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Determination of Triasulfuron in Soil by Monoclonal Antibody-Based Enzyme Immunoassay

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A panel of monoclonal antibodies (MAbs) specific to triasulfuron was obtained by using two different hapten-protein conjugates as immunogens. MAbs generated with a simple hapten corresponding only to the chloroethoxy sulfonamide moiety of triasulfuron with an additional succinic acid spacer showed much higher affinity for triasulfuron as compared to those obtained with a hapten consisting of the complete molecule with an aminoalkyl spacer attached to the triazine ring. The cross-reactivity of the MAbs was limited to only a few structurally related compounds as determined by competitive ELISA. Most of the MAbs cross-reacted with the phenylhydroxylated degradation products of triasulfuron, whereas a few others cross-reacted with cinosulfuron and a fluoroethylthio analogue of triasulfuron. The competitive enzyme immunoassay based on these MAbs detected triasulfuron in aqueous media ranging from 0.01 to 1 μg/L. The ELISA was tested on fortified soil samples. The extraction procedure was optimized to reduce the soil matrix effects to a minimal level, allowing the detection of triasulfuron down to 0.1 μg/kg of soil.

INTRODUCTION

Triasulfuron, the active ingredient of Logran, is a very potent weed killer of the class of sulfonylureas (Amrein and Gerber, 1985). These herbicides are usually used at very low application rates (10-40 g/ha) (Beyer et al., 1987), and the expected residue concentrations in soils are in the low parts per billion range. In some cases, the residual activity of sulfonylureas may be injurious for highly sensitive broad-leafed rotational crops (Peterson and Arnold, 1985; James et al., 1988). Therefore, monitoring the degradation of sulfonylureas in soil required the development of highly sensitive analytical methods. Current methodologies include HPLC combined with photoconductivity or UV detection (Zahnow, 1982; Iwanzik and Egli, 1989; Bussmann et al., 1990), gas chromatography electron capture detection (Ahmad and Crawford, 1990), immunoassay (Kelley et al., 1985), and bioassays (Hsiao and Smith, 1983; Iwanzik et al., 1988; Günther et al., 1989; Sunderland et al., 1991). Standard analytical methods such as HPLC and GLC require cumbersome cleanup procedures to reach the detection limit of 0.1 μg/kg of soil generally reached by bioassays. On the other hand, bioassays are sensitive but not specific. Therefore, immunoassays represent an alternative approach to residue analysis by standard methods. They provide rapid, sensitive, and cost-effective analyses (Hammock and Mumma, 1980; Vanderlaan et al., 1988; Van Emon et al., 1989; Van Vunakis, 1990; Hammock et al., 1990). A competitive ELISA based on a polyclonal antibody was developed for chlorsulfuron using a diazonium derivative covalently linked to a protein as immunogen (Kelley et al., 1985). A sensitivity of 0.4 ppb was reported for this assay, which was eventually improved to 0.05 ppb (Sharp et al., 1989). The aim of the present study was to develop a monoclonal antibody (MAb) based immunoassay specific for triasulfuron. The minimal requirements of the assay were a detection limit comparable to current methodologies and an absence of cross-reactivity with inactive degradation products of triasulfuron in soils. We designed two haptens to prepare protein-conjugates necessary for immunization. The first hapten corresponded to the sulfonylurea molecule with a functional aminoalkyl group attached to the triazine ring. The second hapten consisted of only the chloroethoxy sulfonamide moiety of triasulfuron with a succinic acid spacer attached to the sulfonamide. Interestingly, this simple hapten appeared to be more suitable to ensure the generation of MAbs with high affinity for triasulfuron, without loss of specificity. The cross-reactivity with other sulfonylureas remained limited. One group of MAbs showed no cross-reactivity with the major triasulfuron degradation products. The experiments performed with fortified soil samples indicate that our MAb-based competitive ELISA represents a valuable detection method for sub parts per billion amounts of triasulfuron in soil.

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EXPERIMENTAL PROCEDURES

Materials. Triasulfuron, 3-(6-methoxy-4-methyl-1,3,5-triazin-2-yl)-1-[2-(2-chloroethoxy)phenylsulfonyl]urea, and the analogues were synthesized in the laboratories of Ciba-Geigy Ltd. (Basle, Switzerland). The compound 3-amino-1-propanol was purchased from Aldrich Chemical Co. All of the other reagents and cell culture media were obtained as described previously (Schlaeppi et al., 1989).

