Environ. Sci. Technol. **1999**, *33*, 358−361

ELISA Determination of the Sulfonylurea Herbicide Metsulfuron-Methyl in Different Water Types

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Sulfonylurea herbicides efficiently control broad-leafed weeds and some grasses in cereals. They are typically applied at rates of <100 g/ha. The application of a recently developed competitive enzyme-linked immunosorbent assay (ELISA) for metsulfuron-methyl to spiked real-world water samples such as tap water, surface water from lakes and rivers, municipal wastewater, and landfill leachate is described. With pure water samples the IC_{50} value for the target analyte is 1.4 μg/L with the LOD being 40 ng/L. Determination of tap water and surface water was possible without any sample preparation such as filtration, extraction, pH adjustment, or dilution. On the contrary, with wastewater and leachate strong matrix interferences were observed, and, therefore, filtration and dilution of these samples prior to the immunochemical determination was necessary. Whereas with wastewater a 100-fold dilution was sufficient to ensure accurate herbicide quantification, even with a 500-fold dilution of leachate a positive bias of the method at lower concentrations (<1 μg/L) was observed. Determination of samples spiked with commercial metsulfuron-methyl formulation (GROPPER) showed no interference due to matrix in the immunological method.

Introduction

Sulfonylurea herbicides were first introduced in 1982 by DuPont Agricultural Products. They efficiently control broad-leafed weeds and some grasses in cereals. They are typically applied at rates of <100 g/ha, have low mammalian toxicity, and degrade to innocuous compounds after application (*1*). Because of their chemical and thermal instability, monitoring of these compounds in environmental samples has been a challenge. Normal and reversed-phase high-performance liquid chromatography (HPLC) with photoconductivity, UV or mass spectrometric detection, gas chromatography (GC) with diazomethane derivatization, and capillary electrophoresis have all been used for the analysis of these herbicides (*1*−*19*). However, only a few of these methods with detection limits in the ppt-range (*1, 18*) meet regulatory requirements and are useful for screening. In Europe, a limit value of 0.1 μg/L was set for pesticides in drinking water by the European Community. Polyclonal and monoclonal antibody-based enzyme immunoassays have been reported for chlorsulfuron (*20, 21*) and triasulfuron (*22*−*25*), with limits of detection (LOD) ranging from 20 to 500 ng/kg in soil and plant tissue. Determination of real-world water samples was described by Brady et al. (*24*) with a LOD of 50 ng/L in groundwater. Strahan reported on the determination of multiple sulfonylurea herbicides (nine compounds) on the same polyantigen coated microwell plate with an LOD of 5 μg/L in the formulated product (*26*).

Recently, we reported on the development of a competitive ELISA method for metsulfuron-methyl (*27*). Similar to previous work with triasulfuron reported by Schlaeppi et al. (*22*) the hapten was the methylester phenylsulfonamide moiety of the target analyte with a succinic acid spacer. The resulting ELISA had a LOD of 40 ng/L as determined with spiked highly purified water. In the present investigation, drinking water, surface water, wastewater, and landfill leachate were included in order to prove the utility of the ELISA for a rapid determination of the herbicide in these real-world water samples.

Experimental Section

Sampling. All water samples were collected in an agricultural area near Landsberg, a district town located in southern Bavaria. Brown glass bottles (1 L) were used to collect samples. Samples were characterized in the field with regard to coloring, clouding, odor, and pH. Surface water was collected near the banks of lakes *Ammersee* and *Wörthsee* as well as the *Lech* and *Windach* rivers. Tap water (nonchlorinated) was from two different local water works using groundwater as the source. Municipal wastewater was collected from the influent directly after the coarse screen. Leaching water was taken from a landfill leachate pipe. Samples were analyzed on the same day or stored overnight in a dark, cool place and analyzed on the following day.

Sample Preparation. Drinking and surface water samples were analyzed without any preparation. In contrast, wastewater and landfill leachate were analyzed both untreated and after filtration (glass fiber filter MN 85/90 BF; Macherey-Nagel, Düren, FRG) to remove particles >0.5 μm. Samples were run directly by the metsulfuron-methyl-ELISA (MSM-ELISA) or, in spiking experiments, after dilution with ultrapure water (Milli-RO 5 Plus, Milli Q_{185} Plus; Millipore, Eschborn, Germany). Stock solution of MSM (methyl 2-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl]aminosulfonyl]-benzoate) (Dr. Ehrenstorfer, Augsburg, FRG) was made with ethyl acetate (1 mg/10 mL) and used in the preparation of MSM-standards (ranging from 0.001 to 1000 μg/L) from undiluted (surface water) and diluted (wastewater, leachate) samples. The concentration of ethyl acetate in each standard was no greater than 1% (v/v). ELISA calibration curves were prepared in diluted water samples. These dilutions were prepared immediately before use. Stock solutions (10 mg/100 mL) of the commercial herbicide formulation GROPPER (20% MSM) from DuPont (Bad Homburg, FRG) were prepared in ethyl acetate or tap water. Standard solutions (10, 100, and 1000 μg/L) were made with tap water.

