/18/10	7:00	11										
NBL	9/18/10	9:00	12	224		28	B		90.8	24			
NBL	9/18/10	11:00	13										
NBL	9/18/10	13:00	14										
NBL	9/18/10	15:00	15	231		25	B						
NBL	9/18/10	17:00	16	231		29	B						
NBL	9/18/10	19:00	17	231		40							
NBL	9/18/10	21:00	18	231		39	C,1						
NBL	9/18/10	23:00	19										
NBL	9/19/10	1:00	20										
NBL	9/19/10	3:00	21	231		29	B						
NBL	9/19/10	5:00	22										
NBL	9/19/10	7:00	23	231		33	B						
NBL	9/19/10	9:00	24										
NBL	9/19/10	11:00	NS										
NBL	9/19/10	12:00	1	229		23	B						
NBL	9/19/10	18:00	2	229		21	B						
NBL	9/20/10	0:00	3	229		27	B						
NBL	9/20/10	6:00	4	229		27	B						
NBL	9/20/10	12:00	5	229		31	B		88.2	24			
NBL	9/20/10	18:00	6										
NBL	9/21/10	0:00	7										
NBL	9/21/10	6:00	8	201		22	B		108.1	24			
NBL	9/24/10	13:45	BF	123		20	B						

(Continued on next page. See notes at end of table.)

DISSOLVED GLYPHOSATE CONCENTRATIONS

Sample Tracking						Sample Results							
									MS/MSD[e]			Field Spike[f]	
Site	Date	Time	#[a]	Days Frozen[b]		SA (ng/L)[c]	Data Flags[d]		%Rec	Spike (≈ng/L)		%Rec	Spike (≈ng/L)
NBL	10/1/10	14:15	BF	315		19	B						
NBL	10/8/10	14:00	BF	308		28	B,C						
NBL	10/8/10	ISCO malfunction											
NBL	10/10/10		NS										
NBL	10/14/10	15:00	BF	103		26	B					95.3	500
NBL	10/22/10	11:00	BF	294		24	B						
NBL	10/23/10	14:00	1	461		19	B					81.2	580
NBL	10/23/10	16:00	2	83		30	B						
NBL	10/23/10	18:00	3										
NBL	10/23/10	20:00	4										
NBL	10/23/10	22:00	5										
NBL	10/24/10	0:00	6										
NBL	10/24/10	2:00	7										
NBL	10/24/10	4:00	8										
NBL	10/24/10	6:00	9										
NBL	10/24/10	8:00	10	83		42							
NBL	10/25/10	10:00	24	83		26	B		103.0	200			
NBL	11/5/10	11:00	BF	280		20	B		92.2	20		92.9	480
NBL	11/18/10	times unknown	1										
NBL			2										
NBL			3										
NBL			4										
NBL			5										
NBL			6										
NBL			7										
NBL			8										
NBL			9										
NBL			10										
NBL			11										
NBL	11/20/10	10:00	BF	271		<18	U						
NBL	12/3/10	12:30	BF	258		<18	U		81.1	20		90.0	490
NBL	12/10/10	times unknown	1										
NBL			2										
NBL			3										
NBL			4										
NBL			5										
NBL			6										
NBL			7										
NBL			8										
NBL			9										
NBL			10										
NBL			11										
NBL			12										
NBL			13										
NBL			14										
NBL			15										

(Continued on next page. See notes at end of table.)

DISSOLVED GLYPHOSATE CONCENTRATIONS

Sample Tracking						Sample Results							
									MS/MSD[e]			Field Spike[f]	
Site	Date	Time	#[a]	Days Frozen[b]		SA (ng/L)[c]	Data Flags[d]		%Rec	Spike (≈ng/L)		%Rec	Spike (≈ng/L)
NBL			16										
NBL			17										
NBL			18										
NBL	12/11/10	times unknown	1										
NBL			2										
NBL			3				C,1						
NBL			4										
NBL			5										
NBL			6				1						
NBL			7										
NBL			8										
NBL			9										

[a] Numerical sequence of sample collection during date-specific storm event (one event can include multiple triggering of ISCO sampler); NS = no samples collected, BF = baseflow grab sample.

[b] Days frozen prior to thawing and analysis.

[c] Results from analysis of pre-freeze filtrates, ng/L (ppt).

[d] Data qualifiers:

B = estimated minimum high bias 50%; i.e., result is less than three times mean background from replicate analyses of pre-application baseflow sample (12.8 ±2.0 ng/L; Appendix C, Sections 1.2.1 and 1.2.2); used only when result >MDL.

C = analysis by HPLC/MS-MS (Appendix C, Section 1.2.6).

U = less than estimated MDL (18.0 ng/L; Appendix C, Section 1.2.2).

1 = analysis of whole sample also performed (Appendix C, Section 1.3).

[e] Percent recovery from MS experiments on thawed sample filtrates with nominal spike level (ng/L); result is mean when MSD were performed.

[f] Percent recovery of spike added immediately prior to initial filtration and freezing with nominal spike level (ng/L).

APPENDIX E

DISSOLVED AMPA CONCENTRATIONS

The study-specific method detection limit (MDL) for dissolved AMPA in Needle Branch samples was determined to be 3.8 ng/L (ppt; Appendix C, Section 1.2.2). This metric was developed via replicate analyses of a single pre-application baseflow sample collected at NBL (the blank control). This sample consistently gave a chromatographic peak co-eluting with AMPA averaging 2.4 ng/L (as AMPA) in the HPLC/FLUOR analysis. All these concentrations are less than the lower calibration level (LCL) of the instrumental calibration (ICAL) used in all quantifications, which was 15 ng/L.

Background interference was observed in the pre-application baseflow sample used as the blank control during sample analysis. As discussed in Appendix C, Section 1.2.5, the magnitude of this background varied from sample to sample, and results showed it to be as high as 7 ng/L (as AMPA) in a baseflow collected at NBU immediately prior to application of herbicides (see results for 8/22/2010 NBU Samples #1 and #3 in the tabulation herein). Because it is known that this background was not stable, none of the measured concentrations were background subtracted.

The fact that samples contributed variable background interference also means that the MDL will vary from sample to sample, indicating that the MDL cited is not universally relevant. However, there are no data allowing calculation of MDLs reflecting different background levels. Thus, the MDL based on the blank control was used to censor all concentration results even though the true MDL for many samples would be higher. In the tabulation herein, concentrations less than this MDL are reported as "<4" (ng/L) and are flagged "U" to signify that the associated result is not statistically different than the concentration found in the blank control. All concentrations between the MDL (4 ng/L) and the LCL (15 ng/L) are flagged "J" to signify that these concentrations fall below the ICAL range and so are considered estimates.

Sample concentrations less than three times the mean background signal from the replicate analyses of frozen blank control (2.4 ±0.6 ng/L, n = 16; Appendix C, Table C1.2) are flagged with a "B" to signify that the associated result is high biased by a minimum of 50%. This level of high bias is considered a minimum because (1) concentrations less than this threshold (7.2 ng/L) will carry >50% bias assuming a stable background at the level found in the blank control (2.4 ng/L); and (2) the background varied from sample to sample and is known to have reached ≈7 ng/L in some samples (i.e., the 50% bias threshold would be as high as 21 ng/L in some samples).

Because of the variability in this background interference, the concentrations given in the tabulation should be considered high biased by some unknown amount, and thus should be considered maximum possible concentrations. Note that this leaves the potential that some of the reported concentrations are true false positives.

The table herein does not identify every sample collected for determination of AMPA and glyphosate, but only those actually analyzed. The tabulation in Appendix D (glyphosate results) lists all samples.

