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PERSISTENCE OF HERBICIDE RESIDUES IN *GAMMARUS LACUSTRIS* (CRUSTACEA: AMPHIPODA) IN PRAIRIE WETLANDS

MICHAEL T. ARTS,* JOHN V. HEADLEY and KERRY M. PERU
National Hydrology Research Institute, Environmental Sciences Division, 11 Innovation Blvd., Saskatoon, Saskatchewan, S7N 3H5 Canada

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Abstract—Tandem mass spectrometry (MS) was used to detect herbicide residues in wild populations of amphipods collected from microcosms spiked with a single pulse of either Avadex®-BW (triallate) or HoeGrass® (diclofop-methyl) at three different concentrations (1.0, 10.0, and 100.0 $\mu g \cdot L^{-1}$). Independent confirmations of both herbicides were obtained by spiking amphipod tissues with 50 pg of authentic standards of triallate or diclofop-acid. The in situ microcosm experiments demonstrated that positive confirmation of triallate could still be made 30 d following an initial single spike of 10 $\mu g \cdot L^{-1}$ triallate. Diclofop-acid could still be detected in lipid-rich tissues of amphipods 10 and 15 d following an initial spike concentration of 10 and 100 $\mu g \cdot L^{-1}$, respectively. The chief advantages of tandem MS include the ability to analyze small quantities of tissue (<1 mg wet weight), the ability to detect picogram levels of target analytes, and user flexibility in the choice of tissue to be analyzed (e.g., lipid-rich storage tissue, muscle, nervous tissue, etc.).

Keywords—Persistence Amphipods Herbicides Mass spectrometry Lipids

INTRODUCTION

Accurate prediction of the potential effects that a particular contaminant may have on an aquatic ecosystem requires detailed knowledge of the fate of the compound, its chemical availability in the environment, and the relative sensitivity of target organisms [1]. The determination of environmental fate depends largely on our analytical abilities and understanding of the behavior of the contaminant in the system both chemically and in relation to the ecological dynamics of the organisms. This article is concerned with maximizing our ability to detect contaminants in aquatic biota, an early and fundamental step in risk assessment [1]. We present an application of a tandem mass spectrometry (MS/MS) technique that capitalizes on the elevated contaminant levels found in lipid-rich tissues and at the same time markedly reduces the number of organisms required for detection of pesticides in the field or laboratory. A series of microcosms stocked with amphipods and spiked with Avadex®-BW (triallate) or HoeGrass® (diclofop-methyl) were used to derive field data which demonstrate the utility of the technique for investigating the persistence of selected herbicides under real- life conditions in a eutrophic prairie pond.

Detection of pesticides in aquatic biota by gas chromatography–mass spectrometry (GC-MS) requires appreciable amounts of tissue, typically 1 to 10 g dry weight. For larger organisms (e.g., fish or clams), this poses few problems; however, for relatively small-bodied invertebrates such as amphipods, there can be a significant time factor involved in obtaining enough individuals to achieve the necessary biomass for GC-MS analysis (e.g., ~2,000 *Hyalella azteca* are required to achieve 10 g dry weight). In addition, the detection limits associated with techniques such as GC-MS imply that even if enough tissue is obtained during the course of an extensive field survey, there is always a risk of producing data sets with numerous ''no detects.'' This is especially likely in field studies where the samples come from aquatic systems exposed to environmentally realistic levels of contaminants, particularly if a significant amount of time has passed since the introduction of the contaminant.

Many of the most persistent contaminants in aquatic systems are lipophilic [2]. Thus, lipid-rich tissue can provide a matrix with a higher contaminant concentration as compared with the entire organism. For example, autoradiography has revealed that the herbicide triallate is preferentially associated with lipid-rich tissue alongside the gut, where energy-rich triacylglycerols are stored, as well as with the nervous system [3]. The incorporation of lipophilic contaminants into discrete lipid droplets within storage tissues of invertebrates likely results in far greater persistence of the precursor compared with the open environment since, within the axenic environs of a lipid droplet, these compounds are isolated from bacterial degradation. Thus, the probability of detecting lipophilic contaminants can be increased if lipid-rich tissues [4] are specifically chosen for analysis.

Amphipods were selected as the study organism because of their propensity to remain near littoral regions receiving direct runoff and because their role as detritivores likely increases their exposure to herbicides. Amphipods use lipid as an energy reserve [3] and are a ubiquitous feature of aquatic systems throughout most of North America. Amphipods are especially abundant (density ≤2,000 per m^2) in small productive pothole lakes and prairie ponds bordering on agricultural land and represent a significant portion of the diet of fish and ducklings [5]. Amphipods are widely used in bioassay studies [6].

