Development of Immunoassays for Type II Synthetic Pyrethroids. 2. Assay Specificity and Application to Water, Soil, and Grain

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Cross-reactions of a panel of immunoassays developed for type II (α-cyano-) synthetic pyrethroids were evaluated with type I and II compounds. Cross-reactions of both groups were affected by alkali treatment. The treatment typically increased sensitivities of type II compounds, and decreased cross-reactions of type I compounds. After isomerization, each of the 12 combinations studied provided more sensitive detection of deltamethrin than the other compounds, even in the case of antisera and enzyme conjugates based on cypermethrin, cyhalothrin, or their fragments. Several immunoassays were selective for deltamethrin. Assays with the broadest specificities detected type I and II compounds with IC_{50} values of 20−400 μg/L. None of the assays detected compounds lacking a cyclopropane ring (fenvalerate, fluvalinate). Spike and recovery studies for deltamethrin in water, soil, and wheat grain indicated that the selected immunoassays quantified it with high precision and good recoveries. Good correlations between immunoassay and gas chromatography/mass spectrometry data were also obtained for incurred residues of deltamethrin and bifenthrin extracts of water and soil samples.

Keywords: *Pyrethroid; immunoassay; specificity; analysis; water; soil; grain*

INTRODUCTION

The greater chemical stability and photostability of the type II (α-cyano-) synthetic pyrethroids have led to their widespread use both in broadacre agriculture and as an insecticidal protectant for stored commodities. Their persistence in soil could also create a means for residues to persist in water, especially as turbid runoff (Leahey, 1985). A laboratory study on the persistence and behavior in soil of four pyrethroid insecticides reported that fenpropanate, permethrin, cypermethrin, and fenvalerate were more persistent than chlorpyrifos in sand (Harris et al., 1981). However, field studies indicate variable persistence due to soil type and other factors (Agnihotri et al., 1986). The half-life of deltamethrin in an organic soil was determined to be 72 days (Zhang et al., 1984), with significant bound residues. Established type II compounds such as deltamethrin, cypermethrin, and λ-cyhalothrin are widely used for control of *Helicoverpa* spp., the major pests of cotton. Some more recently developed type I pyrethroids such as bifenthrin are also finding significant use as they have greater photostability than other members of the group (Shaw, 1994). Given the significant proportion of cotton that is grown under irrigation, the risk of environmental damage to fish and desirable invertebrates from contamination of irrigation drainage water could be significant (Lhost and L'Hotelier, 1982). Much of the toxicological studies focused on fish (Coates and O'Donnell-Jeffery, 1979; Edwards and Millburn, 1985; Hill, 1985; Coates et al., 1989; Haya, 1989; Mian and Mulla, 1992); acute toxicities vary widely, depending on the pyrethroid and the species of fish. The type II pyrethroids are more toxic to fish than corresponding type I compounds. Aquatic invertebrates can also be affected by environmental pyrethroid residues.

Deltamethrin has also found significant use as a grain protectant, especially in Europe. It is typically applied at 1−2 mg/kg onto harvested grain that is intended for elevator storage (Snelson, 1987). Its greater stability to degradation than that of alternative pyrethroids has made it attractive for uses requiring insecticidal activity for long periods (Bengston et al., 1983). However, this stability, which also means that residues are not significantly reduced during baking (Snelson, 1987), brings with it the need to closely monitor residues of the compound in grain before it is processed.

In the preceding paper (Lee et al., 1998), we generated a library of haptens differing in structure, linker, and point of linker attachment. The resulting antibodies provided different specificities for deltamethrin, cypermetrin, and λ-cyhalothrin while detecting isomerized forms more sensitively than unisomerized forms. Although not all of the haptens led to useful antibodies, several of these haptens were useful as enzyme conjugates in immunoassays, providing expanded possibilities for manipulating assay sensitivity and specificity. In the present paper, we characterize the specificity for other pyrethroids and the common metabolites and the analytical performance (precision) of the assays. Since these assays detect isomerized compounds (i.e. racemic mixtures) with greter sensitivity, methods for isomerizing deltamethrin in water, soil, and grain extract were developed. The performance of the assays with environmental and food matrices was established by performing spike and recovery studies with soil, water, and wheat grain and sensitive immunoassays selected for

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Table 1. Antibody and Conjugate Combinations Studied

immunogen	compd no. (in Lee et al., 1998)	peroxidase conjugate	compd no. (in Lee et al., 1998)	conjugate concn (ng/mL)
LHDEL-KLH	**24**	LHDEL	**24**	60
		LHCYP	**25**	30
		DEL	**14**	40
LHCYP-KLH	**25**	CYP	**15**	4000
CYH-KLH	**16**	LHDEL	**24**	60
		LHCYP	**25**	10
		CYP	**15**	40
		PBA-BULKY	**32**	500
DEL-OA	**14**	CYP	**15**	400
DEL-KLH	**14**	CYP	**15**	400
CYP-OA	**15**	DEL	**14**	4000
FULPYR	**20**	4OHDEL	**4**	4900

the detection of incurred residues of deltamethrin and bifenthrin in water and soil.

MATERIALS AND METHODS

Immunoassays. Antisera, conjugates and assay methods were described in the preceding paper (Lee et al., 1998). Twelve assays, which provided sensitive detection of one or more of deltamethrin, cypermethrin, or λ-cyhalothrin and utilized peroxidase conjugates at <5 mg/mL, were studied (Table 1); the full chemical names for the compounds are listed in Lee et al. (1998). Pyrethroid standards and dilutions were prepared as described in the preceding paper (Lee et al., 1998); NaOH treatments were applied to standards immediately before dilution and assay. Because of the large size of the experiments, the pyrethroid standards and samples were routinely prepared in borosilicate tubes pretreated with 5% poly(ethylene glycol) (PEG; MW 20 000, Fluka, Buchs, Switzerland) to prevent the compounds adsorbing to the glass sufaces (Sharom and Solomon, 1981; Helmuth et al., 1983). The borosilicate tubes and vials (used in preparation of stock solutions) were filled with 5% PEG and incubated at room temperature overnight. The PEG solution was discarded and the glassware dried at 110 °C overnight.