DISSOLVED AMPA CONCENTRATIONS

Sample Tracking					Sample Results						
							MS/MSD[e]		Field Spike[f]		
Site	Date	Time	#[a]	Days Frozen[b]	SA (ng/L)[c]	Data Flags[d]	%Rec	Spike (≈ng/L)	%Rec	Spike (≈ng/L)
NBH	8/22/10	9:00	1	231	<4	U				
NBH	8/22/10	10:00	2	224	<4	U				
NBH	8/22/10	11:00	3	245	<4	U				
NBH	8/22/10	12:00	4	224	<4	U				
NBH	8/22/10	13:00	5	224	<4	U				
NBH	8/22/10	14:00	6	231	<4	U				
NBH	8/22/10	15:00	7	231	<4	U				
NBH	8/22/10	16:00	8	141	<4	U				
NBH	8/22/10	20:00	12	224	<4	U				
NBH	8/23/10	0:00	16	141	7	B,J				
NBH	8/23/10	8:00	24	231	<4	U				
NBH	8/25/10	10:30	BF	153	7	C				
NBH	8/29/10	23:00	1	187	7	B,C,J				
NBH	8/30/10	0:00	2	215	8	1,J				
NBH	8/30/10	1:00	3	68	4	B,J				
NBH	8/30/10	2:00	4	215	5	B,J	90.0	50		
NBH	8/30/10	3:00	5	187	5	B,J				
NBH	8/30/10	4:00	6	166	7	B,J				
NBH	8/30/10	5:00	7	173	5	B,J				
NBH	8/30/10	6:00	8	132	<4	U				
NBH	8/30/10	7:00	9	166	5	B,C,J				
NBH	8/30/10	8:00	10	132	7	J			83.1	9000
NBH	8/30/10	9:00	11	196	<4	U				
NBH	8/30/10	10:00	12	132	5	B,J				
NBH	8/30/10	13:00	15	196	4	B,J				
NBH	8/30/10	16:00	18	196	4	B,J				
NBH	8/30/10	20:00	22	173	9	J				
NBH	8/31/10	23:00	1	185	6	B,J				
NBH	9/1/10	0:00	2	171	5	B,J				
NBH	9/1/10	1:00	3	194	9	J				
NBH	9/1/10	2:00	4	185	7	J				
NBH	9/1/10	3:00	5	179	9	J				
NBH	9/1/10	4:00	6	164	10	C,J				
NBH	9/1/10	5:00	7	171	9	1,J				
NBH	9/1/10	6:00	8	130	7	J				
NBH	9/1/10	7:00	9	179	6	B,J				
NBH	9/1/10	8:00	10	171	6	B,J				
NBH	9/1/10	11:00	13	220	4	B,J				
NBH	9/1/10	14:00	16	130	7	B,J				
NBH	9/1/10	22:00	24	171	6	B,J	93.2	50		
NBH	9/10/10	14:30	BF	137	<4	U				
NBH	9/14/10	15:15	BF	190	<4	U				
NBH	9/15/10	17:00	1	241	9	J				
NBH	9/15/10	19:00	2	241	7	J				
NBH	9/15/10	21:00	3	241	7	J				
NBH	9/15/10	23:00	4	241	<4	U				

(Continued on next page. See notes at end of table.)

DISSOLVED AMPA CONCENTRATIONS

Sample Tracking						Sample Results						
								MS/MSD[e]			Field Spike[f]	
Site	Date	Time	#[a]	Days Frozen[b]		SA (ng/L)[c]	Data Flags[d]		%Rec	Spike (≈ng/L)		%Rec	Spike (≈ng/L)
NBH	9/16/10	1:00	5	241		4	B,J						
NBH	9/16/10	17:00	13	241		<4	U						
NBH	9/17/10	3:00	18	241		4	B,J						
NBH	9/17/10	5:00	19	241		<4	U						
NBH	9/17/10	7:00	20	241		4	B,J						
NBH	9/17/10	9:00	21	241		5	B,J						
NBH	9/17/10	11:00	22	241		4	B,J						
NBH	9/17/10	13:00	1	239		6	B,J		99.2	48			
NBH	9/17/10	15:00	2	319		<4	U						
NBH	9/17/10	19:00	4	319		<4	U						
NBH	9/18/10	7:00	10	319		<4	U						
NBH	9/18/10	11:00	12	319		<4	U						
NBH	9/18/10	13:00	13	319		<4	U						
NBH	9/18/10	15:00	14	319		<4	U						
NBH	9/18/10	17:00	15	319		4	B,C,J,1						
NBH	9/18/10	19:00	16	319		<4	U						
NBH	9/18/10	21:00	17	319		<4	U						
NBH	9/19/10	3:00	20	319		<4	U						
NBH	9/19/10	7:00	22	319		<4	U,C						
NBH	9/19/10	11:00	24	319		<4	U		102.4	50			
NBH	9/21/10	6:00	8	201		<4	U						
NBH	9/24/10	15:30	BF	123		<4	U						
NBH	10/1/10	12:30	BF	315		<4	U						
NBH	10/8/10	12:00	BF	308		<4	U						
NBH	10/9/10	0:00	3	476		4	B,J					63.1	484
NBH	10/14/10	13:00	BF	103		<4	U		96.3	48			
NBH	10/22/10	13:30	BF	294		<4	U						
NBH	10/23/10	14:00	1	461		<4	U					67.5	553
NBH	10/24/10	4:00	8	77		4	B,J						
NBH	10/24/10	6:00	9				C						
NBH	10/24/10	20:00	16	77		4	B,J						
NBH	10/25/10	10:00	23	77		6	B,J		93.4	400			
NBH	11/5/10	12:30	BF	81		4	B,J						
NBH	11/20/10	12:00	BF	271		<4	U						
NBH	12/3/10	14:00	BF	258		<4	U						
NBH	12/10/10	unknown	17				1						
NBU	8/22/10	9:00	1	134		7	J						
NBU	8/22/10	11:00	3	134		7	J						
NBU	8/23/10	10:15	24	224		6	B,J						
NBU	8/25/10	12:35	BF	125		7	B,J		91.7	800			
NBU	8/29/10	23:00	1	215		<4	U						
NBU	8/30/10	0:00	2	68		<4	U		92.7	4000			
NBU	8/30/10	2:00	4	118		<4	U						
NBU	8/30/10	4:00	6	111		<4	U						
NBU	8/30/10	5:00	7	125		<4	U,1					77.9	9600

(Continued on next page. See notes at end of table.)

DISSOLVED AMPA CONCENTRATIONS

Sample Tracking					Sample Results						
							MS/MSD[e]		Field Spike[f]		
Site	Date	Time	#[a]	Days Frozen[b]	SA (ng/L)[c]	Data Flags[d]	%Rec	Spike (≈ng/L)	%Rec	Spike (≈ng/L)
NBU	8/30/10	6:00	8	125	4	B,J				
NBU	8/30/10	7:00	9	111	6	B,C,J,1			73.8	9000
NBU	8/30/10	8:00	10	125	4	B,J				
NBU	8/30/10	9:00	11	118	4	B,J				
NBU	8/30/10	10:00	12	125	<4	U				
NBU	8/30/10	11:00	13	27	4	B,J				
NBU	8/30/10	13:00	15	118	<4	U				
NBU	8/30/10	15:00	17	111	<4	U	91.4	200		
NBU	8/30/10	18:00	20	111	4	B,J				
NBU	8/30/10	22:00	24	68	4	J,1			84.0	5000
NBU	8/31/10	23:00	1	179	4	B,J				
NBU	9/1/10	1:00	3	59	<4	U	83.5	400		
NBU	9/1/10	3:00	5	116	<4	U				
NBU	9/1/10	4:00	6	164	8	J	91.2	200		
NBU	9/1/10	5:00	7	116	4	B,J				
NBU	9/1/10	6:00	8	164	7	B,C,J,1				
NBU	9/1/10	7:00	9	109	<4	U				
NBU	9/1/10	11:00	13	116	<4	U			82.2	10000
NBU	9/1/10	13:00	15	109	<4	U				
NBU	9/1/10	19:00	21	109	<4	U				
NBU	9/10/10	13:45	BF	109	<4	U			84.8	2000
NBU	9/14/10	14:45	BF	190	<4	U,C				
NBU	9/15/10	17:00	1	164	<4	U				
NBU	9/15/10	19:00	2	170	<4	U				
NBU	9/15/10	21:00	3	170	4	B,J				
NBU	9/15/10	23:00	4	179	<4	U				
NBU	9/16/10	1:00	5	164	4	B,J	105.5	48		
NBU	9/16/10	3:00	6	170	<4	U				
NBU	9/16/10	17:00	13	10	5	B,J				
NBU	9/16/10	23:00	16	186	<4	U				
NBU	9/17/10	5:00	19	186	<4	U				
NBU	9/17/10	7:00	20	186	<4	U				
NBU	9/17/10	9:00	21	186	<4	U				
NBU	9/17/10	11:00	22	186	<4	U				
NBU	9/17/10	13:00	1	184	<4	U				
NBU	9/17/10	15:00	2	184	<4	U				
NBU	9/17/10	17:00	3	184	<4	U				
NBU	9/18/10	13:00	13	8	<4	U	91.8	20000		
NBU	9/18/10	15:00	14	217	<4	U				
NBU	9/18/10	17:00	15	217	<4	U				
NBU	9/18/10	19:00	16	217	<4	U				
NBU	9/18/10	21:00	17	217	<4	U				
NBU	9/19/10	3:00	20	217	<4	U				
NBU	9/19/10	7:00	22	217	<4	U				
NBU	9/19/10	18:00	2	215	<4	U				
NBU	9/20/10	0:00	3	215	<4	U				

(Continued on next page. See notes at end of table.)