Hapten Synthesis. As shown in Figure 1, two different haptens (A and B) were designed to covalently link triasulfuron to the carrier proteins. The synthesis of hapten A (CGA 222335) was carried out in the following two steps.

6-(N-Benzyloxycarbonyl-3-aminopropoxy)-4-methyl-2-amino-1,3,5-triazine (**1**). Triethylamine (1.85 g) in 20 mL of acetone was added within 1 min to a suspension containing 2-amino-4-chloro-6-methyl-*s*-triazine (4.12 g), potassium carbonate (3.9 g), and *N*-(benzyloxycarbonyl)-3-aminopropanol (6.3 g) (Schneider et al., 1974) in 80 mL of acetone. The reaction mixture was stirred at 40-45 °C for 15 h. The acetone was evaporated, and the residual product was redissolved in acetic acid ethyl ester. The solution was washed twice with water and dried over Na_2SO_4. The solvent was almost completely evaporated, and the precipitate was filtered, washed, and dried, resulting in white crystals of compound 1 (mp 126-128 °C). The yield of 1 was 3.1 g (34.2%).

3-[6-(3-Amino-n-propoxy)-4-methyl-1,3,5-triazin-2-yl]-1-[2-(2-chloroethoxy)phenylsulfonyl]urea (**2**) (*CGA 222335*).

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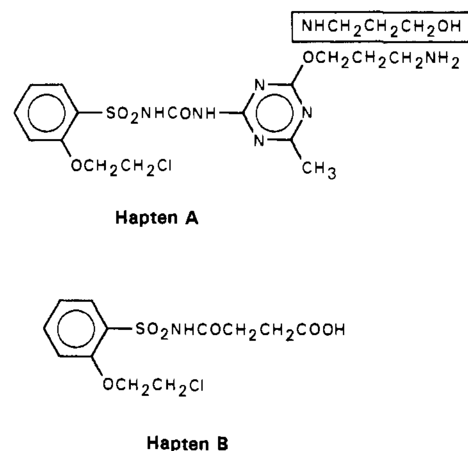

Figure 1. Structures of the two haptens used for preparing the protein conjugates. The correct structure of the spacer group of hapten A, as determined by high-resolution NMR, is shown in the insert.

Compound 1 (3.35 g) and 2-(2-chloroethoxy)phenylsulfonyl isocyanate (2.75 g) were stirred in 20 mL of dioxane for 5 h at 80–85 °C. The solvent was evaporated, and the residual product was cleaned on a silica gel column. The eluent was toluene/acetic acid ethyl ester (1:2 v/v). The eluate was directly hydrogenated in 200 mL of water and Na_2CO_3 (1.83 g) over Pd/C 5% (0.5 g). The catalyst was removed by filtration, and the colorless solution was acidified with 2 M HCl to yield pH 5.5–6.0. The resulting precipitate was filtered and dried. The yield of **2** was 2.6 g (mp 146–147 °C). The structure of compound **2** was confirmed by elemental analysis, 60-MHz ^1H NMR, and mass spectrometry. The electron impact mass spectrum showed no molecular ion at *m/e* 444 (0, M^+). Fragments were shown at *m/e* 261 [5, $M^+ - 183$, $H_2NC_3N_3(CH_3)NHCH_2CH_2OH$], 235 [12, $M^+ - 209$, $OCNC_3N_3(CH_3)NHCH_2CH_2OH$], 173 (20, 235 − 62, CH_2CHCl), and 156 (100, 235 − 79, OCH_2CH_2Cl). The positive chemical ionization mode showed no molecular ion. Fragments were shown at *m/e* 236 (25, $M^+ - 208$), 219 [75, $M^+ - 225$, $HNCOC_3N_3(CH_3)NH(CH_2)_3OH$], and 184 (100, $M^+ - 260$, C_6H_5-SO_2NHCO). The negative chemical ionization mass spectrum showed a weak deprotonated molecular ion at *m/e* 443 (1, $M^+ - H$) and fragments at *m/e* 303 (100, $M^+ - 141$, $C_6H_{11}N_3O$), 277 [22, $M^+ - 167$, $C_3N_3(CH_3)NH(CH_2)_3OH$], 234 [28, $M^+ - 210$, $CONHC_3N_3(CH_3)NH(CH_2)_3OH$], and 225 (28, $M^+ - 219$ $ClCH_2$-$CH_2OC_6H_4SO_2$). During the course of the study, the structure of hapten A was further investigated by high-resolution ^1H NMR. The NMR spectra showed all of the expected protons of the sulfonylurea structure. The triplet signal at 8.0 ppm (t_b, 1 H, NH) showed typically the triazinyl NH proton, and the triplet at 4.5 ppm (t, 1 H, OH alkyl) was attributed to the alkylhydroxy group. These unexpected findings indicated a free hydroxymethyl group and an amino group attached to the triazine, whereas the expected reaction product with a free primary alkylamino group at the end of the spacer should have shown a signal in the range of the aliphatic amino groups (0–4.0 ppm) equivalent to two protons. The exchange experiments in DMSO showed two protons, one at 8.0 ppm and one at 4.5 ppm, confirming that a rearrangement of the spacer took place during the catalytic cleavage of the amino protective group. It follows that hapten A has a hydroxy group at the end of the alkyl spacer and the correct structure corresponds to 3-[6-[(3-hydroxypropyl)amino]-4-methyl-1,3,5-triazin-2-yl]-1-[2-(2-chloroethoxy)-phenylsulfonyl]urea (Figure 1).