MSM-ELISA. The ELISA was run as described elsewhere (*27*). Briefly, microtiter plates (96 flat-bottom wells with high binding capacity, Greiner, Frickenhausen, FRG) were coated with the coating antigen (1-[(2-methylester)phenylsulfonyl]-monoamidosuccinic acid-bovine serum albumin conjugate) in coating buffer (sodium carbonate buffer, pH 9.6; 200 μL/well; 0.5 ng/mL). The plates were covered with adhesive plate sealers to prevent evaporation. After overnight incubation at 4 °C, the plates were washed with PBS-Tween using an

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10.1021/es9807486 CCC: $18.00
Published on Web 12/05/1998

TABLE 1. Characterization of Investigated Water Samples

sample	odor	coloring	clouding	pH
tap water 1	odorless	colorless	clear	6.8
tap water 2	odorless	colorless	clear	6.7
surface water (river *Windach*)	slight fish odor	colorless	clear	7.2
surface water (river *Lech*)	odorless	colorless	clear	7
surface water (lake *Ammersee*)	slight fish odor	colorless	slightly cloudy	7.3
surface water (lake *Wörthsee*)	slight fish odor	colorless	slightly cloudy	7.1
waste water	rotten	dark yellow	very cloudy	7.8
landfill leachate	very mouldly	dark brown	opaque	8.5

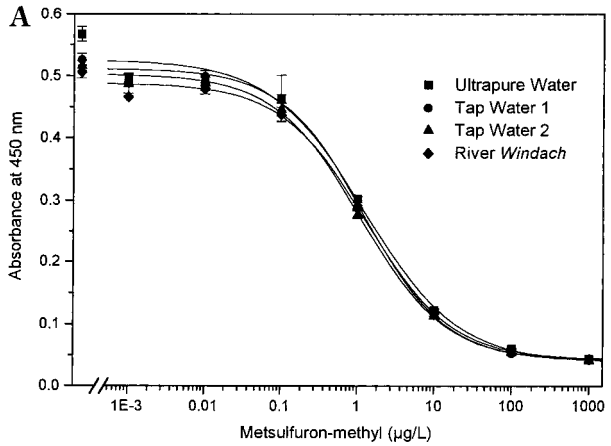
automatic plate washer (Easy Washer 812 SW1, SLT Labinstruments, Crailsheim, FRG). Binding sites not occupied by the coating antigen were blocked with blocking buffer (PBS containing 3% ovalbumin; 300 μL/well) for 1 h at 37 °C. Plates were then washed as before. Samples or standard solutions (100 μL/well) and diluted rabbit antiserum (1:200 000 in PBS; 50 μL/well) were added and incubated at room temperature with agitation for 30 min. After washing with PBS-Tween goat anti-rabbit IgG-horseradish peroxidase conjugate (Pierce, Rockford, USA) was added (1:20000 in PBS; 150 μL/well), incubated at room temperature for 1 h and washed as before. Substrate solution (tetramethylbenzidine/H_2O_2) was added (150 μL/well). Finally, the enzyme reaction was stopped with sulfuric acid (5%; 100 μL/well), and the absorbance was read at 450 nm (Easy Reader 340 ATC, SLT Labinstruments). All determinations were made at least in triplicate. The sigmoidal standard curve was set up using Rodbard's four-parameter function (*28*).

Results and Discussion

The accuracy and precision of an ELISA has to be determined by conducting standard addition experiments with samples obtained from field test locations. This should be done with samples spiked at various concentration levels. In the present investigation, complete calibration curves were run with standards prepared in different water samples for the experimental evaluation of this MSM-ELISA. Drinking water, surface water, wastewater, and landfill leachate were included in order to prove the utility of the immunoassay for a rapid determination of the herbicide in water samples.