DISSOLVED AMPA CONCENTRATIONS

Sample Tracking					Sample Results						
							MS/MSD[e]		Field Spike[f]		
Site	Date	Time	#[a]	Days Frozen[b]	SA (ng/L)[c]	Data Flags[d]	%Rec	Spike (≈ng/L)	%Rec	Spike (≈ng/L)
NBU	9/20/10	6:00	4	215	<4	U				
NBU	9/20/10	12:00	5	215	<4	U	96.7	48		
NBU	9/21/10	6:00	8	201	<4	U				
NBU	9/24/10	14:30	BF	4	<4	U			88.4	5000
NBU	10/1/10	13:30	BF	315	<4	U				
NBU	10/8/10	13:00	BF	308	<4	U				
NBU	10/14/10	13:00	BF	302	<4	U				
NBU	10/22/10	12:30	BF	95	<4	U			87.7	500
NBU	10/23/10	14:00	1	461	<4	U			60.0	533
NBU	10/23/10	16:00	2	70	<4	U				
NBU	10/24/10	0:00	6	70	<4	U				
NBU	10/24/10	2:00	7	13	<4	U			80.0	2000
NBU	10/24/10	10:00	11	13	<4	U				
NBU	10/24/10	22:00	17	70	<4	U				
NBU	10/25/10	10:00	23	70	4	B,J	93.2	400		
NBU	11/5/10	11:40	BF	286	<4	U				
NBU	11/18/10	unknown	5	31	<4	U			74.0	500
NBU	11/20/10	11:00	BF	271	<4	U			77.97	950
NBU	12/3/10	13:00	BF	258	<4	U,1				
NBU	12/10/10	unknown	10			1				
NBL	8/22/10	9:00	1	231	<4	U				
NBL	8/22/10	10:00	2	224	<4	U				
NBL	8/22/10	12:00	4	224	<4	U,1				
NBL	8/22/10	13:00	5	224	<4	U				
NBL	8/22/10	14:00	6	231	<4	U				
NBL	8/22/10	15:00	7	231	<4	U				
NBL	8/22/10	16:00	8	147	<4	U				
NBL	8/22/10	20:00	12	224	<4	U				
NBL	8/23/10	0:00	16	147	<4	U				
NBL	8/23/10	8:00	24	231	<4	U				
NBL	8/25/10	14:45	BF	153	6	B,C,J				
NBL	8/29/10	23:00	1	181	9	J				
NBL	8/30/10	1:00	3	196	9	J				
NBL	8/30/10	3:00	5	196	10	J				
NBL	8/30/10	4:00	6	166	10	J				
NBL	8/30/10	5:00	7	173	11	J				
NBL	8/30/10	6:00	8	138	12	J				
NBL	8/30/10	7:00	9	166	9	J				
NBL	8/30/10	8:00	10	138	10	J				
NBL	8/30/10	9:00	11	173	9	C,J				
NBL	8/30/10	10:00	12	187	12	J				
NBL	8/30/10	11:00	13	187	8	J	96.4	96		
NBL	8/30/10	12:00	14	138	9	J			70.9	4800
NBL	8/30/10	13:00	15	516	10	J,1	86.7	200		
NBL	8/30/10	19:00	21	181	10	J				

(Continued on next page. See notes at end of table.)

DISSOLVED AMPA CONCENTRATIONS

Sample Tracking					Sample Results						
							MS/MSD[e]		Field Spike[f]		
Site	Date	Time	#[a]	Days Frozen[b]	SA (ng/L)[c]	Data Flags[d]	%Rec	Spike (≈ng/L)	%Rec	Spike (≈ng/L)
NBL	8/31/10	23:00	1	179	4	B,J				
NBL	9/1/10	0:00	2	164	<4	U				
NBL	9/1/10	2:00	4	185	4	B,J				
NBL	9/1/10	3:00	5	171	6	B,C				
NBL	9/1/10	4:00	6	179	5	B,J				
NBL	9/1/10	5:00	7	164	6	B,J				
NBL	9/1/10	6:00	8	136	8	J				
NBL	9/1/10	7:00	9	171	5	B,J				
NBL	9/1/10	8:00	10	194	<4	U				
NBL	9/1/10	10:00	12	164	5	B,J				
NBL	9/1/10	12:00	14	194	<4	U	93.7	95		
NBL	9/1/10	14:00	16	136	8	J				
NBL	9/1/10	22:00	24	179	<4	U				
NBL	9/10/10	12:30	BF	137	4	B,J				
NBL	9/14/10	14:00	BF	190	8	C,J	90.5	95		
NBL	9/15/10	19:00	2	226	4	B,J,1				
NBL	9/15/10	21:00	3	226	6	B,J				
NBL	9/15/10	23:00	4	226	6	B,J				
NBL	9/16/10	1:00	5	226	7	B,J				
NBL	9/16/10	3:00	6	226	6	B,J				
NBL	9/16/10	11:00	10	334	<4	U				
NBL	9/16/10	19:00	14	334	<4	U				
NBL	9/17/10	5:00	19	226	5	B,J				
NBL	9/17/10	7:00	20	226	4	B,J				
NBL	9/17/10	9:00	21	226	4	B,J				
NBL	9/17/10	11:00	1	224	5	B,J				
NBL	9/17/10	13:00	2	224	5	B,J				
NBL	9/17/10	15:00	3	224	4	B,J				
NBL	9/18/10	9:00	12	224	6	B,J	91.0	24		
NBL	9/18/10	15:00	15	231	4	B,J				
NBL	9/18/10	17:00	16	231	<4	U				
NBL	9/18/10	19:00	17	231	5	B,J				
NBL	9/18/10	21:00	18	231	<4	U,C,1				
NBL	9/19/10	3:00	21	231	<4	U				
NBL	9/19/10	7:00	23	231	<4	U				
NBL	9/19/10	12:00	1	229	<4	U				
NBL	9/19/10	18:00	2	229	<4	U				
NBL	9/20/10	0:00	3	229	<4	U				
NBL	9/20/10	6:00	4	229	<4	U				
NBL	9/20/10	12:00	5	229	<4	U	93.2	24		
NBL	9/21/10	6:00	8	201	<4	U	93.3	24		
NBL	9/24/10	13:45	BF	123	7	J				
NBL	10/1/10	14:15	BF	315	6	B,J				
NBL	10/8/01	14:00	BF	308	6	B,C,J				
NBL	10/14/01	15:00	BF	103	4	B,J			87.1	490
NBL	10/22/10	11:00	BF	294	6	B,J				

(Continued on next page. See notes at end of table.)

DISSOLVED AMPA CONCENTRATIONS

Sample Tracking						Sample Results							
									MS/MSD[e]			Field Spike[f]	
Site	Date	Time	#[a]	Days Frozen[b]		SA (ng/L)[c]	Data Flags[d]		%Rec	Spike (≈ng/L)		%Rec	Spike (≈ng/L)
NBL	10/23/10	14:00	1	461		6	B,J					46.9	570
NBL	10/23/10	16:00	2	83		<4	U						
NBL	10/24/10	8:00	10	83		4	B,J						
NBL	10/25/10	10:00	24	83		4	B,J		91.1	200			
NBL	11/5/10	11:00	BF	280		6	B,J		90.7	20		78.8	480
NBL	11/20/10	10:00	BF	271		<4	U						
NBL	12/3/10	12:30	BF	258		<4	U		84.8	20		78.0	490
NBL	12/11/10	unknown	3				C,1						
NBL	12/11/10	unknown	6				1						

[a] Numerical sequence of sample collection during date-specific storm event (one event can include multiple triggering of ISCO sampler); NS = no samples collected, BF = baseflow grab sample.

[b] Days frozen prior to thawing and analysis.

[c] Results from analysis of pre-freeze filtrates, ng/L (ppt).

[d] Data qualifiers:

B = estimated minimum high bias 50%; i.e., result is less than three times mean background from replicate analyses of pre-application baseflow sample (2.4 ±0.6 ng/L; Appendix C, Sections 1.2.1 and 1.2.2); used only when result >MDL.

C = analysis by HPLC/MS-MS (Appendix C, Section 1.2.6).

J = estimated concentration (>MDL but <ICAL LCL).

U = less than estimated MDL (3.8 ng/L; Appendix C, Section 1.2.2).

1 = analysis of whole sample also performed (Appendix C, Section 1.3).

[e] Percent recovery from MS experiments on thawed sample filtrates with nominal spike level (ng/L); result is mean when MSD were performed.

[f] Percent recovery of spike added immediately prior to initial filtration and freezing with nominal spike level (ng/L).

APPENDIX F

DISSOLVED IMAZAPYR CONCENTRATIONS

The study-specific method detection limit (MDL) for dissolved imazapyr in Needle Branch samples was determined to be 0.2 µg/L (ppb; Appendix C, Section 2.2.2). This metric was developed via replicate analyses of a single baseflow sample collected at NBL (the blank control). This sample consistently gave a chromatographic peak co-eluting with imazapyr averaging 0.095 µg/L (as imazapyr) in the HPLC/UV analysis. All these concentrations are less than the lower calibration level (LCL) of the instrumental calibration (ICAL) used in all quantifications, which was 0.625 µg/L.