The synthesis of hapten B (CGA 246131), 1-[2-(2-chloroethoxy)phenylsulfonyl]monoamidosuccinic acid, was carried out by dissolving 2-(2-chloroethoxy)phenylsulfonamide (14.1 g) together with succinic anhydride (6.0 g) in 150 mL of dioxane. Then, 1,8-diazobicyclo[5.4.0]undec-7-ene (18.3 g in 60 mL of dioxane) was added drop by drop for 30 min under slight cooling conditions (22 °C). The resulting white suspension was further stirred for 2 h at 22 °C. The mixture was acidified with 2 M HCl and evaporated to dryness. The residual oily product was redissolved in acetic acid ethyl ester and washed twice with water. The solution was then dried over Na_2SO_4, and the solvent was evaporated. The residual product was recrystallized from ethanol, giving 7.1 g (35.3%) of white crystals of hapten B (mp 166–168 °C). The structure of hapten B was confirmed by mass spectrometry. The electron impact mass spectrum showed no molecular ion at *m/e* 335 (0, M^+). Fragments were found at *m/e* 317 (2, $M^+ - 18$, H_2O), 235 (15, $M^+ - 100$, $COCHCH_2COOH$), 174 (90, $M^+ - 161$), 172 (86, 235 − 63, CH_2CH_2Cl), and 156 (100, 235 − 79, OCH_2CH_2Cl). The positive chemical ionization mass spectrum with methane as reagent gas showed the protonated molecular ion *m/e* 336 (30, $M^+ + H$) and fragments at *m/e* 318 (10, $M^+ + H - 18$, H_2O) and 236 (100, $M^+ + H - 100$, $COCHCH_2$-$COOH$). The negative chemical ionization mass spectrum showed the deprotonated molecular ion *m/e* 334 (33, $M^+ - H$) and fragments at *m/e* 272 (10, $M^+ - 63$, CH_2CH_2Cl), 270 (17, $M^+ - 65$), and 234 (20, $M^+ - 101$, $COCH_2CH_2COOH$).

Preparation of the Hapten–Protein Conjugates. Hapten A was covalently linked to bovine serum albumin (BSA) or keyhole limpet hemocyanin (KLH) by the diazonium method described previously by Kelley et al. (1985) for the preparation of chlorsulfuron–protein conjugates. Hapten A (100 mg) was suspended in a mixture of acetic acid (10 mL) and propionic acid (5 mL). The mixture was cooled to 0 °C, and concentrated HCl (0.2 mL) was added together with sodium nitrite (0.05 g). The temperature of the reaction was maintained at 0 °C for 30 min. The yellow solution was divided into two portions of 7.5 mL each. They were added slowly to KLH and BSA, respectively (0.1 g in 20 mL of 0.05 M borate buffer, pH 9.0). The pH of the solution was maintained at 9.0 by adding 1 M NaOH. The final product was extensively dialyzed against phosphate-buffered saline (PBS) (0.01 M sodium phosphate and 0.145 M NaCl, pH 7.0), before using it for immunization.