Spiking of Soil and Grain. The soil used for pyrethroid spiking was a gray-black swelling soil (Vertisol), containing predominantly montmorillonite (~62%) and ~20% of both silt and sand, with pH in the range of 7.5−8.8 (collected from Narrabri, NSW, Australia; Ward et al., 1988). Subsamples (10 g for immunoassay and 20 g for GLC) of soil, verified by gas chromatography to be free of pesticide residues, were spiked with ≤200 μL of freshly prepared 10 and 1000 μg/mL unisomerized deltamethrin or bifenthrin standards in methanol, to obtain concentrations between 0.1 and 10 mg/kg. The soil was mixed thoroughly with a stainless steel spatula for 3 min and then allowed to stand at room temperature for 1−3 days prior to extraction. Hard wheat (11.5% protein) from Geraldton, WA, Australia, was weighed as 10-g subsamples into glass bottles and spiked with 0.01−1 mL of freshly prepared 5 and 50 μg/mL unisomerized deltamethrin standard in methanol. The samples were loosely capped, shaken, and left in a fume cupboard overnight for the methanol to evaporate. To ensure even distribution of the residues, the containers were then sealed and stored for 14 days at room temperature, with daily shaking.

Extraction and Isomerization of Pyrethroid Residues from Soil, Grain, and Water for Immunoassay. The optimal isomerization treatments for deltamethrin residues in these matrices were established in preliminary experiments. For soil samples, 20 mL of 90% methanol was added to a jar containing 10 g of soil and the mixture was mechanically shaken for 3 h, and then allowed to stand overnight. For the analysis of bifenthrin, the supernatant was diluted 1:20 in purified water for immunoassay. For the analysis of deltamethrin, the residue was isomerized by adding NaOH in water to the methanol extract after decantation from the soil (1:200 water/methanol extract ratio, 5 mM NaOH final) and incubating for 1 h at room temperature. The extract was diluted 1:20 in purified water for immunoassay. Water samples were made 5% with respect to methanol. NaOH was added to the methanol/water solution to 5 mM final and incubated for 1 h at room temperature.

Deltamethrin residues in the wheat samples were extracted either by adding of 25 mL of methanol to 10 g of grain and standing for 48 h at room temperature with intermittent shaking (standard extraction) or by using 50 mL of methanol per 10 g of grain and blending in a Waring blender (Dynamics Corp., New Hartford, CT) at full speed for 1 min (rapid extraction). The deltamethrin residues were isomerized by adding NaOH in water to the methanol extract after decantation from the grain (1:100 water/methanol extract ratio, 10 mM NaOH final) and incubating for 30 min at room temperature. The extracts were diluted 1:10 in 50 mM sodium phosphate−0.9% NaCl, pH 7.2 (PBS)−1% bovine serum albumin−0.05% Tween 20 for immunoassay; extracts of grain from the high-level spikes (1 and 5 mg/kg) were first diluted 1:10 in methanol before isomerization and analysis.

Gas−Liquid Chromatographic Analysis. A jar containing 20 g of soil was shaken overnight with 80 mL of hexane−acetone (4:1). The extract was filtered through a Büchner funnel using no. 1 filter paper (Whatman, Maidstone, U.K.), and the filter paper was washed with 10 mL of hexane. The combined extracts were dried over anhydrous sodium sulfate and concentrated to 10 mL using a Turbo Vap 500 (Zymark). Florisil (6 g) was added to a 40-cm-long chromatographic tube plugged with cotton wool at one end, and then the column was topped with 1 g of anhydrous sodium sulfate. After the column was prewet with 20 mL of hexane, 10 mL of soil extract was added. The vial was rinsed with 10 mL of toluene/hexane (2:8), and the rinsate was also added to the column. The column was eluted with 190 mL of toluene/hexane (2:8). The first 90 mL of eluate was discarded. The next 100 mL was collected and concentrated to 10 mL for GLC/MS analysis. GC conditions were as described in Nolting et al. (1992). Water samples were extracted using liquid−liquid extraction (Nolting et al., 1992). This method was claimed by the authors to provide >94% recovery for deltamethrin and bifenthrin. Dichloromethane, however, was used instead of hexane as an organic phase. Sodium chloride (10 g) was added to a separating funnel containing 500 mL of water sample, and the solution was extracted once with 100 mL of dichloromethane for 4 min and twice with 50 mL of dichloromethane for 2 min. The combined extracts were concentrated to 5 mL (for spiked water) or 10 mL (for field water) with hexane using a Turbo Vap. For the field water, cleanup on a Florisil column was performed.

RESULTS AND DISCUSSION

Selection of Immunoassays for Further Evaluation. Twelve immunoassays were selected for further evaluation on the basis of their dynamic behavior, the limits of detection for deltamethrin, cypermethrin, and λ-cyhalothrin [see Lee et al. (1998)], and the concentrations of enzyme conjugate required in the immunoassay. Although the concentration of enzyme conjugate required in the FULPYR-KLH/4OHDEL-HRP combination was rather high, this immunoassay was also included in a selection for comparison with other immunoassays of the cross-reactions, precision, and performance with spiked and field samples, because the antiserum was derived from a "generic" immunogen hapten. The selected immunoassays had comparable sensitivities, with IC_{50} values between 2 and 4 μg/L of isomerized deltamethrin (Tables 2−4) and with limits of detection between 0.2 and 0.7 μg/L. Three of these immunoassays, LHDEL-KLH/DEL-HRP, LHCYP-KLH/CYP-HRP and CYH-KLH/CYP-HRP, were capable of detecting the unisomerized deltamethrin relatively

Table 2. Specificity of Different Immunoassays Using Antisera to a Hapten Based on Deltamethric Acid (LHDEL-KLH) and Different Enzyme Conjugates[a]