Although it is expected that the background interferent impacting imazapyr will vary from sample to sample (e.g., storm event runoff vs. baseflow), there are limited data addressing this potential. As noted in Appendix C, Section 2.2.4, analysis of the three samples collected at NBU immediately prior to application of herbicides showed that background in those samples was equivalent to 0.17 ±0.05 µg/L (n = 3) dissolved imazapyr. Although not authoritative by any measure, these results show that background interference in samples did in fact vary, and can be as high as nominally 0.2 µg/L (as imazapyr). Because it is known that this background was not stable, none of the measured concentrations were background subtracted.

The fact that samples contributed variable background interference also means that the MDL will vary from sample to sample, indicating that the MDL cited is not universally relevant. However, there are no data allowing calculation of MDLs reflecting different background levels. Thus, the MDL based on the blank control was used to censor all concentration results even though the true MDL for many samples could be higher or lower. In the tabulation herein, concentrations less than this MDL are reported as "<0.2" (µg/L) and are flagged "U" to signify that the associated result is not statistically different than the concentration found in the blank control. All concentrations between the MDL (0.2 µg/L) and the LCL (0.6 µg/L) are flagged "J" to signify that these concentrations fall below the ICAL range and so are considered estimates.

Sample concentrations less than three times the mean background signal from the replicate analyses of the blank control (0.095 ±0.037 µg/L, n = 23; Appendix C, Table C2.4) are flagged with a "B" to signify that the associated result is high biased by a minimum of 50%. This level of high bias is considered a minimum because (1) concentrations less than this threshold (0.28 µg/L) will carry >50% bias assuming a stable background at the level found in the blank control (0.095 µg/L); and (2) the background varied from sample to sample and is known to have reached 0.17 µg/L in some samples (i.e., the 50% bias threshold would be as high as 0.51 µg/L in some samples).

Because of the variability in this background interference, the concentrations given in the tabulation should be considered high biased by some unknown amount, and thus should be considered maximum possible concentrations. Note that this leaves the potential that some of the reported concentrations are true false positives.

The table herein lists all samples collected for determination of imazapyr, sulfometuron methyl, and metsulfuron methyl during the course of this study. However, not all samples were analyzed (Section 2.3 in the main text), so there are many samples for which no concentration results are given. These samples are included here for completeness only.

All concentrations are acid equivalents (a.e.) of imazapyr.

DISSOLVED IMAZAPYR CONCENTRATIONS

Sample Tracking						Sample Results							
									MS/MSD[e]			Field Spike[f]	
Site	Date	Time	#[a]	Days Frozen[b]		SA (µg/L)[c]	Data Flags[d]		%Rec	Spike (≈µg/L)		%Rec	Spike (≈µg/L)
NBH	8/23/10	ISCO malfunction											
NBH	8/25/10	10:30	BF	715		0.2	J,B						
NBH	8/29/10	23:00	1	702		0.2	J,B						
NBH	8/30/10	0:00	2										
NBH	8/30/10	1:00	3	709		0.3	J,B					96.1	12
NBH	8/30/10	2:00	4										
NBH	8/30/10	3:00	5	749		0.2	J,B						
NBH	8/30/10	4:00	6										
NBH	8/30/10	5:00	7	749		<0.2	U						
NBH	8/30/10	6:00	8										
NBH	8/30/10	7:00	9	756		<0.2	U						
NBH	8/30/10	8:00	10										
NBH	8/30/10	9:00	11										
NBH	8/30/10	10:00	12										
NBH	8/30/10	11:00	13										
NBH	8/30/10	12:00	14										
NBH	8/30/10	13:00	15										
NBH	8/30/10	14:00	16										
NBH	8/30/10	15:00	17										
NBH	8/30/10	16:00	18										
NBH	8/30/10	17:00	19										
NBH	8/30/10	18:00	20										
NBH	8/30/10	19:00	21										
NBH	8/30/10	20:00	22										
NBH	8/30/10	21:00	23										
NBH	8/30/10	22:00	24										
NBH	8/31/10	23:00	1	754		0.2	J,B						
NBH	9/1/10	0:00	2										
NBH	9/1/10	1:00	3	754		0.4	J						
NBH	9/1/10	2:00	4										
NBH	9/1/10	3:00	5										
NBH	9/1/10	4:00	6	754		< 0.2	U						
NBH	9/1/10	5:00	7										
NBH	9/1/10	6:00	8										
NBH	9/1/10	7:00	9	754		< 0.2	U						
NBH	9/1/10	8:00	10										
NBH	9/1/10	9:00	11										
NBH	9/1/10	10:00	12										
NBH	9/1/10	11:00	13										
NBH	9/1/10	12:00	14										
NBH	9/1/10	13:00	15										
NBH	9/1/10	14:00	16										
NBH	9/1/10	15:00	17										
NBH	9/1/10	16:00	18										
NBH	9/1/10	17:00	19										
NBH	9/1/10	18:00	20										

(Continued on next page. See notes at end of table.)

DISSOLVED IMAZAPYR CONCENTRATIONS

Sample Tracking						Sample Results							
									MS/MSD[e]			Field Spike[f]	
Site	Date	Time	#[a]	Days Frozen[b]		SA (µg/L)[c]	Data Flags[d]		%Rec	Spike (≈µg/L)		%Rec	Spike (≈µg/L)
NBH	9/1/10	19:00	21										
NBH	9/1/10	20:00	22										
NBH	9/1/10	21:00	23										
NBH	9/1/10	22:00	24										
NBH	9/10/10	14:30	BF	699		< 0.2	U					92.8	6
NBH	9/14/10	15:15	BF	695		< 0.2	U						
NBH	9/15/10	17:00	1										
NBH	9/15/10	19:00	2										
NBH	9/15/10	21:00	3										
NBH	9/15/10	23:00	4										
NBH	9/16/10	1:00	5										
NBH	9/16/10	3:00	6										
NBH	9/16/10	5:00	7										
NBH	9/16/10	7:00	8										
NBH	9/16/10	9:00	9										
NBH	9/16/10	11:00	10										
NBH	9/16/10	13:00	11										
NBH	9/16/10	15:00	12										
NBH	9/16/10	17:00	13										
NBH	9/16/10	19:00	14										
NBH	9/16/10	21:00	15										
NBH	9/16/10	23:00	16										
NBH	9/17/10	1:00	17										
NBH	9/17/10	3:00	18										
NBH	9/17/10	5:00	19										
NBH	9/17/10	7:00	20										
NBH	9/17/10	9:00	21										
NBH	9/17/10	11:00	22										
NBH	9/17/10	13:00	1										
NBH	9/17/10	15:00	2										
NBH	9/17/10	17:00	3										
NBH	9/17/10	19:00	4										
NBH	9/17/10	21:00	5										
NBH	9/17/10	23:00	6										
NBH	9/18/10	1:00	7										
NBH	9/18/10	3:00	8										
NBH	9/18/10	5:00	9										
NBH	9/18/10	7:00	10										
NBH	9/18/10	9:00	11										
NBH	9/18/10	11:00	12										
NBH	9/18/10	13:00	13										
NBH	9/18/10	15:00	14										
NBH	9/18/10	17:00	15										
NBH	9/18/10	19:00	16										
NBH	9/18/10	21:00	17										
NBH	9/18/10	23:00	18										
NBH	9/19/10	1:00	19										

(Continued on next page. See notes at end of table.)

DISSOLVED IMAZAPYR CONCENTRATIONS

Sample Tracking						Sample Results							
									MS/MSD[e]			Field Spike[f]	
Site	Date	Time	#[a]	Days Frozen[b]		SA (µg/L)[c]	Data Flags[d]		%Rec	Spike (≈µg/L)		%Rec	Spike (≈µg/L)
NBH	9/19/10	3:00	20										
NBH	9/19/10	5:00	21										
NBH	9/19/10	7:00	22										
NBH	9/19/10	9:00	23										
NBH	9/19/10	11:00	24										
NBH	9/19/10	12:00	1										
NBH	9/19/10	18:00	2										
NBH	9/20/10	0:00	3										
NBH	9/20/10	6:00	4										
NBH	9/20/10	12:00	5										
NBH	9/20/10	18:00	6										
NBH	9/21/10	0:00	7										
NBH	9/21/10	6:00	8										
NBH	9/24/10	15:30	BF	739		< 0.2	U		89.6	1			
NBH	10/1/10	12:30	BF	732		< 0.2	U						
NBH	10/8/10	12:00	BF	725		< 0.2	U					94.4	5
NBH	10/8/10	18:00	1										
NBH	10/8/10	21:00	2										
NBH	10/9/10	0:00	3										
NBH	10/9/10	3:00	4										
NBH	10/9/10	6:00	5										
NBH	10/9/10	9:00	6										
NBH	10/9/10	12:00	7										
NBH	10/9/10	15:00	8										
NBH	10/9/10	18:00	9										
NBH	10/9/10	21:00	10										
NBH	10/10/10	0:00	11										
NBH	10/10/10	3:00	12										
NBH	10/10/10	6:00	13										
NBH	10/10/10	9:00	14										
NBH	10/10/10	12:00	1										
NBH	10/10/10	18:00	2										
NBH	10/10/10	18:00	2										
NBH	10/11/10	0:00	3										
NBH	10/11/10	6:00	4										
NBH	10/11/10	12:00	5										
NBH	10/14/10	13:00	BF										
NBH	10/22/10	13:30	BF										
NBH	10/23/10	14:00	1										
NBH	10/23/10	16:00	2										
NBH	10/23/10	18:00	3										
NBH	10/23/10	20:00	4										
NBH	10/23/10	22:00	5										
NBH	10/24/10	0:00	6										
NBH	10/24/10	2:00	7										
NBH	10/24/10	4:00	8										
NBH	10/24/10	6:00	9										

(Continued on next page. See notes at end of table.)