In general, application of herbicides to control broadleaf weeds and wild oats has increased by 40 to 55% in the prairie region from 1978 to 1986 [7]. The area under cultivation, however, has increased by only ~11.5% over the same period [7,8]. In 1989, 3,295,000 kg (a.i.) of triallate was applied in the province of Saskatchewan, Canada [9]. Triallate is the common name for the herbicide with the International Union of Pure and Applied Chemistry (IUPAC) name of *S*-2,3,3-trichloroallyl diisopropyl (thiocarbamate) [10]. It is an amber oil with a molecular formula of $C_{10}H_{16}Cl_3NOS$. Triallate was introduced in Canada in 1962 by Monsanto Agricultural Co. and is currently marketed under the trade names Avadex-BW and Fortress®. Triallate is

* To whom correspondence may be addressed.

a moderately lipophilic (log K_{ow} = 4.6) preemergence herbicide recommended for control of wild oats in barley, lentils, durum wheat, spring wheat, and dry peas [11]. It is also recommended for wild-oat control on rapeseed, flax, sugarbeets, and mustard [12]. Triallate can persist from several weeks to several months in soils depending on the organic matter content [13]. Triallate entering aquatic systems may be acutely toxic to some aquatic invertebrates [10,14,15], which in turn are critical for ducks, especially during their reproductive period [16].

Diclofop-methyl is the common name for the herbicide with the IUPAC name of methyl 2-[4-(2,4-dichlorophenoxyl)phenoxy]propionic acid. Diclofop-methyl (log K_{ow} = 4.57) is used to control gramineous annual weeds such as wild oats, green foxtail, and witch grass primarily in wheat, barley, and soybeans [17]. At application rates ranging from 67 to 132 kg·km^{-2} a.i., there is strong adsorption of the herbicide to soil and low potential for volatilization [18,19]. Under field conditions, the herbicide ester, diclofop-methyl, is known to undergo hydrolysis to its corresponding acid, diclofop, 2-[4(2,4-dichlorophenoxy)-phenoxy]propionic acid [20–22]. Two other metabolites, 4-(2,4-dichloro-phenoxy)phenetole and 4-(2,4-dichlorophenoxy)phenol, have been observed at trace levels relative to the concentration of diclofop [23,24]. Diclofop-methyl has been detected in shallow groundwater, surface waters, and air samples [25]. This apparent recalcitrance has resulted in the herbicide being listed for postregistration monitoring in Canada.

We describe an application of a tandem MS procedure [26] that complements conventional analytical methods which require solvent extractions, preconcentration, or derivatization steps for the determination of herbicide residues. This tandem MS [26–28] method can be used for both detection and determination of persistence of picogram levels of herbicide residues in small quantities (< 1 mg wet weight) of amphipod tissue.

MATERIALS AND METHODS

Microcosms

The microcosms were deployed in a large pond (Gursky Pond, surface area = 0.13 km^2) located 40 km east (52°08′N, 106°07′W) of Saskatoon, Saskatchewan, Canada. The design and construction of the field microcosms was reported earlier [3]. The microcosms contained a volume of 1 m^3 and were composed of a wooden frame and polyethylene side walls. The lower edges of the polyethylene film were wrapped around wooden battens which were then fastened to galvanized steel sheets cut and bent to form a 15-cm-tall square frame around the lower margin of each microcosm. The 15-cm frame formed a ''knife-edge'' which was embedded in the pond bottom, ensuring a good seal. The microcosms were open to the atmosphere. A total of seven microcosms were deployed. Three of the microcosms were spiked with Avadex-BW (triallate) and three with HoeGrass (diclofop-methyl) at concentrations of 1, 10, and 100 μg·L^{-1} (a.i.). The remaining microcosm served as a control. We were careful to ensure that the herbicides were accurately measured and that all the herbicides were added to the microcosms with no spillover or other losses. Furthermore, the water in the microcosms was well mixed following the addition of the herbicides. We expected that triallate would disappear quickly from the water column since previous work with large (20 × 40 m) field mesocosms spiked with 240 μg·L^{-1} triallate demonstrated that triallate concentrations in the water returned to control concentrations after ~10 d (J. Désy, personal communication).

Amphipod collection, tissue preparation, and tandem mass spectrometry analysis

Amphipods (*Gammarus lacustris* Sars) were collected from Gursky Pond with a sweep net and added (200 adults) to each enclosure. Ten amphipods were removed from the microcosms at day 1, 3, 15, and 30 and stored frozen (−75°C) in the dark. Several (two to three from each microcosm) of the frozen amphipods were cut in half longititudinally with a sterile razor blade and stained with Nile Red (0.25 mg in 10 ml acetone) to reveal lipid-rich tissues. This stain has been shown to bind specifically to tissues rich in neutral lipid such as triacylglycerol [4]. A small piece of lipid-rich tissue (approx. 1 mg wet weight) was then transferred, via the needle of a hypodermic syringe, directly to shallow quartz specimen holders (Fisons Instruments, Manchester, UK, Type C 7021901). The tissue samples were dried in the quartz holders for 30 min at room temperature (approx. 23°C) prior to introduction to the ion source via the solids probe of the Autospec Q (Fisons Instruments, Manchester, UK). Heating applied to the probe was limited to radiant source heat. This was found to be optimal for the analysis of these tissues for two reasons: (1) the analytes of interest desorbed from the matrix at this temperature and (2) nonvolatile matrix constituents remained in the quartz cup, so less source cleaning was necessary. The tissue samples were directly analyzed with no sample extraction, cleanup, or preconcentration steps.