		IC_{50} (μg/L)							
	immunogen:	LHDEL-KLH		LHDEL-KLH		LHDEL-KLH		LHCYP-KLH	
	conjugate:	LHDEL		LHCYP		DEL		CYP	
compd	isomerized?:	no	yes	no	yes	no	yes	no	yes
type II pyrethroids									
deltamethrin		–	3.5	–	4	70	2	60	2
α-cypermethrin		–	35	–	–	–	300	300	55
cypermethrin		–	20	–	20	1000	10	300	15
λ-cyhalothrin		–	–	–	–	–	1000	–	70
cyfluthrin		–	350	650	400	230	80	850	20
esfenvalerate		–	–	–	–	–	–	–	–
fluvalinate		–	–	–	–	–	–	–	–
type I pyrethroids									
bifenthrin		–	–	750	–	110	–	30	1000
resmethrin		–	–	–	–	–	–	–	–
bioresmethrin		–	–	–	–	–	–	–	–
tetramethrin		–	–	350	–	30	250	80	200
allethrin		–	–	–	–	600	–	–	–
bioallethrin		–	–	750	–	150	1000	–	–
S-bioallethrin		–	–	800	500	800	800	–	–
permethrin		1000	700	–	–	300	400	–	120
phenothrin		–	–	–	–	–	–	–	–
trans-phenothrin		–	–	–	–	–	–	300	–
common metabolites									
PB-cyanohydrin		–	–	–	–	–	–	–	–
PB-alcohol		–	–	–	–	–	–	–	–
PB-aldehyde		–	–	–	–	–	–	–	–
PB-acid		–	–	–	–	–	–	–	–
chrysanthemic acid		–	–	–	–	–	–	–	–
deltamethric acid		–	–	–	–	360	–	200	35
cypermethric acid		–	–	250	–	350	–	100	–
cyhalothric acid		–	–	–	–	600	–	–	550

[a] PB, phenoxybenzyl. Dash indicates IC_{50} > 1000 μg/L; data shown are means of two determinations.

Table 3. Specificity of Different Immunoassays Using Antisera to a Hapten Based on the Full Cyhalothrin Molecule, Coupled through the Middle of the Molecule (CYH-KLH), and Different Enzyme Conjugates

		IC_{50} (μg/L)							
	immunogen:	CYH-KLH		CYH-KLH		CYH-KLH		CYH-KLH	
	conjugate:	LHDEL		LHCYP		CYP		PBA-BULKY	
compd	isomerized?:	no	yes	no	yes	no	yes	no	yes
type II pyrethroids									
deltamethrin		–	4	930	2	50	4	240	4
α-cypermethrin		–	45	300	6	180	90	–	250
cypermethrin		–	60	80	10	70	20	320	30
λ-cyhalothrin		–	50	–	10	1000	10	–	–
cyfluthrin		560	250	280	140	160	200	550	800
esfenvalerate		–	–	–	–	–	–	–	–
fluvalinate		–	–	–	–	–	–	–	–
type I pyrethroids									
bifenthrin		220	–	250	450	30	270	800	–
resmethrin		–	–	–	–	–	–	–	–
bioresmethrin		–	–	–	–	400	–	–	–
tetramethrin		–	–	220	–	30	350	–	–
allethrin		–	–	–	–	110	–	–	–
bioallethrin		–	–	–	–	300	–	–	–
S-bioallethrin		–	–	–	–	140	460	–	–
permethrin		630	360	320	17	20	140	600	480
phenothrin		–	–	–	–	–	–	–	–
trans-phenothrin		–	–	–	–	–	–	–	–
common metabolites									
PB-cyanohydrin		–	–	–	–	–	–	–	–
PB-acohol		–	–	–	–	–	–	–	–
PB-aldehyde		–	–	–	–	–	–	–	–
PB-acid		–	–	–	–	–	–	–	–
chrysanthemic acid		–	–	–	–	–	–	–	–
deltamethric acid		300	–	200	–	180	–	–	–
cypermethric acid		250	–	100	–	200	–	–	–
cyhalothric acid		–	–	700	–	–	–	–	–

[a] PB, phenoxybenzyl. Dash indicates IC_{50} >1000 μg/L; data shown are means of two determinations.

sensitively, with IC_{50} values of 70, 60, and 50 μg/L, respectively, and limits of detection 5−10-fold lower. However, since these immunoassays were far more sensitive for isomerized deltamethrin than for uni-

Table 4. Specificity of Different Immunoassays Using Antisera to Haptens Based on the Full Deltamethrin (DEL-OA and KLH) and Cypermethrin Structures Coupled through the Middle of the Molecule (CYP-OA) and a Generic Pyrethroid Structure Coupled through the Cyclopropane Moiety (FULPYR-KLH), Using Different Enzyme Conjugates[a]

		IC_{50} (μg/L)							
	immunogen:	DEL-OA		DEL-KLH		CYP-OA		FULPYR-KLH	
	conjugate:	CYP		CYP		DEL		40HDEL	
compd	isomerized?:	no	yes	no	yes	no	yes	no	yes
type II pyrethroids									
deltamethrin		130	4	50	5	180	2	210	4
α-cypermethrin		–	20	40	–	–	–	160	340
cypermethrin		1000	80	60	–	1000	30	160	80
λ-cyhalothrin		–	7	40	–	–	–	650	–
cyfluthrin		600	–	140	1000	400	210	650	200
esfenvalerate		–	–	–	–	–	–	–	–
fluvalinate		–	–	–	–	–	–	–	–
type I pyrethroids									
bifenthrin		–	–	80	–	–	–	30	800
resmethrin		–	–	–	–	–	–	–	–
bioresmethrin		–	–	–	–	–	–	–	–
tetramethrin		100	–	220	1000	170	800	150	–
allethrin		160	–	70	–	–	–	400	–
bioallethrin		350	–	210	–	–	–	500	–
S-bioallethrin		230	60	–	1000	–	–	550	–
permethrin		400	–	30	750	950	320	80	–
phenothrin		–	–	–	–	–	–	–	–
trans-phenothrin		–	–	–	–	–	–	–	–
common metabolites									
PB-cyanohydrin		–	–	–	–	–	–	–	–
PB-alcohol		–	–	–	–	–	–	–	–
PB-aldehyde		–	–	–	–	–	–	–	–
PB-acid		–	–	–	–	–	–	–	–
chrysanthemic acid		630	–	–	–	–	250	–	–
deltamethric acid		–	–	400	–	–	560	–	–
cypermethric acid		–	160	–	–	800	–	–	–
cyhalothric acid		550	240	–	–	700	–	–	–

[a] PB, phenoxybenzyl. Dash indicates IC_{50} >1000 μg/L; data shown are means of two determinations.

somerized compound, quantitation of deltamethrin in samples that contained "aged" residues would only be possible if the deltamethrin isomers in the mix that would have arisen were isomerized to a consistent mixture before analysis.