The BSA and KLH-conjugated hapten B was prepared according to the active ester method (Schlaeppi et al., 1989). Briefly, hapten B (7.2 mg dissolved in 200 μL of DMF) was reacted with a 4 M excess of *N*-hydroxysuccinimide (9.1 mg/200 μL of DMF) and *N,N'*-dicyclohexylcarbodiimide (16 mg/200 μL of DMF). The reaction mixture was stirred for 1 h at 22 °C and then for 18 h at 4 °C. The active ester was then added to BSA or KLH (12 mg in 3.3 mL of PBS) and incubated for 4 h at 4 °C. After extensive dialysis, the protein concentration was determined according to the Lowry assay (Lowry et al., 1951). The extent of coupling of hapten B to BSA was determined by UV absorbance at 280 nm, which corresponded approximately to the peaks of both BSA and hapten B. By assuming additive absorbance values, we determined that the molar ratio of hapten to BSA was 12/1.

Immunization and Fusion Protocol. Following previous practice (Schlaeppi et al., 1989), BALB/c female mice (4–6 weeks old) were given a series of three injections every 2 weeks with KLH conjugated to either hapten A or hapten B and mixed with Freund's adjuvant (80 and 60 μg/injection, respectively). One week after the last injection, serum titers were determined by ELISA using the corresponding BSA-conjugated hapten as coating antigen. After a rest period, the mice were boosted intraperitoneally with either 830 (KLH–hapten A) or 540 μg (KLH–hapten B) in PBS (200 μL). Three to four days later, the mouse spleen cells were fused with the murine myeloma cell line PAI (Stocker et al., 1982) as described previously (Schlaeppi et al., 1989). After 2–4 weeks, growing hybridoma cultures were analyzed by indirect ELISA for MAbs binding to the homologous hapten conjugated to BSA (same hapten used for immunization). Positive hybridoma cultures were then tested by competitive ELISA, and only those showing more than 50% inhibition by 0.1 μg/mL triasulfuron were selected for further cloning by limited dilutions. Pure MAbs were prepared from mouse ascitic fluid (Schlaeppi et al., 1989).

Competitive ELISA for Triasulfuron Determination. The competitive indirect ELISA was performed as previously described (Schlaeppi et al., 1989, 1991). The 96-well microtiter plates were coated with, respectively, BSA–hapten A (MAb 4134-40-1) or BSA–hapten B (MAb 4149-1-1 and MAb 4147-19-4), at 2 μg/mL (100 μL/well) in 50 mM sodium carbonate buffer, pH 9.6. After overnight incubation, the plates were washed with PBS supplemented with 0.1% Tween 20 and blocked with PBS supplemented with 1% BSA. In separate test tubes, 50 μL of

purified MAbs (0.1–0.2 μg/mL) was incubated with 950 μL of standard solutions containing increasing amounts of triasulfuron solutions or with the diluted soil extracts (both in 0.1% PBS–Tween). After a 1-h incubation at 22 °C, 200 μL of the antibody–antigen mixture was transferred to each well and incubated for an additional hour. After washing, 100 μL of goat anti-mouse antibody conjugated to alkaline phosphatase (diluted 1:1500) was added to each well and incubated for 1.5 h. After washing, 150 μL of the substrate p-nitrophenyl phosphate (1 mg/mL) in diethanolamine buffer (1 M, pH 9.8, supplemented with 0.5 mM $MgCl_2·6H_2O$) was added to each well. The change of color was measured after 2 h at 22 °C. Controls, including samples without antibody and samples with antibodies and excess of triasulfuron (1 μg/mL), gave absorbance values below 0.01. All samples were done in triplicate. The concentration of triasulfuron or analogues reducing the ELISA signal to 50% of the control (I_{50}) was calculated as described previously (Schlaeppi et al., 1989).

Soil Samples Analysis. Standard soil samples from various locations were extracted according to three procedures developed previously for HPLC analysis.

Procedure A (Iwanzik and Egli, 1989). The soil samples (100 g) were extracted for 2 h with 300 mL of a 2:1 (v/v) methanol and aqueous phosphate buffer mixture (pH 7, total phosphate concentration of 0.07 M). After filtration and acidification with phosphoric acid, the samples were reextracted three times with 75 mL of CH_2Cl_2 and evaporated. The residual soil extracts were dissolved in 10 mL of phosphate buffer and filtered.