A sample always consisted of tissue from a single animal. Typically, one or two animals were analyzed from samples collected during the first 10 d in the 10- and 100-μg·L^{-1} treatments. For the 1- μg·L^{-1} treatment and for the later dates in the 10- and 100-μg·L^{-1} treatments, we analyzed two to three separate animals. We required complete agreement among all the samples within each treatment level and date before we designated that treatment cell as showing a positive detection (precursor and both product ions present), trace detection (precursor and one product ion), or no detection.

For laboratory experiments, spiked amphipod tissue (2 mg) was prepared by injecting amphipods with 50 pg of triallate or diclofop-acid. The isolated pieces of lipid tissue were spiked by adding 1 μl of herbicide solution with a hypodermic microsyringe. The spiking solution consisted of the herbicides dissolved in methanol. Authentic standards were obtained from Monsanto and Hoechst Canada for triallate and diclofop-acid, respectively. These standards were used to obtain library spectra for confirmation of herbicide identity in amphipod tissues.

Mass spectrometry experiments were conducted using a Fisons Instruments AutospecQ mass spectrometer with EBEQ geometry, equipped with a 4000–60 VAX data system (Digital Equipment Co., Maynard, MA, USA) and Opus V3.1x software. The acronym EBEQ refers to the sequential order of the two electrostatic analyzers (E), the magnet mass analyzer (B), and the quadrupole analyzer (Q) of the mass spectrometer. For the tandem MS experiments, the precursor ions were selected manually, and the ion-beam transmission was reduced to 50% using xenon as the collision gas while transmitting *m/z* 331 for perfluorokerosene. The precursor ion is defined in this case as the ion selected from the ion source for collision-induced dissociations or, more generally, as the decomposing ion in any reaction [29]. Xenon has a high center of mass and thus exhibits excellent properties for collision-induced dissociation experiments. A more detailed description of the instrument settings and procedures used are provided elsewhere [24,30].

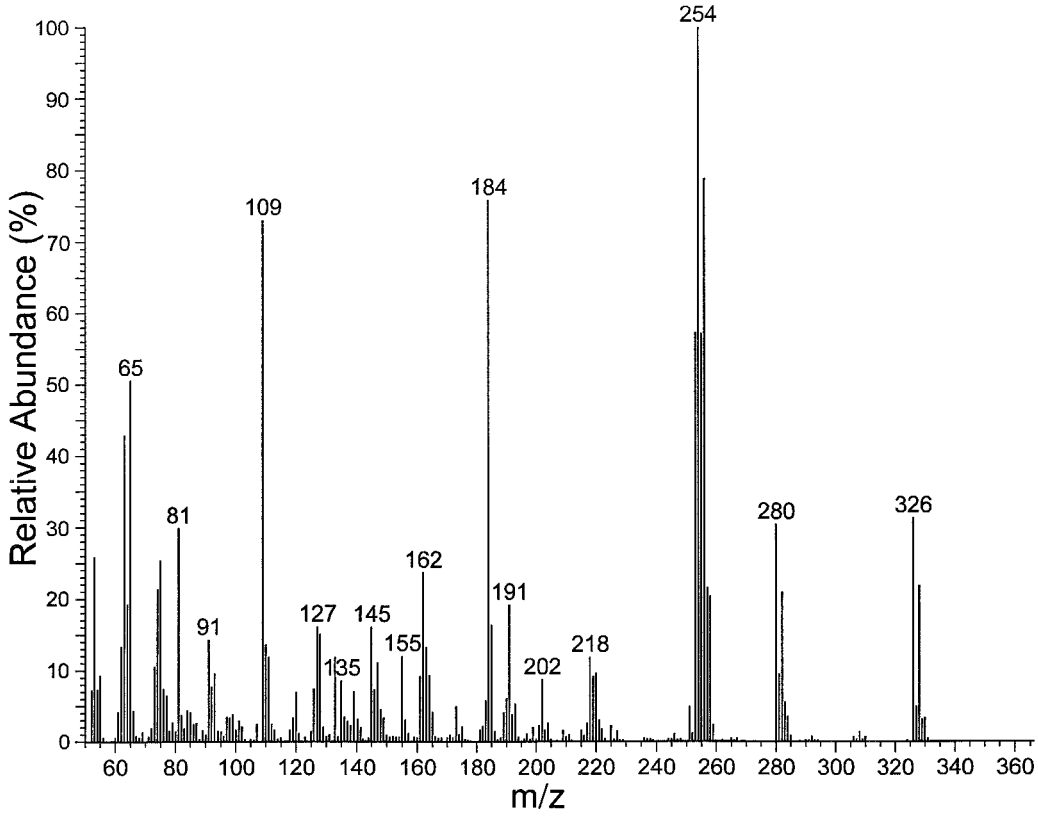

Fig. 1. Example of an electron-impact mass spectrum for an authentic standard of diclofop-acid.