Specificity of Immunoassays. The IC_{50} values of the 12 selected immunoassays for the alkali-treated ("isomerized") and unisomerized pyrethroids and their common metabolites are shown in Tables 2−4. After the alkali treatment used for isomerization of the type II compounds, each of the 12 combinations studied detected deltamethrin more sensitively than the other compounds, even in the case of assays using antisera and enzyme conjugates based on cypermethrin, cyhalothrin, or their fragments. The cross-reactions of both groups of compounds were affected by the dilute alkali treatment used for isomerization of type II compounds; the treatment typically increased the detection sensitivities of the type II compounds, while the cross-reactions of type I compounds often decreased. It is probable that the decrease observed in sensitivity for certain compounds was due to hydrolysis of these type I compounds. The difference in the detection of a single isomer and a mixture of isomers can be examined by comparison of the data obtained using α-cypermethrin (α*S*, 1*R cis* isomer of cypermethrin) and cypermethrin (eight isomers); differences in the detection sensitivities were obtained in several cases both before and after the alkali treatment, which would suggest that in this case the treatment did not convert both forms into the same isomer mixture.

Several immunoassays were selective for deltamethrin, even though these assays had employed quite different haptens in the immunogen, including conjugates of deltamethrin or cyhalothrin through the middle of the molecule, deltamethric or cypermethric acids, or a hapten based on the common type II pyrethroid structure but lacking halogenated substituents. Others exhibited selectivity for two or three of deltamethrin, cypermethrin, and cyhalothrin, and a few assays recognized both type I and type II compounds. The pattern of detection of these compounds varied among immunoassays using the same antibody with different enzyme conjugates or different antibodies with the same enzyme conjugate. For example, immunoassays utilizing an antibody to LHDEL-KLH and LHDEL-HRP conjugates showed similar sensitivities for isomerized α-cypermethrin and cypermethrin. The same antibody could be made specific to isomerized cypermethrin by using LHCYP-HRP conjugate, while the sensitivities for unisomerized compounds were enhanced by using CYP-HRP conjugate. The CYH-KLH antisera /LHCYP-HRP combination was the most sensitive for both isomerized α-cypermethrin and cypermethrin, providing IC_{50} values of 6 and 10 μg/L respectively.

Only three immunoassays displayed IC_{50} values <10 μg/L for λ-cyhalothrin (in isomerized form): CYH-KLH antibody/LHCYP-HRP, CYH-KLH antibody/CYP-HRP, and DEL-OA antibody/CYP-HRP. Although the assay using antibody to DEL-KLH and CYP-HRP was able to detect unisomerized cypermethrin and λ-cyhalothrin more sensitively than isomerized compounds, the detection sensitivities were insufficient for quantitation of these compounds in water. Detection of bifenthrin was of special interest, given its increasing use in cotton agriculture. It is a type I pyrethroid with an acid group [3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate] identical with that of cyhalo-

thrin. The antibody to CYH-KLH detected bifenthrin with each of the conjugates used, and detection with the CYP-HRP conjugate was especially sensitive. Two other antibody/conjugate combinations detected bifenthrin more sensitively that any other pyrethroid in unisomerized samples: LHCYP-KLH/CYP-HRP and FULPYR-KLH/4OHDEL-HRP. Isomerization did not enhance the sensitivity of detection of bifenthrin.

Most of the selected immunoassays exhibited narrow specificities, significantly cross-reacting with fewer than five synthetic pyrethroids. The broadest specificity pyrethroid assays detected group I and II compounds with IC_{50} values in the 20−400 μg/L range, but none detected the compounds that lacked a cyclopropane ring (fenvalerate, fluvalinate), and reaction with phenothrin and resmethrin/bioresmethrin was also poor. Several organochlorine (aldrin, chlordane, dicofol, dieldrin, DDE, DDT, endosulfan, heptachlor, lindane, methoxychlor), organophosphorus (chlorpyrifos, methylparathion, monocrotophos, parathion, profenofos, sulprofos), and carbamate (methomyl, thiodicarb) insecticides and herbicides (2,4-dichlorophenoxyacetic acid, diuron, molinate, triclopyr) were assessed at 1000 μg/L for cross-reaction with each of the antibody combinations. No cross-reaction was found, indicating these assays were very specific to pyrethroids.

The cross-reaction data suggested that some of these immunoassays may also be useful for screening of other pyrethroids such as cyfluthrin, tetramethrin, permethrin, allethrin, and bifenthrin, although detection would, of course, not be compound-specific (Tables 2−4). Cyfluthrin and permethrin are used as stored product protectants, and the limits of detection required for analyses are relatively high (Tomlin, 1994). The immunoassay using an antibody to LHCYP-KLH and CYP-HRP conjugates could quantify cyfluthrin with an IC_{50} of 20 μg/L of isomerized cyfluthrin. Quantification of tetramethrin would not require isomerization, with an IC_{50} of 30 μg/L using LHDEL-KLH/DEL-HRP or CYH-KLH/CYP-HRP combinations. Permethrin and allethrin could be detected at low micrograms per liter concentrations without isomerization using the DEL-KLH/CYP-HRP combination (IC_{50} values of 30 and 70 μg/L, respectively). The sensitivity for the former compound is similar to that of the monoclonal antibody assay developed by Stanker et al. (1989).

Detection of pyrethroid metabolites (Hill, 1985) was usually less sensitive than detection of the parent compounds. With the exception of antisera to phenoxybenzyl alcohol haptens [see preceding paper, Lee et al. (1997)], the range of phenoxybenzyl metabolite analogues (alcohol, aldehyde, acid, cyanohydrin) were not detected by the immunoassays. Pyrethric acid moieties were detected more sensitively than phenoxybenzyl analogues but, in the vast majority of cases, less sensitively than the corresponding parent compound. Specificity for parent molecules over metabolites (which are also significantly less toxic) is important if the results of the immunoassays are to correlate with instrumental methods, which typically detect the parent molecules. Even the antisera raised to deltamethric acid and cypermethric acid haptens (LHDEL and LH-CYP) detected the parent pyrethroids more sensitively than the acid breakdown products (Table 2). This is possibly because the acids would be negatively charged under the assay conditions, while both the immunogens used and the pyrethroids exist as (uncharged) esters.