DISSOLVED IMAZAPYR CONCENTRATIONS

Sample Tracking						Sample Results							
									MS/MSD[e]			Field Spike[f]	
Site	Date	Time	#[a]	Days Frozen[b]		SA (µg/L)[c]	Data Flags[d]		%Rec	Spike (≈µg/L)		%Rec	Spike (≈µg/L)
NBH	10/24/10	8:00	10										
NBH	10/24/10	10:00	11										
NBH	10/24/10	12:00	12										
NBH	10/24/10	14:00	13										
NBH	10/24/10	16:00	14										
NBH	10/24/10	18:00	15										
NBH	10/24/10	20:00	16										
NBH	10/24/10	22:00	17										
NBH	10/25/10	0:00	18										
NBH	10/25/10	2:00	19										
NBH	10/25/10	4:00	20										
NBH	10/25/10	6:00	21										
NBH	10/25/10	8:00	22										
NBH	10/25/10	10:00	23										
NBH	10/25/10	12:00	24										
NBH	11/5/10	12:30	BF										
NBH	11/18/10	times	1										
NBH		unknown	2										
NBH			3										
NBH			4										
NBH			5										
NBH			6										
NBH			7										
NBH			8										
NBH			9										
NBH			10										
NBH			11										
NBH			12										
NBH			13										
NBH			14										
NBH			15										
NBH	11/20/10	12:00	BF										
NBH	12/3/10	14:00	BF										
NBH	12/11/10	times	1										
NBH		unknown	2										
NBH			3										
NBH			4										
NBH			5										
NBH			6										
NBH			7										
NBH			8										
NBH			9										
NBH			10										
NBH			11										
NBH			12										
NBH			13										

(Continued on next page. See notes at end of table.)

DISSOLVED IMAZAPYR CONCENTRATIONS

Sample Tracking						Sample Results							
									MS/MSD[e]			Field Spike[f]	
Site	Date	Time	#[a]	Days Frozen[b]		SA (µg/L)[c]	Data Flags[d]		%Rec	Spike (≈µg/L)		%Rec	Spike (≈µg/L)
NBH			14										
NBH			15										
NBH			16										
NBH			17										
NBH			18										
NBH	12/11/10	times unknown	1										
NBH			2										
NBH			3										
NBH			4										
NBH			5										
NBH			6										
NBH			7										
NBH			8										
NBH			9										
NBH			10										
NBH			11										
NBH			12										
NBU	8/22/10	9:00	1	710		< 0.2	U		98.7	2			
NBU	8/22/10	10:00	2	757		< 0.2	U						
NBU	8/22/10	11:00	3	710		0.2	J,B						
NBU	8/22/10	12:00	4	710		0.2	J,B						
NBU	8/22/10	13:00	5	710		0.2	J,B						
NBU	8/22/10	14:00	6	710		< 0.2	U						
NBU	8/22/10	15:00	7										
NBU	8/22/10	16:00	8	710		0.2	J,B						
NBU	8/22/10	17:00	9	757		0.2	J,B						
NBU	8/22/10	18:00	10	757		0.3	J						
NBU	8/22/10	19:00	11										
NBU	8/22/10	20:00	12										
NBU	8/22/10	21:00	13										
NBU	8/22/10	22:00	14										
NBU	8/22/10	23:00	15										
NBU	8/23/10	0:00	16										
NBU	8/23/10	1:00	17										
NBU	8/23/10	2:00	18										
NBU	8/23/10	3:00	19										
NBU	8/23/10	4:00	20										
NBU	8/23/10	5:00	21										
NBU	8/23/10	6:00	22										
NBU	8/23/10	7:00	23										
NBU	8/23/10	8:00	24	710		< 0.2	U					95.8	5
NBU	8/25/10	12:35	BF	715		< 0.2	U						
NBU	8/29/10	23:00	1										

(Continued on next page. See notes at end of table.)

DISSOLVED IMAZAPYR CONCENTRATIONS

Sample Tracking					Sample Results						
							MS/MSD[e]		Field Spike[f]		
Site	Date	Time	#[a]	Days Frozen[b]	SA (µg/L)[c]	Data Flags[d]	%Rec	Spike (≈µg/L)	%Rec	Spike (≈µg/L)
NBU	8/30/10	0:00	2							
NBU	8/30/10	1:00	3	756	< 0.2	U			88.7	9
NBU	8/30/10	2:00	4	702	< 0.2	U				
NBU	8/30/10	3:00	5							
NBU	8/30/10	4:00	6							
NBU	8/30/10	5:00	7	702	< 0.2	U				
NBU	8/30/10	6:00	8	678	<0.2	U	100.2	25		
NBU	8/30/10	7:00	9	702	<0.2	U				
NBU	8/30/10	8:00	10	709	<0.2	U				
NBU	8/30/10	9:00	11	709	0.2	J,B				
NBU	8/30/10	10:00	12	749	0.4	J				
NBU	8/30/10	11:00	13	749	0.2	J,B				
NBU	8/30/10	12:00	14	749	0.3	J,B				
NBU	8/30/10	13:00	15	749	0.2	J,B				
NBU	8/30/10	14:00	16	749	0.2	J,B				
NBU	8/30/10	15:00	17	749	< 0.2	U	93.0	1		
NBU	8/30/10	16:00	18	749	0.3	J,B				
NBU	8/30/10	17:00	19							
NBU	8/30/10	18:00	20							
NBU	8/30/10	19:00	21							
NBU	8/30/10	20:00	22							
NBU	8/30/10	21:00	23							
NBU	8/30/10	22:00	24							
NBU	8/31/10	23:00	1	754	< 0.2	U				
NBU	9/1/10	0:00	2							
NBU	9/1/10	1:00	3							
NBU	9/1/10	2:00	4							
NBU	9/1/10	3:00	5							
NBU	9/1/10	4:00	6	754	0.2	J,B				
NBU	9/1/10	5:00	7							
NBU	9/1/10	6:00	8	754	<0.2	U	83.4	1		
NBU	9/1/10	7:00	9							
NBU	9/1/10	8:00	10							
NBU	9/1/10	9:00	11							
NBU	9/1/10	10:00	12							
NBU	9/1/10	11:00	13							
NBU	9/1/10	12:00	14							
NBU	9/1/10	13:00	15							
NBU	9/1/10	14:00	16							
NBU	9/1/10	15:00	17							
NBU	9/1/10	16:00	18							
NBU	9/1/10	17:00	19							
NBU	9/1/10	18:00	20							
NBU	9/1/10	19:00	21							
NBU	9/1/10	20:00	22							
NBU	9/1/10	21:00	23							
NBU	9/1/10	22:00	24							

(Continued on next page. See notes at end of table.)

DISSOLVED IMAZAPYR CONCENTRATIONS

Sample Tracking					Sample Results						
							MS/MSD[e]		Field Spike[f]		
Site	Date	Time	#[a]	Days Frozen[b]	SA (µg/L)[c]	Data Flags[d]	%Rec	Spike (≈µg/L)	%Rec	Spike (≈µg/L)
NBU	9/10/10	13:45	BF	699	0.2	J,B				
NBU	9/14/10	14:45	BF	749	< 0.2	U				
NBU	9/15/10	17:00	1							
NBU	9/15/10	19:00	2							
NBU	9/15/10	21:00	3							
NBU	9/15/10	23:00	4							
NBU	9/16/10	1:00	5							
NBU	9/16/10	3:00	6							
NBU	9/16/10	5:00	7							
NBU	9/16/10	7:00	8							
NBU	9/16/10	9:00	9							
NBU	9/16/10	11:00	10							
NBU	9/16/10	13:00	11							
NBU	9/16/10	15:00	12							
NBU	9/16/10	17:00	13							
NBU	9/16/10	19:00	14							
NBU	9/16/10	21:00	15							
NBU	9/16/10	23:00	16							
NBU	9/17/10	1:00	17							
NBU	9/17/10	3:00	18							
NBU	9/17/10	5:00	19							
NBU	9/17/10	7:00	20							
NBU	9/17/10	9:00	21							
NBU	9/17/10	11:00	22							
NBU	9/17/10	13:00	1							
NBU	9/17/10	15:00	2							
NBU	9/17/10	17:00	3							
NBU	9/17/10	19:00	4							
NBU	9/17/10	21:00	5							
NBU	9/17/10	23:00	6							
NBU	9/18/10	1:00	7							
NBU	9/18/10	3:00	8							
NBU	9/18/10	5:00	9							
NBU	9/18/10	7:00	10							
NBU	9/18/10	9:00	11							
NBU	9/18/10	11:00	12							
NBU	9/18/10	13:00	13							
NBU	9/18/10	15:00	14							
NBU	9/18/10	17:00	15							
NBU	9/18/10	19:00	16							
NBU	9/18/10	21:00	17							
NBU	9/18/10	23:00	18							
NBU	9/19/10	1:00	19							
NBU	9/19/10	3:00	20							
NBU	9/19/10	5:00	21							
NBU	9/19/10	7:00	22							
NBU	9/19/10	9:00	23							

(Continued on next page. See notes at end of table.)