Water and sediment samples

Water and sediment samples were collected for herbicide analyses during the course of the microcosm experiments. Four water samples were collected on day 0 (June 1) from the control microcosm. Subsequent water samples were collected from the experimental and control microcosms on June 6 and again on June 15. A single sample was collected on each occasion from each microcosm. Water samples were collected by immersing a 1-L amber-colored glass bottle (level 3) approx. 50 cm below the surface. The bottles were filled by moving them in a slow spiral from 50 cm to the surface in an attempt to evenly sample different microgradients that may have occurred within the microcosms. Ten milliliters of dichloromethane (DCM) was added to each bottle in order to prepartition the analytes into an organic solvent and to safeguard against losses from bacterial degradation. Water samples were stored at 4°C in the dark prior to extraction. Nominal concentrations were assumed on day 0 based on the measured amounts added to each microcosm.

Herbicides in water samples were extracted using a multiresidue method [31]. Our extraction method differed from the previously published method [31] in that (1) methylene chloride was used in the solvent exchange step, (2) extractions were carried out at pH 12.0 or 2.0 for the determination of triallate and diclofop-acid, respectively, (3) isooctane was used as a keeper during the solvent evaporation steps, and (4) no Fluorosil® (J.T. Baker, Phillipsburg, NJ, USA) was required for cleanup. Recoveries averaged 93 and 121% for triallate and diclofop-acid, respectively. The rapid (<12 h) hydrolysis of diclofop-methyl ($C_{16}H_{14}Cl_2O_4$) to diclofop-acid ($C_{15}H_{12}Cl_2O_4$) is well known [32]. Diclofop-acid was therefore chosen as the analyte for the tissue samples. For the analysis of diclofop-acid, the extracts were derivatized using diazomethane. The final extracts were analyzed using capillary GC-MS.

Surficial (0 to 20 cm) sediment was collected from a series of dispersed and haphazardly chosen locations around the margins of Gursky Pond [3]. The sediment samples were thoroughly composited by mixing in a plastic pail. Four aliquots of this composited sediment were reserved for background-level herbicide analyses (day 0), and the remainder was divided into acid-cleaned and solvent-washed glass Petri dishes which were attached by plastic strapping to the end of a 1.5-m wooden stick. Three Petri dishes containing an aliquot of the composited sediment were placed in each microcosm so that the Petri dish came to rest on the sediment surface within each microcosm. On days 15 and 30 a sediment sample was retrieved from each microcosm by slowly raising the wooden stick with the attached Petri dish to the surface. The sediments were stored frozen in the dark in precleaned (level 2) amber-colored jars.

Herbicide residues in sediments were quantified following a multiresidue extraction method [33] with some modifications as follows. For triallate, 30 g of wet sediment, to which a surrogate standard (4-terphenyl d_{14}) was added, was extracted with 120 ml acetone:water (80:20). For diclofop-acid, 30 g of wet sediment, to which a surrogate standard (2,4,6- tribromophenol) was added, was extracted with 120 ml acetone:water:sulfuric acid (80:19:1). Sediment samples were shaken for 24 h during the extraction procedure.

In each case the sediment was centrifuged and the supernatant decanted into a 1-L separator funnel. An additional 120 ml acetone/water or acetone/water/sulfuric acid was added to the sediment containing triallate or diclofop-acid, respectively. The sediments were then reextracted by shaking for 1 h. The

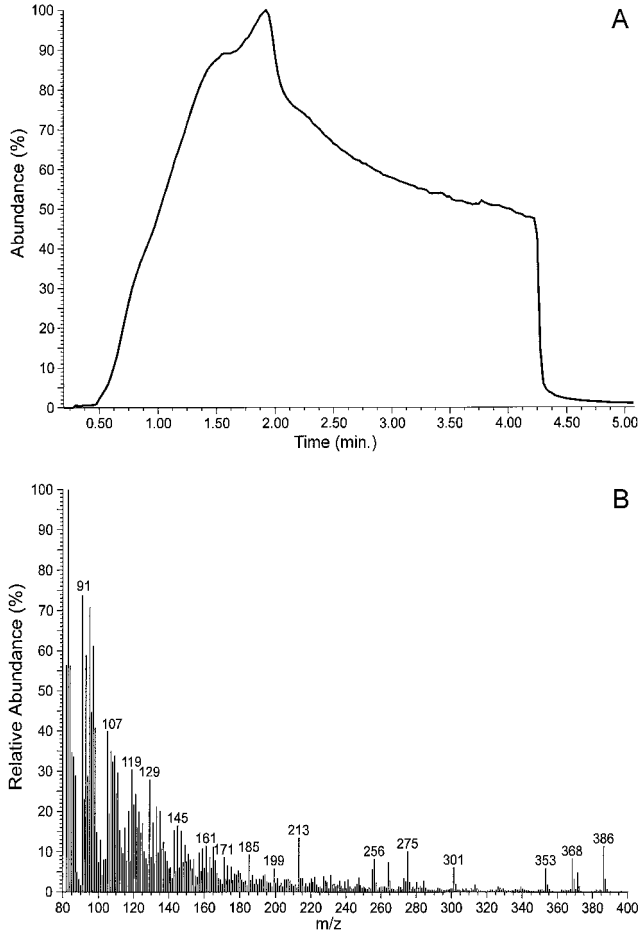

Fig. 2. (**A**) A typical mass spectrometer total ion chromatogram from an injection of amphipod tissue spiked with diclofop-acid into the mass spectrometer via the solids probe. (**B**) Full-scan mass-spectrum of amphipod tissue showing that the presence of diclofop-acid is masked by high background chemical noise caused by interfering ions from the matrix. Figure 2B represents the averaged mass spectrum obtained from 0.75 to 2.0 min in the total ion chromatogram (Fig. 2A).