Isomerization of Pyrethroid Residues in Matrices. Since the immunoassays exhibited a greater sensitivity for isomerized compounds than for unisomerized compounds, methods were developed for the isomerization of pyrethroid in these matrices, using deltamethrin as a model compound and an immunoassay using an antibody to CYH-KLH and LHCYP-HRP conjugates. The effects of soil extract on the isomerization with 5 mM NaOH were determined by preparing deltamethrin standards in methanol and soil extract (90% methanol) prepared from the soil known to be free of contamination by pyrethroids. Isomerization was initially slightly slower in soil extract than in 90% methanol but was optimal after 60 min of incubation (Figure 2A); this procedure was routinely used for soil analysis. Several factors may have influenced the rate of isomerization, including the presence of water in the soil extract and the extracted soil components retarding the isomerization. A similar approach was used for deltamethrin in grain extracts, although a slightly higher final NaOH concentration (10 mM) and shorter isomerization time (30 min) were optimal.

Isomerization of deltamethrin in water was more difficult than in soil or grain extracts, due to the competing effects of hydrolysis. Hydrolysis of cypermethrin in 1 M NaOH has been reported to occur without *cis*/*trans*-isomerization at the cyclopropane ring (Takahashi et al., 1985). Deltamethrin, which is the dibromo analogue, would be expected to behave in a similar manner. Initially, the isomerization of deltamethrin in water was attempted by adding NaOH to 1 mM in water samples and observing the rate of isomerization. The assay sensitivity reached a maximum after 1 h; however, the sensitivity of the assay was still insufficient. Increasing the amount of NaOH to 10 mM improved sensitivity, although a reduction in the assay sensitivity was observed with overnight incubation, probably due to hydrolysis (Takahashi et al., 1985). Thus, milder treatments involving buffering with 1, 10, and 100 mM Tris (pH 8.2, 9.0, and 9.8, respectively) and 0.5, 1, and 5 mM sodium 3-(cyclohexylamino)-1-propanesulfonate (pH 10.2) were examined. However, none of these treatments increased assay sensitivities to the same extent as 10 mM NaOH.

Addition of methanol to the water samples prior to NaOH addition improved assay sensitivity (Figure 2B), since methanol addition alone did not significantly alter the assay sensitivity. The isomerization increased with increasing amounts of added methanol. The assay sensitivity (determined as the IC_{50} value for deltamethrin) increased by 6 times at a final concentration of 5% methanol and 5 mM NaOH, compared with 5 mM NaOH alone. The isomerization of deltamethrin reached a maximum after 1 h with the 5% methanol/5 mM NaOH treatment. The effects of isomerization of deltamethrin in turbid water were examined (Figure 2C). The deltamethrin standards were prepared in purified water and in three samples with turbidities of 27, 36, and 228 NTU. The standard curves prepared in the field water samples were superimposable, indicating that the turbidities of the water samples did not influence the isomerization with 5 mM NaOH/5% methanol. However, a slight difference between the sensitivities of standard curves prepared in purified water and field water samples indicated that isomerization of deltamethrin is slightly lower in the field water samples than in purified water. Deltamethrin