DISSOLVED IMAZAPYR CONCENTRATIONS

Sample Tracking						Sample Results							
									MS/MSD[e]			Field Spike[f]	
Site	Date	Time	#[a]	Days Frozen[b]		SA (µg/L)[c]	Data Flags[d]		%Rec	Spike (≈µg/L)		%Rec	Spike (≈µg/L)
NBU	9/19/10	11:00	24										
NBU	9/19/10	12:00	1										
NBU	9/19/10	18:00	2										
NBU	9/20/10	0:00	3										
NBU	9/20/10	6:00	4										
NBU	9/20/10	12:00	5										
NBU	9/20/10	18:00	6										
NBU	9/21/10	0:00	7										
NBU	9/21/10	6:00	8										
NBU	9/24/10	14:30	BF	739		< 0.2	U						
NBU	10/1/10	13:30	BF	732		< 0.2	U					97.0	10
NBU	10/8/10	13:00	BF	725		< 0.2	U						
NBU	10/8/10	18:00	1										
NBU	10/8/10	21:00	2										
NBU	10/9/10	0:00	3										
NBU	10/9/10	3:00	4										
NBU	10/9/10	6:00	5										
NBU	10/9/10	9:00	6										
NBU	10/9/10	12:00	7										
NBU	10/9/10	15:00	8										
NBU	10/9/10	18:00	9										
NBU	10/9/10	21:00	10										
NBU	10/10/10	0:00	11										
NBU	10/10/10	3:00	12										
NBU	10/10/10	6:00	13										
NBU	10/10/10	9:00	14										
NBU	10/10/10	12:00	1										
NBU	10/10/10	18:00	2										
NBU	10/10/10	18:00	2										
NBU	10/11/10	0:00	3										
NBU	10/11/10	6:00	4										
NBU	10/11/10	12:00	5										
NBU	10/14/10	13:00	BF										
NBU	10/22/10	12:30	BF										
NBU	10/23/10	14:00	1										
NBU	10/23/10	16:00	2										
NBU	10/23/10	18:00	3										
NBU	10/23/10	20:00	4										
NBU	10/23/10	22:00	5										
NBU	10/24/10	0:00	6										
NBU	10/24/10	2:00	7										
NBU	10/24/10	4:00	8										
NBU	10/24/10	6:00	9										
NBU	10/24/10	8:00	10										
NBU	10/24/10	10:00	11										
NBU	10/24/10	12:00	12										
NBU	10/24/10	14:00	13										

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DISSOLVED IMAZAPYR CONCENTRATIONS

Sample Tracking						Sample Results							
									MS/MSD[e]			Field Spike[f]	
Site	Date	Time	#[a]	Days Frozen[b]		SA (µg/L)[c]	Data Flags[d]		%Rec	Spike (≈µg/L)		%Rec	Spike (≈µg/L)
NBU	10/24/10	16:00	14										
NBU	10/24/10	18:00	15										
NBU	10/24/10	20:00	16										
NBU	10/24/10	22:00	17										
NBU	10/25/10	0:00	18										
NBU	10/25/10	2:00	19										
NBU	10/25/10	4:00	20										
NBU	10/25/10	6:00	21										
NBU	10/25/10	8:00	22										
NBU	10/25/10	10:00	23										
NBU	11/5/10	11:40	BF										
NBU	11/18/10	times unknown	1										
NBU			2										
NBU			3										
NBU			4										
NBU			5										
NBU			6										
NBU			7										
NBU			8										
NBU			9										
NBU			10										
NBU			11										
NBU			12										
NBU			13										
NBU			14										
NBU	11/20/10	11:00	BF										
NBU	12/3/10	13:00	BF										
NBU	12/10/10	times unknown	1										
NBU			2										
NBU			3										
NBU			4										
NBU			5										
NBU			6										
NBU			7										
NBU			8										
NBU			9										
NBU			10										
NBU			12										
NBU			13										
NBU			14										
NBU			15										
NBU			16										
NBU			17										

(Continued on next page. See notes at end of table.)

DISSOLVED IMAZAPYR CONCENTRATIONS

Sample Tracking					Sample Results						
							MS/MSD[e]		Field Spike[f]		
Site	Date	Time	#[a]	Days Frozen[b]	SA (µg/L)[c]	Data Flags[d]	%Rec	Spike (≈µg/L)	%Rec	Spike (≈µg/L)
NBU			18							
NBU	12/11/12	times unknown	1							
NBU			2							
NBU			3							
NBU			4							
NBU			5							
NBU			6							
NBU			7							
NBL	8/22/10	9:00	1							
NBL	8/22/10	10:00	2							
NBL	8/22/10	11:00	3							
NBL	8/22/10	12:00	4							
NBL	8/22/10	13:00	5							
NBL	8/22/10	14:00	6							
NBL	8/22/10	15:00	7							
NBL	8/22/10	16:00	8							
NBL	8/22/10	17:00	9							
NBL	8/22/10	18:00	10							
NBL	8/22/10	19:00	11							
NBL	8/22/10	20:00	12							
NBL	8/22/10	21:00	13							
NBL	8/22/10	22:00	14							
NBL	8/22/10	23:00	15							
NBL	8/23/10	0:00	16							
NBL	8/23/10	1:00	17							
NBL	8/23/10	2:00	18							
NBL	8/23/10	3:00	19							
NBL	8/23/10	4:00	20							
NBL	8/23/10	5:00	21							
NBL	8/23/10	6:00	22							
NBL	8/23/10	7:00	23	764	< 0.2	U			96.1	5
NBL	8/23/10	8:00	24							
NBL	8/25/10	14:45	BF	715	< 0.2	U	99.1	2	92.9	5
NBL	8/29/10	ISCO malfunction								
NBL	9/1/10									
NBL	9/10/10	12:30	BF	699	< 0.2	U				
NBL	9/14/10	14:00	BF	749	< 0.2	U				
NBL	9/15/10	17:00	1							
NBL	9/15/10	19:00	2							
NBL	9/15/10	21:00	3							
NBL	9/15/10	23:00	4							
NBL	9/16/10	1:00	5							

(Continued on next page. See notes at end of table.)

DISSOLVED IMAZAPYR CONCENTRATIONS

Sample Tracking						Sample Results							
									MS/MSD[e]			Field Spike[f]	
Site	Date	Time	#[a]	Days Frozen[b]		SA (µg/L)[c]	Data Flags[d]		%Rec	Spike (≈µg/L)		%Rec	Spike (≈µg/L)
NBL	9/16/10	3:00	6										
NBL	9/16/10	5:00	7										
NBL	9/16/10	7:00	8										
NBL	9/16/10	9:00	9										
NBL	9/16/10	11:00	10										
NBL	9/16/10	13:00	11										
NBL	9/16/10	15:00	12										
NBL	9/16/10	17:00	13										
NBL	9/16/10	19:00	14										
NBL	9/16/10	21:00	15										
NBL	9/16/10	23:00	16										
NBL	9/17/10	1:00	17										
NBL	9/17/10	3:00	18										
NBL	9/17/10	5:00	19										
NBL	9/17/10	7:00	20										
NBL	9/17/10	9:00	21										
NBL	9/17/10	11:00	22										
NBL	9/17/10	13:00	1										
NBL	9/17/10	15:00	2										
NBL	9/17/10	17:00	3										
NBL	9/17/10	19:00	4										
NBL	9/17/10	21:00	5										
NBL	9/17/10	23:00	6										
NBL	9/18/10	1:00	7										
NBL	9/18/10	3:00	8										
NBL	9/18/10	5:00	9										
NBL	9/18/10	7:00	10										
NBL	9/18/10	9:00	11										
NBL	9/18/10	11:00	12										
NBL	9/18/10	13:00	13										
NBL	9/18/10	15:00	14										
NBL	9/18/10	17:00	15										
NBL	9/18/10	19:00	16										
NBL	9/18/10	21:00	17										
NBL	9/18/10	23:00	18										
NBL	9/19/10	1:00	19										
NBL	9/19/10	3:00	20										
NBL	9/19/10	5:00	21										
NBL	9/19/10	7:00	22										
NBL	9/19/10	9:00	23										
NBL	9/19/10	11:00	24										
NBL	9/19/10	12:00	1										
NBL	9/19/10	18:00	2										
NBL	9/20/10	0:00	3										
NBL	9/20/10	6:00	4										
NBL	9/20/10	12:00	5										
NBL	9/20/10	18:00	6										

(Continued on next page. See notes at end of table.)