sediments were then centrifuged and the supernatants pooled. Organic free water (600 ml) was added to each of the separator funnels containing the pooled extracts. The aqueous extract was then back-extracted with two 75-ml portions of DCM. The DCM extracts were combined, dried with either anhydrous sodium sulfate or acidified anhydrous sodium sulfate in the case of extracts from the triallate or diclofop treatments, respectively, and then evaporated to approx. 1 ml using a rotary evaporator. The 1-ml extracts from the diclofop treatments were transferred to test tubes and methylated using diazomethane [34]. These methylated extracts were then transferred to a 1-ml volumetric flask, and solvent exchange to DCM was performed under a gentle stream of nitrogen.

The final extracts (both triallate and diclofop-acid) were then transferred to a 1-ml volumetric flask, and an internal standard (phenanthrene d_{10}) was added. Both sets of extracts were adjusted to exactly 1.0 ml using a gentle stream of nitrogen and analyzed using capillary GC-MS.

Diclofop-methyl was used in the calibration procedures for the sediment and water analyses. Any diclofop-acid in the sediment or water samples would have been derivatized back to diclofop-methyl as part of the analysis procedure.

Detection and persistence of herbicides in amphipod tissues

The electron impact mass spectra of the authentic standards (Fig. 1) were first examined to select diagnostic ions suitable for evaluating the presence of the target analytes in the full-scan spectra of amphipod tissue. The precursor ions *m/z* 268 (triallate) and *m/z* 326 (diclofop-acid) were chosen for the subsequent tandem MS experiments. These ions were selected based on (1) their low probability of formation from fragmentation or rearrangement in the ion source, (2) their relative abundances, and (3) their occurrence at relatively high mass.

Precursors were selected from total-ion spectra and corresponding mass spectra of amphipod tissue containing each herbicide (Fig. 2A and B). For example, a full-scan MS spectrum of amphipod tissue showed that the presence of diclofop- acid is masked by high background chemical noise caused by interfering ions from the matrix. However, these precursors can be dissociated to produce product-ion spectra. These product-ion spectra served to verify that the selected ions were diagnostic for the respective analytes and were used to enhance the sensitivity of the method for identification of transformation products relative to the full-scan MS procedure. Diagnostic product ions resulting from the collision of the *m/z* 268 and 326 fragments with xenon were *m/z* 184 and 226 and *m/z* 253 and 281 for triallate and diclofop-acid, respectively.

RESULTS AND DISCUSSION

Tissue samples

Amphipod tissues spiked with authentic standards and amphipod tissues from wild specimens exposed to the test herbicides had nearly identical product-ion spectra (Fig. 3A and B), demonstrating that the precursor herbicides existed intact in the lipid-rich tissues of amphipods collected from the pond. For full assessment of the sensitivity of the procedure, a rigorous examination of the factors affecting the quantification of the analytes is required. Although development of the procedure for quantification was outside the scope of the present work, preliminary studies estimated the sensitivity of the tandem MS technique. As reported earlier for work with biofilm samples [21], good product-ion tandem MS spectra were obtained for tissue spiked with 20 pg of the herbicides. In this study, a spike of 20 pg was masked by chemical noise in the MS prescreening experiments (Fig. 2).

The identification detection limit of diclofop-acid (based on signal : noise ratio of 3:1 for *m/z* 326) was approx. 300 pg in the MS experiments compared to 20 pg (based on observation of product ions at *m/z* 281 and 253) for the tandem MS procedure. Similarly, the detection limit for triallate using tandem MS was also 20 pg. The inner ion source had to be cleaned after approx. 30 to 50 successive samples in order to maintain this level of performance.

Detection of the herbicides in the tissue was confirmed by the presence of the product ions obtained from the precursor ions *m/z* 268 (triallate) and *m/z* 326 (diclofop-acid) (Fig. 3A and B). The latter were not diagnostic in the full-scan probe-MS (Fig. 2B). These results provide confirmation of the herbicide residues in minute quantities of lipid-rich tissue obtained from individual amphipods. Typical results of the tandem MS product-ion scans for spiked tissue and field-derived amphipod tissue samples are given in Table 1. The difference in the relative abundance of the product ions observed between the spiked tissues and field samples can be attributed to matrix interference of the same nominal mass when transmitting the precursor ion.