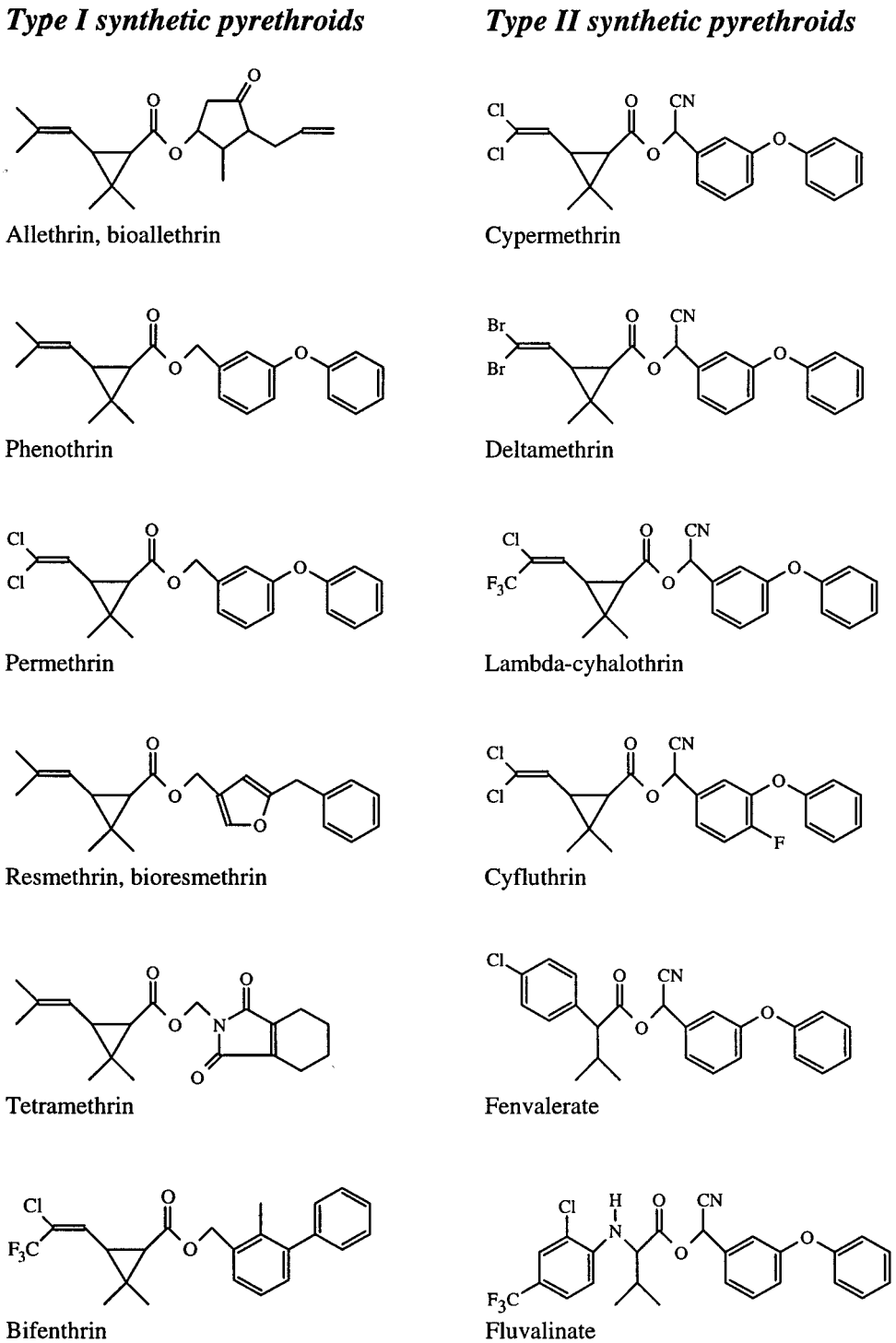

Figure 1. Structures of synthetic pyrethroids used in this study. Systematic chemical names are as follows: allethrin, (*RS*)-3-allyl-3-methyl-4-oxocyclopent-2-enyl (1*RS*)-*cis*-*trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate; bioallethrin, (*RS*)-3-allyl-3-methyl-4-oxocyclopent-2-enyl (1*R*)-*trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate; *S*-bioallethrin, (*S*)-3-allyl-3-methyl-4-oxocyclopent-2-enyl (1*R*)-*trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate; phenothrin, (1*RS*)-*cis*-*trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate; permethrin, 3-phenoxybenzyl (1*RS*)-*cis*-*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate; resmethrin, 5-benzyl-3-furylmethyl (1*RS*)-*cis*-*trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate; bioresmethrin, 5-benzyl-3-furylmethyl (1*R*-*trans*)-2,2-dimethyl-3-(2-methylprop-1-enyl)-cyclopropanecarboxylate; tetramethrin; bifenthrin, 2-methylbiphenyl-3-ylmethyl (*Z*)-(1*RS*)-*cis*-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate; cyfluthrin, (*RS*)-α-cyano-4-fluoro-3-phenoxybenzyl (1*RS*)-*cis*-*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate; fenvalerate, (*RS*)-α-cyano-3-phenoxybenzyl-(*RS*)-2-(4-chlorophenyl)-3-methylbutyrate; fluvalinate, (*RS*)-α-cyano-3-phenoxybenzyl N-(2-chloro-α,α,α-trifluoro-*p*-tolyl)-D-valinate].

may have adsorbed onto the particles (Katagi, 1993), becoming resistant to isomerization (Edwards, 1972; Weber, 1972; Carringer et al., 1975; Peterson and Batley, 1993). Analysis of deltamethrin in water samples, if possible, should be conducted after deltamethrin is isomerized both in standards prepared in field water and in samples concurrently.

Precision of Immunoassays. The interassay precision of the immunoassay standard curves was evaluated as the variation in percentage inhibition of antibody