DISSOLVED IMAZAPYR CONCENTRATIONS

Sample Tracking						Sample Results							
									MS/MSD[e]			Field Spike[f]	
Site	Date	Time	#[a]	Days Frozen[b]		SA (µg/L)[c]	Data Flags[d]		%Rec	Spike (≈µg/L)		%Rec	Spike (≈µg/L)
NBL	9/21/10	0:00	7										
NBL	9/21/10	6:00	8										
NBL	9/24/10	13:45	BF	739		< 0.2	U						
NBL	10/1/10	14:15	BF										
NBL	10/8/10	14:00	BF										
NBL	10/8/10	18:00	1										
NBL	10/8/10	21:00	2										
NBL	10/9/10	0:00	3										
NBL	10/9/10	3:00	4										
NBL	10/9/10	6:00	5										
NBL	10/9/10	9:00	6										
NBL	10/9/10	12:00	7										
NBL	10/9/10	15:00	8										
NBL	10/9/10	18:00	9										
NBL	10/9/10	21:00	10										
NBL	10/10/10	0:00	11										
NBL	10/10/10	3:00	12										
NBL	10/10/10	6:00	13										
NBL	10/10/10	9:00	14										
NBL	10/10/10	12:00	1										
NBL	10/10/10	18:00	2										
NBL	10/10/10	18:00	2										
NBL	10/11/10	0:00	3										
NBL	10/11/10	6:00	4										
NBL	10/11/10	12:00	5										
NBL	10/14/10	15:00	BF										
NBL	10/22/10	11:00	BF										
NBL	10/23/10	14:00	1										
NBL	10/23/10	16:00	2										
NBL	10/23/10	18:00	3										
NBL	10/23/10	20:00	4										
NBL	10/23/10	22:00	5										
NBL	10/24/10	0:00	6										
NBL	10/24/10	2:00	7										
NBL	10/24/10	4:00	8										
NBL	10/24/10	6:00	9										
NBL	10/24/10	8:00	10										
NBL	10/24/10	10:00	11										
NBL	10/24/10	12:00	12										
NBL	10/24/10	14:00	13										
NBL	10/24/10	16:00	14										
NBL	10/24/10	18:00	15										
NBL	10/24/10	20:00	16										
NBL	10/24/10	22:00	17										
NBL	10/25/10	0:00	18										
NBL	10/25/10	2:00	19										
NBL	10/25/10	4:00	20										

(Continued on next page. See notes at end of table.)

DISSOLVED IMAZAPYR CONCENTRATIONS

Sample Tracking						Sample Results							
									MS/MSD[e]			Field Spike[f]	
Site	Date	Time	#[a]	Days Frozen[b]		SA (µg/L)[c]	Data Flags[d]		%Rec	Spike (≈µg/L)		%Rec	Spike (≈µg/L)
NBL	10/25/10	6:00	21										
NBL	10/25/10	8:00	22										
NBL	10/25/10	10:00	23										
NBL	11/5/10	11:00	BF										
NBL	11/18/10	times unknown	1										
NBL			2										
NBL			3										
NBL			4										
NBL			5										
NBL			6										
NBL			7										
NBL			8										
NBL			9										
NBL			10										
NBL			11										
NBL			12										
NBL			13										
NBL			14										
NBL			15										
NBL	11/20/10	10:00	BF										
NBL	12/3/10	12:30	BF										
NBL	12/10/10	times unknown	1										
NBL			2										
NBL			3										
NBL			4										
NBL			5										
NBL			6										
NBL			7										
NBL			8										
NBL			9										
NBL			10										
NBL			11										
NBL			12										
NBL			13										
NBL			14										
NBL			15										
NBL			16										
NBL			17										
NBL			18										
NBL	12/11/10	times unknown	1										
NBL			2										
NBL			3										
NBL			4										
NBL			5										
NBL			6										
NBL			7										
NBL			8										

(Continued on next page. See notes at end of table.)

DISSOLVED IMAZAPYR CONCENTRATIONS

Sample Tracking						Sample Results							
									MS/MSD[e]			Field Spike[f]	
Site	Date	Time	#[a]	Days Frozen[b]		SA (µg/L)[c]	Data Flags[d]		%Rec	Spike (≈µg/L)		%Rec	Spike (≈µg/L)
NBL			9										
NBL			10										
NBL			11										
NBL			12										
NBL			13										
NBL			14										
NBL			15										
NBL			16										
NBL			17										
NBL			18										

[a] Numerical sequence of sample collection during date-specific storm event (one event can include multiple triggering of ISCO sampler); NS = no samples collected, BF = baseflow grab sample.
[b] Days frozen prior to thawing and analysis.
[c] Results in µg/L (ppb).
[d] Data qualifiers:
B = estimated minimum high bias 50%; i.e., result is less than three times mean background from replicate analyses of pre-application baseflow sample (0.10 µg/L; Appendix C, Sections 2.2.1 and 2.2.2); used only when result >MDL.
J = estimated concentration (>MDL but <ICAL LCL).
U = less than estimated MDL (0.2 µg/L; Appendix C, Section 2.2.2).
[e] Percent recovery from MS experiments with nominal spike level (µg/L); result is mean when MSD were performed.
[f] Percent recovery of spike added immediately prior to freezing with nominal spike level (µg/L).

APPENDIX G

DISSOLVED SULFOMETURON METHYL CONCENTRATIONS

The study-specific method detection limit (MDL) for dissolved sulfometuron methyl in Needle Branch samples was determined to be 0.5 µg/L (ppb; Appendix C, Section 2.2.2). This metric was developed via replicate analyses of a single baseflow sample collected at NBL (the blank control). This sample consistently gave a chromatographic peak co-eluting with sulfometuron methyl averaging 0.23 µg/L (as sulfometuron methyl) in the HPLC/UV analysis. All these concentrations are less than the lower calibration level (LCL) of the instrumental calibration (ICAL) used in all quantifications, which was 0.625 µg/L.

The table herein does not identify every sample collected for determination of imazapyr, sulfometuron methyl, and metsulfuron methyl, but only those actually analyzed. The tabulation in Appendix F (imazapyr results) lists all samples.

All concentrations are active ingredient (a.i.) of sulfometuron methyl.

DISSOLVED SULFOMETURON METHYL CONCENTRATIONS

Sample Tracking					Sample Results						
							MS/MSD[e]		Field Spike[f]		
Site	Date	Time	#[a]	Days Frozen[b]	SA (µg/L)[c]	Data Flags[d]	%Rec	Spike (≈µg/L)	%Rec	Spike (≈µg/L)
NBH	8/25/10	10:30	BF	715	< 0.5	U				
NBH	8/29/10	23:00	1	702	< 0.5	U				
NBH	8/30/10	1:00	3	709	< 0.5	U			74.0	12
NBH	8/30/10	3:00	5	749	< 0.5	U				
NBH	8/30/10	5:00	7	749	< 0.5	U				
NBH	8/30/10	7:00	9	756	< 0.5	U				
NBH	8/31/10	23:00	1	754	< 0.5	U				
NBH	9/1/10	1:00	3	754	< 0.5	U				
NBH	9/1/10	4:00	6	754	< 0.5	U				
NBH	9/1/10	7:00	9	754	< 0.5	U				
NBH	9/10/2010	14:30	BF	699	< 0.5	U			71.3	6
NBH	9/14/2010	15:15	BF	695	< 0.5	U				
NBH	9/24/10	15:30	BF	739	< 0.5	U	92.3	2		
NBH	10/1/10	12:30	BF	732	< 0.5	U				
NBH	10/8/10	12:00	BF	725	< 0.5	U			70.0	5
NBU	8/22/10	9:00	1	710	< 0.5	U	94.3	3		
NBU	8/22/10	10:00	2	757	< 0.5	U				
NBU	8/22/10	11:00	3	710	< 0.5	U				
NBU	8/22/10	12:00	4	710	< 0.5	U				
NBU	8/22/10	13:00	5	710	< 0.5	U				
NBU	8/22/10	14:00	6	710	< 0.5	U				
NBU	8/22/10	16:00	8	710	< 0.5	U				
NBU	8/22/10	17:00	9	757	< 0.5	U				
NBU	8/22/10	18:00	10	757	< 0.5	U				
NBU	8/23/10	8:00	24	710	< 0.5	U			72.3	5
NBU	8/25/10	12:35	BF	715	< 0.5	U				
NBU	8/30/10	1:00	3	756	< 0.5	U			71.4	9

(Continued on next page. See notes at end of table.)