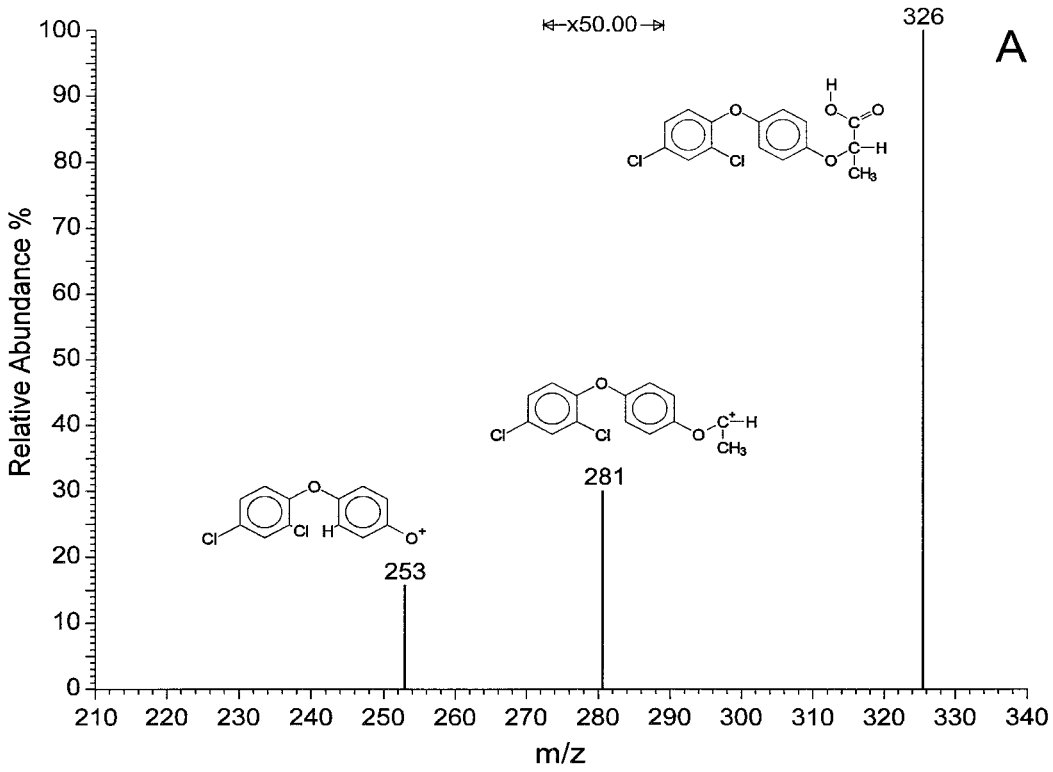

Fig. 3. Typical product ion spectra of (**A**) amphipod tissue spiked with 50 pg of authentic diclofop-acid standard.

It is expected that different samples will have different amounts of matrix interference due to the complexity of the sample being analyzed. Although this may result in apparently different product-ion abundances in relation to the precursor ion, the abundance ratios of the product ions obtained from the spiked tissue to that of the field samples are comparable, allowing for confident analyte confirmation (Table 1).

Results from the tandem MS analyses of the lipid-rich amphipod tissues from the microcosm experiments demonstrated that triallate could be detected after 30 d in the 10- and 100-

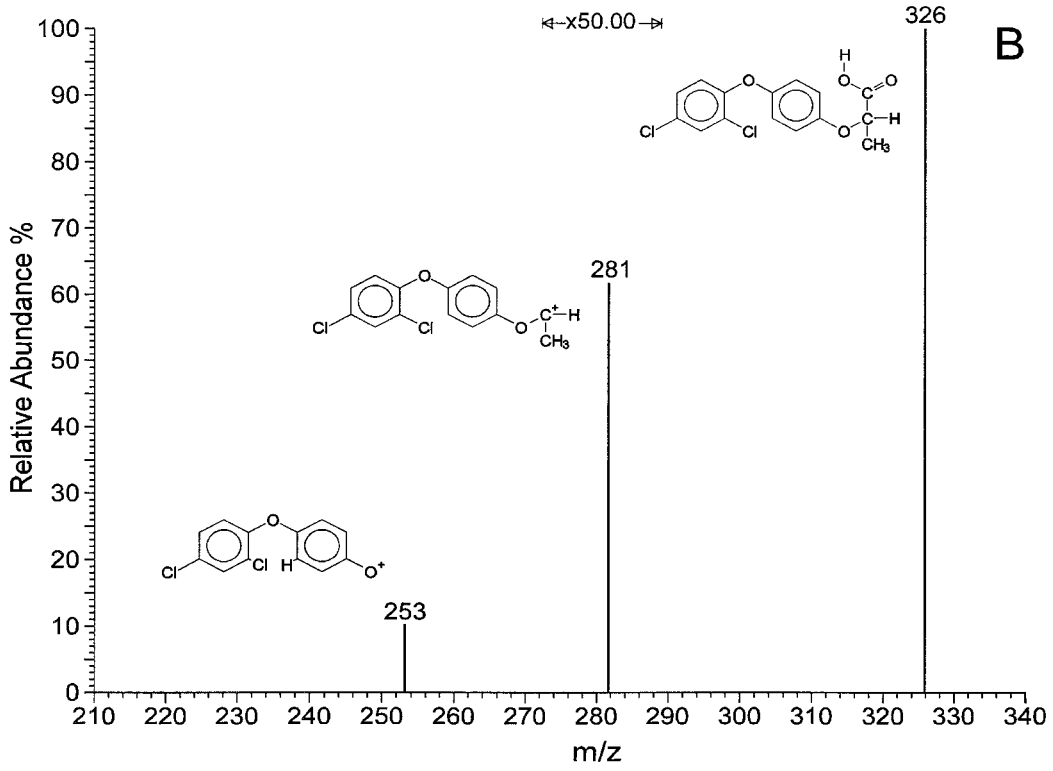

Fig. 3. Continued. (**B**) Typical product ion spectra of field amphipod tissue containing dicofop-acid.