Procedure B. The extraction described above was repeated with two standard soil samples. However, the final organic phase was further cleaned up by ion-pair partition into aqueous hydrogen carbonate solution (5%) (Iwanzik and Egli, 1989). After addition of tetrabutylammonium hydrogen carbonate, the samples were reextracted with dichloromethane/n-hexane (80:20). The organic phase was evaporated, and the residue was dissolved in 10 mL of phosphate buffer.

Procedure C. The ion-partition step with tetrabutylammonium hydroxide was performed directly on a 30-mL aliquot (corresponding to 10 g of soil) of the MeOH/aqueous phosphate extract described in procedure A. After the addition of 3% (v/v) tetrabutylammonium hydroxide (pH 7–8), the aqueous phase was transferred onto a liquid–liquid partitioning cartridge (ChemElut, Analytichem International) and washed with 30 mL of n-hexane. The sample was eluted with dichloromethane/n-hexane (60:40). The organic phase was evaporated, and the residue was dissolved in 1 mL of phosphate buffer.

Prior to the ELISA determination, the soil extracts were further diluted 1:20 (MAb 4147-19-4) and 1:40 (MAb 4149-1-1) in 0.1% PBS–Tween (giving 0.5 and 0.25 g of soil/mL of PBS–Tween, respectively).

RESULTS AND DISCUSSION

Preparation and Characterization of the MAbs. The first hapten (hapten A) was originally designed to ensure the generation of MAbs capable of distinguishing triasulfuron from other sulfonylureas. By substituting the methoxy group on the triazine ring by a functional amino-n-propoxy group (Figure 1), we assumed that the chloroethoxy substituent specific to triasulfuron would remain free to interact with the antibodies, allowing maximum specificity. Indeed, it was shown previously by Kelley et al. (1985) that the diazonium derivative of chlorsulfuron used for immunization generated antibodies recognizing principally the heterocycle and the bridge structures common to several other sulfonylureas. The resulting immunoassay was class rather than compound specific. Of five fusions using the KLH–hapten A conjugate as immunogen, we obtained 22 hybridomas (6% of total hybridoma cultures) binding to the solid-phase antigen by indirect ELISA. However, only one MAb (MAb 4134-40-1), an IgG_1, bound triasulfuron competitively (I_{50} of 7.2 ng/mL) (Figure 2). The MAb showed no cross-reactivity with most of the other sulfonylureas or triasulfuron degradation products (Table I). However, the competitive ELISA based on MAb 4134-40-1 was not sensitive enough to measure triasulfuron at low parts per billion levels. The failure to obtain other MAbs binding triasulfuron competitively was very likely due to the nonspecific conjugation of the hapten to the protein as a result of the rearrangement of the spacer group (Figure 1). This unexpected rearrangement was recognized only by high-resolution NMR at a later stage of the study and may explain our failure to obtain appropriate protein–hapten A conjugates using other coupling reagents such as glutaraldehyde, the heterobifunctional cross-linker SPDP, or water-soluble carbodiimide.

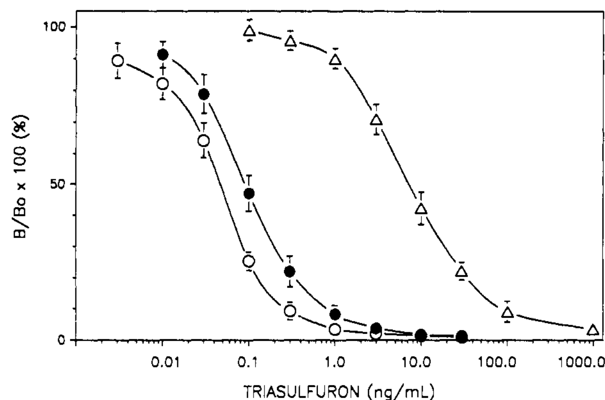

Figure 2. Inhibition of binding of MAb 4149-1-1 (○), MAb 4147-19-4 (●), and MAb 4134-40-1 (△) by triasulfuron in the competitive ELISA. The coating antigens were BSA–hapten A for MAb 4134-40-1 and BSA–hapten B for MAb 4149-1-1 and MAb 4147-19-4. $B/B_0 \times 100$ (%) represents the percentage of MAb bound to the plate. Vertical bars represent the standard deviations between assays.