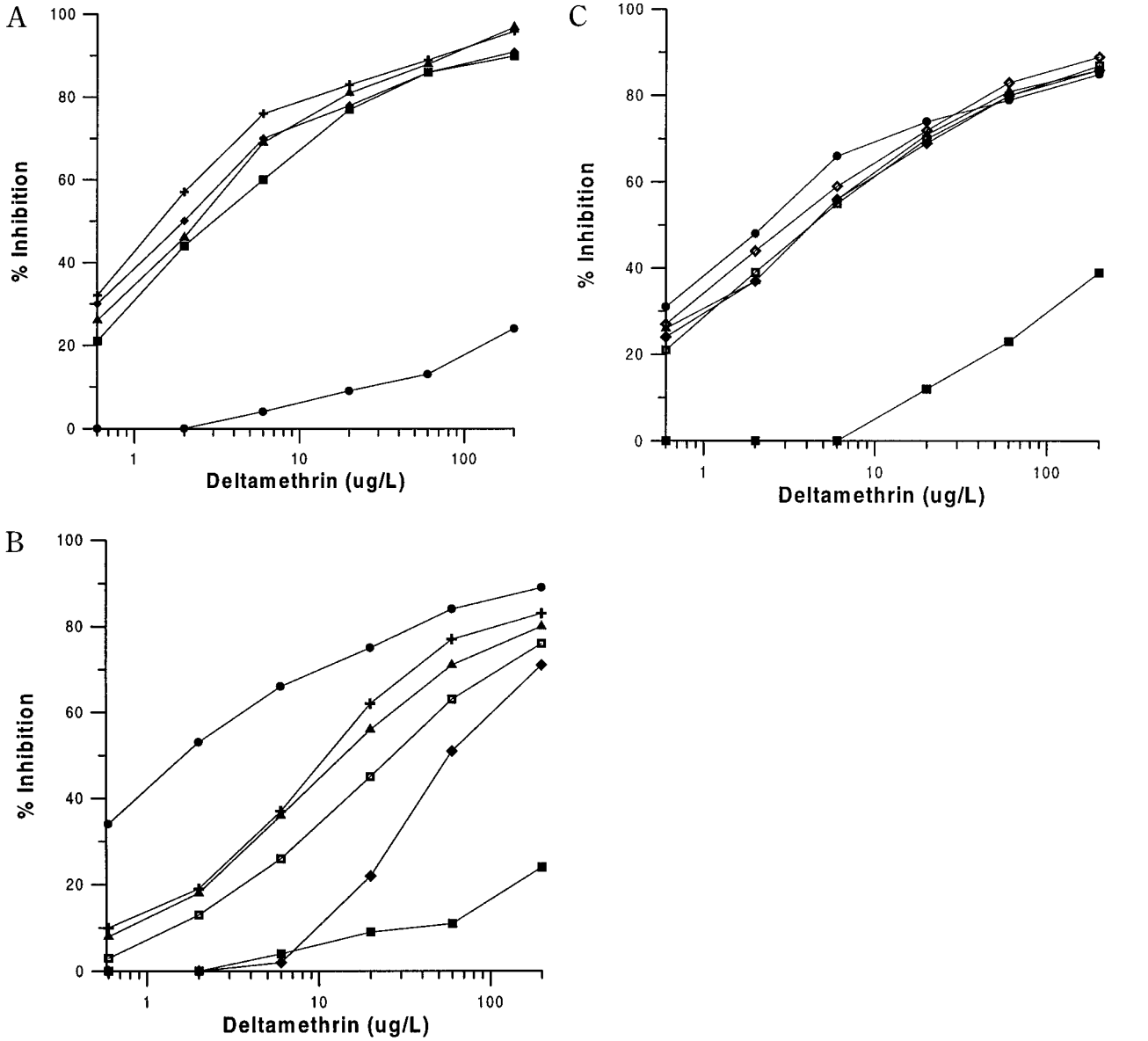

Figure 2. Isomerization of deltamethrin: (A) spikes in methanol extract of soil, treated with 5 mM NaOH for 15 (■), 30 (◆), and 60 (▲), and in pure methanol, treated with 5 mM NaOH for 15 min (+) (unisomerized deltamethrin indicated by ●); (B) effects of methanol concentration on isomerization under aqueous conditions [5 mM NaOH only (◆), 1% methanol/5 mM NaOH (□), 2% methanol/5 mM NaOH (▲), 5% methanol/5 mM NaOH (+)] (isomerized deltamethrin indicated by ● and unisomerized deltamethrin by ■); (C) isomerization of deltamethrin in purified water (◇) and three turbid field water samples (36 NTU, ◆; 27 NTU, ▲; 228 NTU, □) (unisomerized deltamethrin indicated by ■). Data were generated using antibody to CYH-OA and LHCYP-HRP conjugate.

binding at the six deltamethrin concentrations used for preparation of standard curves, in 2−3 assays for each of 10 antibody/enzyme conjugate combinations. The coefficients of variation (CV) were higher for lower deltamethrin concentrations, as previously noted for molinate immunoassays (Harrison et al., 1989). The percent CV values for the 10 assays varied as follows: at 200 μg/L, 1−4% (mean 2%); at 60 μg/L, 2−5% (mean 3%); at 20 μg/L, 2−14% (mean 5%); at 6 μg/L, 1−14% (mean 7%); at 2 μg/L, 1−30% (mean 14%); at 0.6 μg/L, 6−54% (mean 24%). The assays with poorer precision were not necessarily less sensitive than the others but did include examples such as FULPYR-KLH/4OH-HRP, for which the required conjugate concentration was high. Assays with the highest standard curve precision included those with the LHDEL-KLH antiserum and DEL-HRP conjugate, the CYH-KLH antiserum and each of the LHCYP, LHDEL, and CYP conjugates, DEL-OA antiserum and CYP conjugate, LHCYP-KLH antiserum and CYP conjugate, and CYP-OA antiserum and DEL conjugate.

Spike and Recovery of Pyrethroids in Soil. The inhibition curves for deltamethrin standards prepared in either methanol or in methanol extract of soil before isomerization and dilution 1:10 were not significantly different (Figure 2A), indicating the absence of matrix interference from the soil type used in this study. The limit of detection of deltamethrin standards in methanol soil extract after 1:10 dilution in water was <0.5 μg/L, equivalent to 10 μg/kg in the original soil. Soil was spiked with unisomerized deltamethrin at 0.5, 1, 2, 5, and 10 mg/kg. The recoveries of deltamethrin in soil were evaluated by extracting duplicate samples with 90% methanol and treatment of the extract with 5 mM NaOH and then dilution in water to provide between 25 and 75% inhibition of antibody binding. The stan-

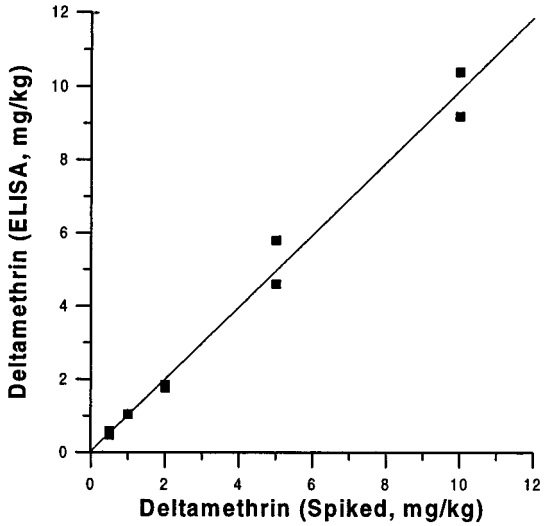

Figure 3. Spike (*x* axis) and recovery (*y* axis) of deltamethrin in soil, using an antibody to CYH-KLH and LHCYP-HRP. Regression line: deltamethrin (recovered, mg/kg) = 0.99 × deltamethrin (spiked, mg/kg) − 0.02 (n = 10, r = 0.99, P < 0.001).

Table 5. Recoveries of Deltamethrin in Spiked Soil and Water Samples[a,b]

antibody	enzyme conjugate	sample	recovery (%)
LHDEL-KLH	LHCYP	soil	66
		water	95
LHDEL-KLH	LHDEL	soil	103
		water	103
LHDEL-KLH	DEL	soil	74
		water	105
CYH-KLH	LHDEL	soil	77
		water	105
CYH-KLH	CYP	soil	117
		water	100
DEL-OA	CYP	soil	116
		water	100
FULPYR-KLH	4OHDEL	soil	77
		water	94
LHCYP-KLH	CYP	soil	78
		water	105
CYP-OA	DEL	soil	110
		water	80

[a] Data shown are mean recoveries for deltamethrin at four levels (1, 2, 5, and 10 mg/L) in four field water samples of turbidities <5, 27, 36, and 228 NTU. [b] Data shown are mean recoveries obtained for five duplicate soil samples spiked with deltamethrin at 0.5, 1, 2, 5, and 10 mg/kg

dard curve was prepared in a methanol extract of soil known to be free of pesticide residues and treated in the same manner as the spiked samples. Initial evaluation using the antibody to CYH-KLH and LHCYP-HRP (Figure 3) provided a mean recovery of 100 ± 6% (assessed as the ratio of deltamethrin determined by ELISA to the spike level) and a mean coefficient of variation between the immunoassay data from the duplicate assays of 9%. The regression data from the spike/recovery studies for nine antibody/conjugate combinations are shown in Table 5. Other immunoassays also provided good recoveries: LHDEL-KLH/LHDEL-HRP (101 ± 17%), FULPYR-KLH/4OHDEL-HRP (107 ± 27%), CYP-OA/DEL-HRP (112 ± 13%), DEL-OA/CYP-HRP (111 ± 18%), LHDEL-KLH/DEL-HRP (85 ± 27%), LHCYP-KLH/CYP-HRP (89 ± 16%) and for CYH-KLH/LHDEL-HRP (84 ± 13%). Only two immunoassays, CYH-KLH/CYP-HRP (124 ± 16%) and LHDEL-KLH/LHCYP-HRP (71 ± 11%), gave recoveries that averaged