Table 1.Relative abundance of product ions of triallate and diclofop-acid quantified by tandem mass spectrometry

	Mass to charge (*m*/*z*) ratio					
	Triallate			Diclofop-acid		
	184	226	268	253	281	326
Spiked tissue	2.5	3.8	100.0	16.0	30.9	100.0
Field tissue	5.1	10.1	100.0	30.9	61.7	100.0

Data are from representative scans from amphipod tissues spiked with 50 pg of analytical standard and from field specimens obtained from microcosms treated with 100 $\mu g \cdot L^{-1}$ of either herbicide.

$\mu g \cdot L^{-1}$ treatments and up to day 3 in the 1-$\mu g \cdot L^{-1}$ treatment but was not detected in the control microcosm (Table 2). Diclofop-acid could be detected in amphipod tissues after 3, 10, and 15 d in the 1-, 10-, and 100-$\mu g \cdot L^{-1}$ treatments, respectively, and was not detected in tissues from the control microcosm (Table 2).

Water and sediments

The concentrations of diclofop-acid and especially triallate decreased rapidly in the water column of the experimental microcosms. By day 6 triallate and diclofop-acid in the 100-$\mu g \cdot L^{-1}$ treatment had decreased to 5.5 and 62.3 $\mu g \cdot L^{-1}$, respectively (Fig. 4). The rapid disappearance of triallate from the water column of the 100- $\mu g \cdot L^{-1}$ treatment is not surprising given the strong adsorption of triallate from aqueous solution onto soil particles [35]. Although aquatic fate studies are scarce, adsorption of triallate onto particulate matter in the aquatic environment is suspected as being a major fate process [10]. There is some suggestion that herbicide concentrations may have increased slightly in some of the treatments on day 15 compared with day 6; we suspect that this may have been due to subsequent desorption from the sediments and/or the polyethylene walls of the microcosms.

Significant amounts of triallate could be seen in the sediments by day 15 in the 100-$\mu g \cdot L^{-1}$ treatment, whereas the 1- and the 10-$\mu g \cdot L^{-1}$ treatments had relatively low levels comparable to the controls (Table 3). By day 30 concentrations of triallate in the sediments of the 100-$\mu g \cdot L^{-1}$ treatment had decreased from 24.27 to 10.29 $ng \cdot g^{-1}$. This indicates that there had been either some degradation or remobilization of triallate from the sediments. Concentrations of triallate in both the 1- and 10-$\mu g \cdot L^{-1}$ treatments had increased on day 30 relative to day 15. Diclofop was also found in the sediments in the 100-$\mu g \cdot L^{-1}$ treatment on day 15, but at much lower concentration compared with triallate (Table 3), suggesting that triallate had a higher affinity for surficial pond sediment than diclofop. This is confirmed by the water concentration data, which showed the more rapid disappearance of triallate as compared with diclofop-acid in the 100- $\mu g \cdot L^{-1}$ treatment (Fig. 4). Diclofop concentrations continued to increase in the 1- and the 10-$\mu g \cdot L^{-1}$ treatments; however, unlike triallate, the concentration of diclofop in the 100- $\mu g \cdot L^{-1}$

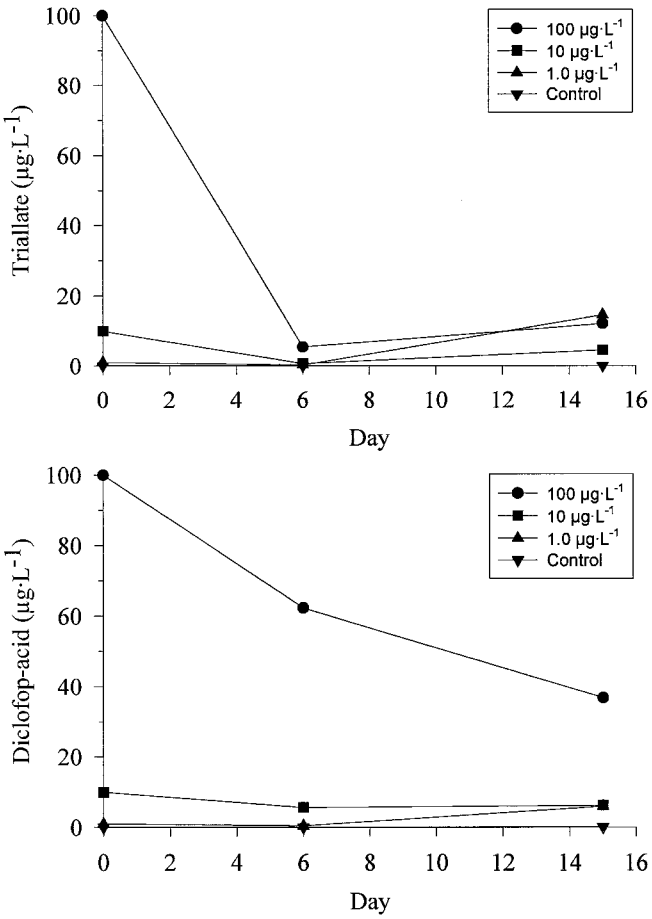

Fig. 4. Concentration of triallate (upper panel) and diclofop-acid (lower panel) in the water of the microcosms at the various treatment concentrations for a 15-d period.