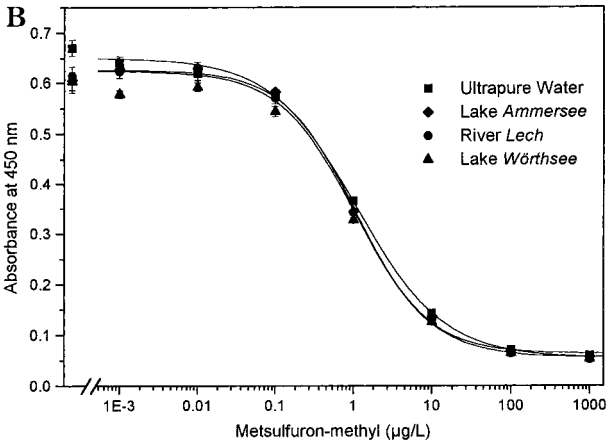
Gross Characterization of Water Samples. By personal observation the water samples showed clear differences (Table 1). Drinking water samples and water from the river *Lech* could be characterized as clear, colorless, and odorless. Surface water samples from the two lakes and the river *Windach* were slightly cloudy with slight fish odor but still colorless. In contrast, wastewater and the landfill leachate was strongly colored, was cloudy, or opaque and had a mouldly or rotten smell. While the latter samples were slightly alkaline, the others were nearly neutral with a pH-value from 6.7 to 7.3. MSM with a pK_a of 3.3 is readily soluble in all of these samples.

MSM-ELISA with Spiked Water Samples. ELISA determination of unspiked tap water, river water, and lake water gave no indication of the presence of MSM in these samples. Based on the 3-fold standard deviation (defined by IUPAC for the calculation of the LOD), the ELISA results of these samples did not differ significantly from the blank which was prepared from highly purified water. Calibration curves which were obtained with spiked samples are outlined in Figure 1. Data from one diagram were measured on a single plate. Standards prepared in ultrapure water served as the reference. Compared to the reference, a nearly identical shape of the calibration curve was found for all samples. If at all, a positive bias of the method was observed only at lower concentrations (<0.1 μg/L) of MSM. From this we concluded



Calculations from 4-parameter model:

	A	B	C	D
■	0.039	-0.728	1.63	0.524
●	0.041	-0.805	1.60	0.512
▲	0.040	-0.771	1.27	0.503
◆	0.042	-0.781	1.88	0.488



Calculations from 4-parameter model:

	A	B	C	D
■	0.055	-0.799	1.27	0.650
◆	0.059	-0.894	1.22	0.628
●	0.053	-0.876	1.20	0.625
▲	0.054	-0.871	1.16	0.597

FIGURE 1. Comparison of dose−response curves for metsulfuron-methyl calibrators obtained with spiked water samples (diagram A: ultrapure water, tap water 1, tap water 2, river *Windach*; diagram B: ultrapure water, river *Lech*, lake *Ammersee*, lake *Wörthsee*). Error bars represent ±1 standard deviation about the mean.

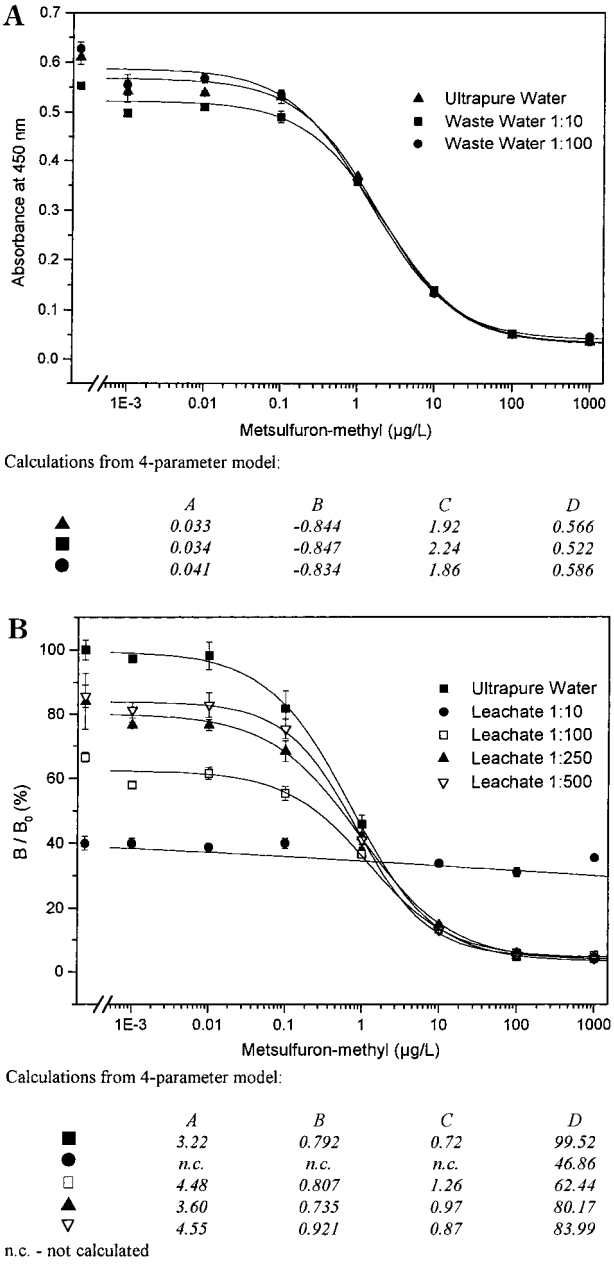
TABLE 2. ELISA Determination of Unspiked Samples from Wastewater and Landfill Leachate[a]