To overcome these problems, we considered the possibility of using the chloroethoxy phenylsulfonamide carboxy moiety instead of the whole triasulfuron molecule to obtain antibodies with high affinity. A small spacer with a functional carboxyl group was added to covalently link the derivative to the carrier proteins (Figure 1). Of five fusion experiments using the KLH–hapten B conjugate as immunogen, we obtained 86 hybridomas (24% of total hybridoma cultures) binding to the solid-phase antigen by indirect ELISA, from which 19 bound triasulfuron by competitive ELISA. The MAbs were of the IgG_1 subclass, except one IgG_{2b}. On the basis of their overall cross-reactivity pattern, the 19 hybridomas could be divided into two groups, one with 2 and the other with the remaining 17 MAbs. One MAb of each group was selected for further detailed study (MAb 4147-19-4 and MAb 4149-1-1, respectively). Both were IgG_1 MAbs. As shown in Table I, both MAbs showed no cross-reactivity with two major breakdown products of triasulfuron, namely compounds C and D. On the other hand, unlike MAb 4147-19-4, MAb 4149-1-1 cross-reacted with two phenylhydroxylated degradation products of triasulfuron, namely compounds A and B. MAb 4147-19-4 cross-reacted with cinosulfuron and a fluoroethylthio analogue of triasulfuron (CGA 168 987), whereas MAb 4149-1-1 showed only 2.2% and 0.3% cross-reactivity, respectively. Both MAbs showed less than 1% cross-reactivity with nine other sulfonylurea herbicides, including chlorsulfuron, bensulfuron-methyl, metsulfuron-methyl, thiameturon-methyl, tribenuron-methyl, and sulfometuron-methyl. The I_{50} values for triasulfuron determined by competitive ELISA were 0.09 and 0.05 ng/mL for MAb 4147-19-4 and MAb 4149-1-1, respectively (Table I; Figure 2). The minimum detectable amounts defined as the concentration required to produce a decrease in the percent of MAb bound equal to 2 times

Table I. Cross-Reactivity of Various Triasulfuron Analogues with MAb 4134-40-1, MAb 4147-19-4, and MAb 4149-1-1

	MAb 4134-40-1		MAb 4147-19-4		MAb 4149-1-1		Structures			
	(a) I_{50}	(b) cross reactivity	(a) I_{50}	(b) cross reactivity	(a) I_{50}	(b) cross reactivity				
compound	(ng/mL)	(%)	(ng/mL)	(%)	(ng/mL)	(%)	R1	R2	R3	R4
Triasulfuron	7.2	100	0.09	100.0	0.05	100.0	$-OCH_2CH_2Cl$	-H	$-OCH_3$	$-CH_3$
Metsulfuron-methyl	>1000	<0.7	81.5	0.1	600	<0.01	$-COOCH_3$	-H	$-OCH_3$	$-CH_3$
Chlorsulfuron	>1000	<0.7	14.3	0.6	420	0.01	-Cl	-H	$-OCH_3$	$-CH_3$
Tribenuron-methyl	>1000	<0.7	>1000	<0.01	>1000	<0.01	$-COOCH_3$	$-CH_3$	$-OCH_3$	$-CH_3$
Cinosulfuron	>1000	<0.7	0.06	150.0	2.3	2.2	$-OCH_2CH_2OCH_3$	-H	$-OCH_3$	$-OCH_3$
CGA 168 987	30	24.0	0.13	69.2	16.2	0.3	$-SCH_2CH_2F$	-H	$-OCH_3$	$-CH_3$
Sulfometuron-methyl	>1000	<0.7	>1000	<0.01	>1000	<0.01	$-COOCH_3$	-H	$-CH_3$	$-CH_3$
Primisulfuron	>1000	<0.7	>1000	<0.01	>1000	<0.01	$-COOCH_3$	-H	$-OCHF_2$	$-OCHF_2$
Nicosulfuron	>1000	<0.7	>1000	<0.01	>1000	<0.01	$-CON(CH_3)_2$	-H	$-OCH_3$	$-OCH_3$
DPX 9636	>1000	<0.7	>1000	<0.01	>1000	<0.01	$-SO_2CH_2CH_3$	-H	$-OCH_3$	$-OCH_3$
Thiameturon-methyl	>1000	<0.7	14.2	0.6	>1000	<0.01				
Bensulfuron-methyl	>1000	<0.7	>1000	<0.01	>1000	<0.01				
							R1	R2		
A	>1000	<0.7	85	0.1	0.04	125.0	-H	-OH		
B	550	1.3	21.4	0.4	0.16	31.3	-OH	-H		
							R1	R2	R3	
C	>1000	<0.7	>1000	<0.01	>1000	<0.01	-OH	-Cl	-H	
D	>1000	<0.7	>1000	<0.01	1000	<0.01	-H	-Cl	-H	
E	>1000	<0.7	>1000	<0.01	>1000	<0.01	-H	-OH	-H	
F	6.0	120	2.6	3.5	0.02	250.0	-H	-Cl	$-CONH_2$	
G	2.1	342.9	1.9	4.7	0.02	250.0	-H	-Cl	$-CONHCH_2CH_2CH_3$	
H	0.9	800.0	17.6	0.5	0.01	500.0	-H	-Cl	$-CON(CH_2CH_3)_2$	
I	>1000	<0.7	>1000	<0.01	>1000	<0.01				