Table 2.Direct-insertion-probe/tandem MS detection results for triallate precursor (*m*/*z* = 268) and product ions (*m*/*z* = 226 and 184) and for diclofop-acid precursor (*m*/*z* = 326) and product ions *m*/*z* 281 and 523) from lipid-rich amphipod tissues collected over a 30-d period from in situ microcosms

			Initial herbicide concn. ($\mu g \cdot L^{-1}$)		
Herbicide	Day	Control	1	10	100
Triallate	1	N	Y	Y	Y
	3	N	T	Y	Y
	15	N	N	Y	Y
	30	N	N	Y	Y
Diclofop-acid	1	N	Y	Y	Y
	3	N	Y	Y	Y
	6	N	N	Y	Y
	10	T	Y	Y	Y
	15	N	N	N	Y
	20	N	N	T	T
	25	N	N	T	T

N = no detection; T = trace detection, precursor and one production; Y = positive detection, precursor and both product ions.

Table 3.Concentrations ($ng \cdot g^{-1}$ dry weight) of triallate and diclofop in sediments collected from in situ microcosms in Gursky Pond treated with a single-spike of either triallate (Avadex® = BW) or diclofop-methyl (HoeGrass®)

	Nominal initial concn. ($\mu g \cdot L^{-1}$)							
	Triallate				Diclofop			
Day	C	1	10	100	C	1	10	100
15	0.59	0.76	0.85	24.27	0.19	0.48	2.03	8.92
30	0.75	1.02	5.58	10.29	0.47	1.01	2.51	11.86

C = control microcosm.

treatment increased somewhat by day 30 (Table 3). Thus, the amphipods present in these microcosms would have been exposed to both triallate and diclofop throughout the course of the experiment at levels that were higher than the controls. Note that both triallate and diclofop were detected in the sediments from the control microcosm at low concentrations. This may in part be due to spray drift from neighboring farms. Farms in this part of the Canadian prairies routinely apply both Avadex-BW and HoeGrass during this time of the year. In addition, these herbicides could enter the aquatic system via dry deposition.

General applicability

We realize that the use of a Fisons Instruments AutospecQ mass spectrometer with EBEQ geometry with a VAX 4000-60 data system to achieve the results presented here precludes universal access; however, alternate, less costly instruments based on similar principles are rapidly becoming available. Other configurations and techniques utilizing ion traps or quadrupoles for tandem MS analysis presently exist. For example, instruments with triple quadrupole geometry or ion traps may be utilized to provide similar results to those presented here for approx. one-fourth to one-third the cost of our instrument, providing the precursor ion can be adequately resolved from the chemical interference.

This technique could be useful in monitoring programs as well as contaminant fate studies. Large-scale field monitoring programs are expensive and usually yield a relatively large proportion of ''no detects.'' Tandem MS could be used to screen samples for the presence or absence of contaminants of interest prior to committing funds to more traditional quantitative techniques such as gas chromatography–electron capture detection or GC-MS. Similarly, in fate studies, tandem MS could be used to identify target organisms for further study.

The examples provided here for two herbicides reveal the sensitivity of the direct-insertion-probe tandem MS technique. The analytes triallate and diclofop-acid were chosen for this study because these compounds are environmentally relevant to the prairie ecozone. However, the technique presented here could be readily extended to other analytes of environmental consequence [24]. This is especially true for lipophilic contaminants because their tendency to accumulate in lipid-rich tissues effectively increases the sensitivity of the method by raising the analyte concentration in the tissue that is to be analyzed.

CONCLUSION

A tandem MS procedure was employed to detect residues of two herbicides in small pieces (≈1 mg wet weight) of amphipod tissue at picogram levels with no sample extraction or preconcentration steps. Field trials were conducted by stocking small (1 m^3) microcosms with amphipods (*Gammarus lacustris*) and spiking them with either Avadex-BW (triallate) or HoeGrass (diclofop-methyl). We found that triallate could be detected in amphipod tissue 30 d after a single spike of 10 $\mu g \cdot L^{-1}$ triallate and that the presence of diclofop-acid in amphipod tissues was confirmed 10 d after an initial spike of 10 $\mu g \cdot L^{-1}$ diclofop-methyl.

We suggest that the technique could be readily extended to other analytes and tissues. Future developments include the addition of a suitable internal standard for quantitative analyses and exploration of negative-ion electron capture for enhancing the sensitivity of the technique for chlorinated herbicides such as bromoxynil (Pardner®, Rhône-Poulenc, Wareham, MA, USA).

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