sample preparation	ELISA result (absorbance ± 1 s)
Municipal Waste Water	
untreated	0.373 ± 0.0033
filtered, undiluted	0.368 ± 0.0114
filtered, 1:10 dilution	0.716 ± 0.0165
filtered, 1:100 dilution	0.791 ± 0.0005
Landfill Leachate	
untreated	0.360 ± 0.0219
filtered, undiluted	0.418 ± 0.0017
filtered, 1:10 dilution	0.422 ± 0.0092
filtered, 1:100 dilution	0.478 ± 0.0013
Blank (Ultrapure Water)	
untreated	0.796 ± 0.0132

[a] $n = 3$, samples were done in triplicate.

the MSM-ELISA may be used for surface water without any sample preparation such as extraction, pH adjustment, or dilution. Further, as shown with the two lake water samples, no filtration of slightly cloudy samples is necessary. In contrast, ELISA determination of untreated wastewater and leachate was not possible as can be taken from Table 2. In this table, ELISA results were outlined as absorbances. With the exception of the 100-fold dilution of wastewater all other samples gave absorbances significantly lower than the corresponding value of ultrapure water which served as the control. This result would clearly indicate the presence of the target analyte at lower ppb-level in these samples. Although it cannot be excluded completely, an interference of matrix constituents with the immunochemical reaction is much more likely. Matrix interference is also suggested by nonlinearity of the ELISA results after serial sample dilution. With both water sources, results were nearly unchanged after preliminary filtration of samples. While the absorbance in the ELISA of the unspiked wastewater after 1:100 dilution prior to immunochemical analysis was the same as the blank (0.791 compared to 0.796), absorbance of the leachate at this dilution was still considerably lower (0.478) indicating stronger matrix interference. This was further demonstrated by the setup of ELISA calibration curves which were prepared with diluted samples. While the calibration with wastewater was run on a single plate (Figure 2A), leachate was run on two different plates. Therefore, for the latter normalized optical density was used (Figure 2B). A dilution of 1:100 was enough in order to ensure accurate determinations of the target analyte in waste water. On the contrary, a dilution of 1:500 was insufficient for diluting out matrix interferences in the leachate. At this dilution, target analyte concentrations below 1 μg/L clearly would give an overestimation of the herbicide in this sample medium. Compared to earlier ELISA results for polycyclic aromatic hydrocarbons (PAHs) in leachates, from both municipal and special waste deposits, the MSM-ELISA is much more sensitive to constituents contained in leachate samples (*29*). However, the results cannot be compared directly as the leachate samples were not from the same landfill sites in both studies. Depending on the material deposited at the landfill, leaching water can constitute a very complex matrix difficult to analyze and in no case will be identical. Minerals and dissolved organic matter (DOM), such as humic acids, will be significant in this simple matrix.

MSM-ELISA of a Commercial Herbicide Formulation. GROPPER was included in the present investigation to look for ELISA interferences due to inert ingredients of the product. For example, this could be relevant when analyzing non-targeted plants after unintentional contamination by spray-drift. In agriculture, this formulation is used after dispersion in water (25−40 g/100 L) at a rate of 100 L/ha. Table 3 shows only marginal differences when using standard reference herbicide (active ingredient only) or commercial product. No interferences were exhibited by this particular formulation. However, this may not apply to all commercial MSM-formulations, as the composition of the individual products can be different.