[a] Inhibitor concentration for 50% inhibition in the competitive ELISA. [b] Cross-reactivity defined as (triasulfuron concentration for 50% inhibition/triasulfuron analogue concentration for 50% inhibition) × 100.

the standard deviation of the blank response were 10 and 5 pg/mL, respectively (Figure 2). With MAb 4147-19-4, the working detection range of the assay in aqueous media was from 0.01 to 1 ng/mL.

Table II. Percentage of Added Triasulfuron Detected in Fortified Soil Extracts[a]

soil	soil composition					triasulfuron added, ppb	triasulfuron recovered			
							MAb 4147-19-4		MAb 4149-1-1	
	% humus	% sand	% silt	% clay	pH		ppb[b]	%[c]	ppb[b]	%[c]
Vetroz (Switzerland)	9.3	18.1	60.4	21.5	7.3	0.1	0.26	90	0.23	60
						0.3	0.44	90	0.47	100
						1	1.11	94	1.10	93
Stein (Switzerland)	5.0	43.0	17.4	34.6	7.1	0.1	0.13	70	0.20	130
						0.3	0.39	110	0.39	107
						1	1.14	108	1.04	97
Collombey (Switzerland)	1.4	83.9	13.6	2.5	7.4	1	1.08	99	1.04	98
Les Evouettes (Switzerland)	2.6	25.7	64.0	10.3	6.2	1	0.91	81	1.00	89
Speyer (Germany)	1.0	93.0	3.1	2.9	7.4	1	1.03	99	0.95	88

[a] Soil extracts were prepared by procedure A (see Experimental Procedures). [b] Calculated according to standards made of triasulfuron in PBS–Tween (average of five determinations). [c] Percentage defined as [(ppb measured after addition − ppb before addition)/ppb added] × 100.

The cross-reactivity analysis revealed a few interesting findings. The absence of cross-reactivity of MAb 4149-1-1 with the phenylsulfonamide moiety of triasulfuron (compound D) indicated that the urea function of the bridge structure was a prerequisite for the binding of the MAb and was confirmed by the strong cross-reactivity with the phenylsulfonylurea structure (compound F). Moreover, the even higher affinity of MAb 4149-1-1 for compound H suggested that the aliphatic chain of this analogue interacted with the antibody combining site, very likely replacing the contribution of the spacer group of the hapten. Minor modifications of the chloroethoxy group considerably reduced the affinity of MAb 4149-1-1. We concluded that this group indeed interacted with the MAb. A more complex situation was observed with MAb 4147-19-4. The low cross-reactivity of the MAb with compound F suggested that not only the bridge structure but also the triazine ring interacted with the MAb, although the triazine structure was present neither in the immunogen nor in the solid-phase antigen used for screening. In contrast to MAb 4149-1-1, the binding of MAb 4147-19-4 was influenced by the presence of hydroxyl groups on the phenyl ring, suggesting that the phenyl structure was part of the antigenic determinant recognized by the MAb. The contribution of the chloroethoxy group in the binding of MAb 4147-19-4 was less important than with MAb 4149-1-1, since the chlorine could be substituted by methoxy as evidenced by the cross-reactivity with cinosulfuron (Table I). Interestingly, these results indicate that antibodies with high affinity to triasulfuron and with a limited cross-reactivity pattern could be obtained using a simple hapten corresponding only to the chloroethoxy phenylsulfonamide carboxy moiety of the triasulfuron molecule (hapten B). Although most herbicides are small molecules, the design of a simple hapten corresponding to a substructure of the molecule may constitute a valuable approach to avoid the cumbersome chemical synthesis of the complete molecule as exemplified with hapten A.