DISSOLVED SULFOMETURON METHYL CONCENTRATIONS

Sample Tracking					Sample Results						
							MS/MSD[e]		Field Spike[f]		
Site	Date	Time	#[a]	Days Frozen[b]	SA (µg/L)[c]	Data Flags[d]	%Rec	Spike (≈µg/L)	%Rec	Spike (≈µg/L)
NBU	8/30/10	2:00	4	702	< 0.5	U				
NBU	8/30/10	5:00	7	702	< 0.5	U				
NBU	8/30/10	6:00	8	678	< 0.5	U	90.1	25		
NBU	8/30/10	7:00	9	702	< 0.5	U				
NBU	8/30/10	8:00	10	709	< 0.5	U				
NBU	8/30/10	9:00	11	709	< 0.5	U				
NBU	8/30/10	10:00	12	749	< 0.5	U				
NBU	8/30/10	11:00	13	749	< 0.5	U				
NBU	8/30/10	12:00	14	749	< 0.5	U				
NBU	8/30/10	13:00	15	749	< 0.5	U				
NBU	8/30/10	14:00	16	749	< 0.5	U				
NBU	8/30/10	15:00	17	749	< 0.5	U	92.6	2		
NBU	8/30/10	16:00	18	749	< 0.5	U				
NBU	8/31/10	23:00	1	754	< 0.5	U				
NBU	9/1/10	4:00	6	754	< 0.5	U				
NBU	9/1/10	6:00	8	754	< 0.5	U	91.1	2		
NBU	9/10/10	13:45	BF	699	< 0.5	U				
NBU	9/14/10	14:45	BF	749	< 0.5	U				
NBU	9/24/10	14:30	BF	739	< 0.5	U				
NBU	10/1/10	13:30	BF	732	< 0.5	U			73.7	10
NBU	10/8/10	13:00	BF	725	< 0.5	U				
NBL	8/23/10	7:00	23	764	< 0.5	U			81.0	5
NBL	8/25/10	14:45	BF	715	< 0.5	U	90.0	3	72.0	5
NBL	9/10/10	12:30	BF	699	< 0.5	U				
NBL	9/14/10	14:00	BF	749	< 0.5	U				
NBL	9/24/10	13:45	BF	739	< 0.5	U				

[a] Numerical sequence of sample collection during date-specific storm event (one event can include multiple triggering of ISCO sampler); NS = no samples collected, BF = baseflow grab sample.

[b] Days frozen prior to thawing and analysis.

[c] Results in µg/L (ppb).

[d] Data qualifiers:

B = estimated minimum high bias 50%; i.e., result is less than three times mean background from replicate analyses of pre-application baseflow sample (0.23 µg/L; Appendix C, Sections 2.2.1 and 2.2.2); used only when result >MDL.

J = estimated concentration (>MDL but <ICAL LCL).

U = less than estimated MDL (0.5 µg/L; Appendix C, Section 2.2.2).

[e] Percent recovery from MS experiments with nominal spike level (µg/L); result is mean when MSD were performed.

[f] Percent recovery of spike added immediately prior to freezing with nominal spike level (µg/L).

APPENDIX H

DISSOLVED METSULFURON METHYL CONCENTRATIONS

The study-specific method detection limit (MDL) for dissolved metsulfuron methyl in Needle Branch samples was determined to be 1 µg/L (ppb; Appendix C, Section 2.2.2). This metric was developed via replicate analyses of a single baseflow sample collected at NBL (the blank control). This sample consistently gave a chromatographic peak co-eluting with metsulfuron methyl averaging 0.23 µg/L (as metsulfuron methyl) in the HPLC/UV analysis. All these concentrations are less than the lower calibration level (LCL) of the instrumental calibration (ICAL) used in all quantifications, which was 0.625 µg/L.

The table herein does not identify every sample collected for determination of imazapyr, sulfometuron methyl, and metsulfuron methyl, but only those actually analyzed. The tabulation in Appendix F (imazapyr results) lists all samples.

All concentrations are active ingredient (a.i.) of metsulfuron methyl.

DISSOLVED METSULFURON METHYL CONCENTRATIONS

Sample Tracking					Sample Results						
							MS/MSD[e]		Field Spike[f]		
Site	Date	Time	#[a]	Days Frozen[b]	SA (µg/L)[c]	Data Flags[d]	%Rec	Spike (≈µg/L)	% Rec	Spike (≈µg/L)
NBH	8/25/10	10:30	BF	715	< 1	U				
NBH	8/29/10	23:00	1	702	< 1	U				
NBH	8/30/10	1:00	3	709	< 1	U			91.9	12
NBH	8/30/10	3:00	5	749	< 1	U				
NBH	8/30/10	5:00	7	749	< 1	U				
NBH	8/30/10	7:00	9	756	< 1	U				
NBH	8/31/10	23:00	1	754	< 1	U				
NBH	9/1/10	1:00	3	754	< 1	U				
NBH	9/1/10	4:00	6	754	< 1	U				
NBH	9/1/10	7:00	9	754	< 1	U				
NBH	9/10/10	14:30	BF	699	< 1	U			83.9	5
NBH	9/14/10	15:15	BF	695	< 1	U				
NBH	9/24/10	15:30	BF	739	< 1	U	88.7	2		
NBH	10/1/10	12:30	BF	732	< 1	U				
NBH	10/8/10	12:00	BF	725	< 1	U			95.5	5
NBU	8/22/10	9:00	1	710	< 1	U	99.3	3		
NBU	8/22/10	10:00	2	757	< 1	U				
NBU	8/22/10	11:00	3	710	< 1	U				
NBU	8/22/10	12:00	4	710	< 1	U				
NBU	8/22/10	13:00	5	710	< 1	U				
NBU	8/22/10	14:00	6	710	< 1	U				
NBU	8/22/10	16:00	8	710	< 1	U				
NBU	8/22/10	17:00	9	757	< 1	U				
NBU	8/22/10	18:00	10	757	< 1	U				
NBU	8/23/10	8:00	24	710	< 1	U			91.4	5
NBU	8/25/10	12:35	BF	715	< 1	U				
NBU	8/30/10	1:00	3	756	< 1	U			91.5	9

(Continued on next page. See notes at end of table.)

DISSOLVED METSULFURON METHYL CONCENTRATIONS

Sample Tracking					Sample Results						
							MS/MSD[e]		Field Spike[f]		
Site	Date	Time	#[a]	Days Frozen[b]	SA (µg/L)[c]	Data Flags[d]	%Rec	Spike (≈µg/L)	% Rec	Spike (≈µg/L)
NBU	8/30/10	2:00	4	702	< 1	U				
NBU	8/30/10	5:00	7	702	< 1	U				
NBU	8/30/10	6:00	8	678	< 1	U	77.6	25		
NBU	8/30/10	7:00	9	702	< 1	U				
NBU	8/30/10	8:00	10	709	< 1	U				
NBU	8/30/10	9:00	11	709	< 1	U				
NBU	8/30/10	10:00	12	749	< 1	U				
NBU	8/30/10	11:00	13	749	< 1	U				
NBU	8/30/10	12:00	14	749	< 1	U				
NBU	8/30/10	13:00	15	749	< 1	U				
NBU	8/30/10	14:00	16	749	< 1	U				
NBU	8/30/10	15:00	17	749	< 1	U	94.6	2		
NBU	8/30/10	16:00	18	749	< 1	U				
NBU	8/31/10	23:00	1	754	< 1	U				
NBU	9/1/10	4:00	6	754	< 1	U				
NBU	9/1/10	6:00	8	754	< 1	U	84.6	2		
NBU	9/10/10	13:45	BF	699	< 1	U				
NBU	9/14/10	14:45	BF	749	< 1	U				
NBU	9/24/10	14:30	BF	739	< 1	U				
NBU	10/1/10	13:30	BF	732	< 1	U			95.4	10
NBU	10/8/10	13:00	BF	725	< 1	U				
NBL	8/23/10	7:00	23	764	< 1	U			95.3	5
NBL	8/25/10	14:45	BF	715	< 1	U	85.5	3	91.7	5
NBL	9/10/10	12:30	BF	699	< 1	U				
NBL	9/14/10	14:00	BF	749	< 1	U				
NBL	9/24/10	13:45	BF	739	< 1	U				

[a] Numerical sequence of sample collection during date-specific storm event (one event can include multiple triggering of ISCO sampler); NS = no samples collected, BF = baseflow grab sample.
[b] Days frozen prior to thawing and analysis.
[c] Results in µg/L (ppb).
[d] Data qualifiers:
B = estimated minimum high bias 50%; i.e., result is less than three times mean background from replicate analyses of pre-application baseflow sample (0.38 µg/L; Appendix C, Sections 2.2.1 and 2.2.2); used only when result >MDL.
J = estimated concentration (>MDL but <ICAL LCL).
U = less than estimated MDL (1 µg/L; Appendix C, Section 2.2.2).
[e] Percent recovery from MS experiments with nominal spike level (µg/L); result is mean when MSD were performed.
[f] Percent recovery of spike added immediately prior to freezing with nominal spike level (µg/L).