Calculations from 4-parameter model:

	A	*B*	*C*	*D*
▲	*0.033*	*-0.844*	*1.92*	*0.566*
■	*0.034*	*-0.847*	*2.24*	*0.522*
●	*0.041*	*-0.834*	*1.86*	*0.586*

Calculations from 4-parameter model:

	A	*B*	*C*	*D*
■	*3.22*	*0.792*	*0.72*	*99.52*
●	*n.c.*	*n.c.*	*n.c.*	*46.86*
□	*4.48*	*0.807*	*1.26*	*62.44*
▲	*3.60*	*0.735*	*0.97*	*80.17*
▽	*4.55*	*0.921*	*0.87*	*83.99*

n.c. - not calculated

FIGURE 2. Comparison of dose−response curves for metsulfuron-methyl obtained with spiked water samples (diagram A: ultrapure water, wastewater dilution 1:10, 1:100; diagram B: ultrapure water, landfill leachate dilution 1:10, 1:100, 1:250, 1:500). Error bars represent ±1 standard deviation about the mean. B/B_0 is the absorbance at 450 nm of a standard divided by the absorbance of the zero standard.

Effect of Hapten Structure on Assay Performance. In ELISA, in some cases the use of a lower affinity coating antigen or tracer compared to the target analyte can result in higher assay sensitivity (*30*, *31*). In the past, cross-reactivity (CR) studies of this MSM-ELISA revealed only about 10% CR with triasulfuron compared to MSM (*27*). Therefore, a preliminary investigation was carried out whether the use of triasulfuron derivative-BSA conjugates instead of the MSM-derivative-

TABLE 3. Determination of Metsulfuron-Methyl in Spiked Tap Water Samples Prepared from a Commercial Herbicide Formulation and Standard Reference Material (SRM)[a]

	ELISA-absorbance (±1 s)		
std concn [μg/L]	SRM (stock in ethyl acetate)	GROPPER (stock in tap water)	GROPPER (stock in ethyl acetate)
1000	0.019 ± 0.0021	0.018 ± 0.0011	0.019 ± 0.0015
100	0.024 ± 0.0017	0.022 ± 0.0011	0.027 ± 0.0016
10	0.063 ± 0.0011	0.073 ± 0.0016	0.077 ± 0.0064

[a] $n = 4$, samples were done in quadruplicate.

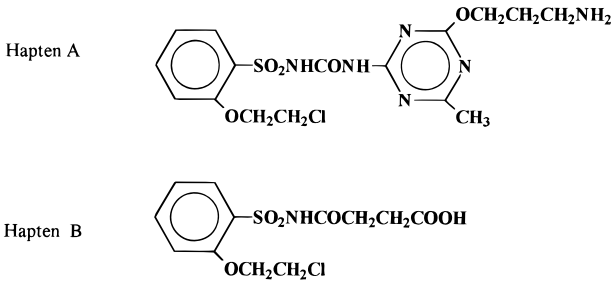


FIGURE 3. Structures of the two haptens used for preparing the bovine serum albumin conjugates (*22*).

BSA conjugate can lead to a more sensitive assay. Two different triasulfuron conjugates were used which were prepared by Schlaeppi's group at Ciba-Geigy (now Novartis, Basel, Switzerland) for the triasulfuron-ELISA (Figure 3). Hapten A corresponded to the sulfonylurea molecule with a functional aminoalkyl group attached to the triazine ring. Hapten B consisted of only the chloroethoxy sulfonamide moiety of triasulfuron with a succinic acid spacer attached to the sulfonamide. Surprisingly, absolutely no binding of MSM-antibodies to both coating antigens was observed which indicates very low, if any, binding affinity. Recommendations for future work in optimizing the sensitivity of the MSM-ELISA may include the use of coating antigens with haptens of higher structural homology to MSM.

Acknowledgments

We are very grateful to Dr. Werner Föry (Novartis, Basel, Switzerland) for providing us with triasulfuron-BSA conjugates. The authors sincerely thank Dr. Jeanette Van Emon of U.S. Environmental Protection Agency Las Vegas for careful review of the manuscript and helpful suggestions. Matthias Schedl is acknowledged for his help in the preparation of the figures. This work was supported by the German Research Council under contract Kn 348/1-2.

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Received for review July 22, 1998. Revised manuscript received October 16, 1998. Accepted October 27, 1998.

ES9807486