Soil Extract and Soil Fortification Experiments. We tested for possible interfering soil coextracted materials, by adding various amounts of triasulfuron to the aqueous extract (obtained with procedure A) of five standard soil samples of known composition. Triasulfuron was measured by competitive ELISA using either MAb 4147-19-4 or MAb 4149-1-1. The measurements were done before and after the addition of triasulfuron to the soil extracts. As shown in Table II, all of the samples tested

Table III. Recovery of Triasulfuron from Various Fortified Soils[a]

soil	triasulfuron added, ppb	triasulfuron recovered (MAb 4147-19-4)			
		ppb[b]	%[c]	SD[d]	CV,[e] %
Vetroz expt 1	0.1	0.14	120	0.023	16.4
	0.5	0.34	64	0.033	9.7
	1	0.68	66	0.115	16.9
	10	6.60	66	0.801	12.1
expt 2	0.1	0.19	120	0.021	11.1
	0.3	0.31	80	0.017	5.5
	1	0.60	53	0.046	7.7
Les Evouettes	0.5	0.37	62	0.074	20.0
	1	0.71	65	0.184	25.9
	10	7.41	74	1.129	15.2
Stein	0.1	0.12	110	0.023	19.2
	0.3	0.25	80	0.008	3.2
	1	0.63	62	0.040	6.4

[a] Fortified soil samples were extracted by procedure C (see Experimental Procedures). [b] Calculated according to standards made of triasulfuron in PBS–Tween (average of four determinations). [c] Percentage defined as [(ppb measured after addition − ppb before addition)/ppb added] × 100. [d] SD, standard deviation. [e] CV, coefficient of variation.

gave acceptable recoveries (93% and 96% mean recoveries for MAb 4147-19-4 and MAb 4149-1-1, respectively). However, the blank soil extracts (without triasulfuron) showed some inhibitory effects on the baseline response of the ELISA. Depending on the soil types analyzed, the absorbance values of the blank extracts represented 66–86% of that of the buffer control with MAb 4147-19-4 and 61–85% with MAb 4149-1-1. These interferences were indicative of soil matrix effects and occurred regardless of the MAb used. No other MAbs showed better ruggedness. It should be pointed out that comparable soil matrix effects were reported by Kelley et al. (1985), when using the chlorsulfuron immunoassay with aqueous alkaline soil extracts. To remove the interfering materials, we performed an additional cleanup step by ion-pair partition (procedure B). This procedure eliminated most of the effects on the baseline response but increased the length and complexity of the sample preparation. When the ion-pair partition was performed directly on the MeOH/aqueous phosphate extract to save time for the sample preparation (procedure C), the soil matrix effects remained minimal (80–97% of buffer control).

A recovery study with fortified soils was performed with MAb 4147-19-4 using soil extraction procedure C (Table

III). The recovery averaged 78% with soil samples fortified with triasulfuron in the range 0.1–10 μg/kg. The sensitivity of the assay using the given extraction and dilution protocol was 0.1 μg/kg. In these experiments, we calculated that the intra- and interassay coefficients of variation were 6.0% (10 assays) and 13.0% (52 assays), respectively (all assays were done in triplicate).

In conclusion, the immunoassays based on MAb 4147-19-4 and MAb 4149-1-1 represent valuable methods for the detection of triasulfuron in soil down to the sub parts per billion level. MAb 4147-19-4 appears to be more suitable for environmental soil monitoring due to the absence of cross-reactivity with the major triasulfuron degradation products.

ABBREVIATIONS USED

BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay; I_{50}, concentration of triasulfuron or analogues reducing the ELISA signal to 50% that of the control; KLH, keyhole limpet hemocyanin; MAb, monoclonal antibody; PBS, phosphate-buffered saline.

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