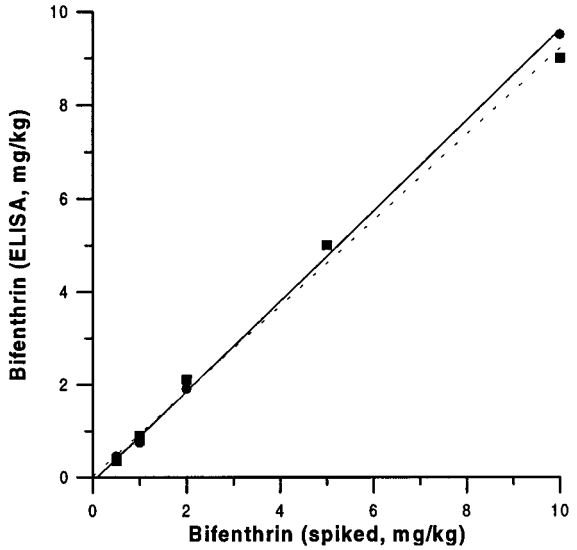

Figure 4. Spike (*x* axis) and recovery (*y* axis) of bifenthrin in seven soil samples, using LHCYP-KLH/CYP-HRP (●) and CYH-KLH/CYP-KLH (■) combinations. Each data point represents a mean of two replicates of soil samples. LHCYP-KLH/CYP-HRP: bifenthrin (recovered, μg/L) = 0.97 × bifenthrin (spiked, μg/L) − 0.09 (r = 0.99, P < 0.001). CYH-KLH/CYP-KLH: bifenthrin (recovered, μg/L) = 0.92 × bifenthrin (spiked, μg/L) − 0.02 (r = 0.99, P < 0.001).

>20% different from 100%. The precision of the soil analyses was typically quite high; for replicate soil samples spiked with 0.5, 1, 2, 5, and 10 mg/kg deltamethrin, the mean CV values were as follows: LHDEL-KLH with LHCYP-HRP, 11%, with LHDEL-HRP, 13%; and with DEL, 29%; CYH-KLH with LHCYP, 9%; with LH-DEL, 9%; with CYP, 7%; DEL-OA with CYP, 12%; FULPYR-KLH with 4OHDEL-HRP, 12%; LHCYP-KLH with CYP, 17%; and CYP-OA with DEL, 12%. Both LHCYP-KLH/CYP-HRP and CYH-KLH/CYP-HRP combinations gave high recoveries for bifenthrin spiked in duplicate soil samples at 0.5, 1, 2, 5, and 10 mg/kg (Figure 4). Although both immunoassays gave comparable recovery for soil samples (average 88 ± 0% for the first assay and 87 ± 13% for the second), the LHCYP-KLH/CYP-HRP assay is more sensitive for bifenthrin, and thus more suitable for its quantitation at lower levels. The superiority of this combination was borne out in studies with residues of bifenthrin, rather than laboratory spiking (Table 6). The slope of the regression plot for the relationship between GC/MS and immunoassay data was also closer to unity; the plot for both assays passed very close to the origin.

Pyrethroids Spiked in Water. The performance of immunoassays was initially examined using antibody to CYH-KLH and LHCYP-HRP. Purified water and two field water samples (36 and 126 NTU) were spiked with unisomerized deltamethrin at 1, 2, 5, and 10 μg/L, and isomerization was conducted with both standard curve and the spiked samples. Taking the recovery data for the 12 samples together, recoveries correlated well with the spiked levels (deltamethrin recovered (mg/L) = 1.05 × deltamethrin spiked (mg/L) − 0.84; r = 0.99, P < 0.001), indicating that this brief isomerization treatment was effective with spiked samples. A similar study was also conducted using other antibody/enzyme conjugate combinations (Table 5). All provided good mean recoveries (>93%), except for the immunoassay using antibody to CYP-OA and DEL-HRP conjugate (76 ± 8%). The regression analyses of the data sets for nine

Table 6. Comparison of Incurred Residues of Synthetic Pyrethroids in Hexane Extracts of 13 Soil and Water Samples Determined by GC/MS and Immunoassay Using Utilizing Different Antibody/Enzyme Conjugate Combinations

pyrethroid	antibody	enzyme conjugate	slope	*Y* intercept (μg/L)	correl coeff (*r*)
deltamethrin	LHDEL-KLH	LHCYP	0.98	−1.0	0.99
deltamethrin	LHDEL-KLH	LHDEL	0.89	13.8	0.98
deltamethrin	LHDEL-KLH	DEL	0.83	23	0.91
deltamethrin	CYH-KLH	LHCYP	1.12	12.7	0.97
deltamethrin	CYH-KLH	LHDEL	1.18	−13.5	0.99
deltamethrin	CYH-KLH	PBA-BULKY	0.65	15.8	0.96
deltamethrin	CYH-KLH	CYP	0.98	−5.1	0.99
bifenthrin	CYH-KLH	CYP	0.83	0.6	0.99
deltamethrin	DEL-OA	CYP	0.8	5.2	0.99
deltamethrin	FULPYR-KLH	4OHDEL	0.7	12	0.97
deltamethrin	LHCYP-KLH	CYP	0.9	8.2	0.99
bifenthrin	LHCYP-KLH	CYP	1.05	−0.1	0.99
deltamethrin	CYP-OA	DEL	0.83	−8.4	0.99

different immunoassays each showed regression coefficients of 0.99 with the *y*-axis intercepts very close to zero. The coefficients of variation at 1, 2, 5, and 10 μg/L deltamethrin in three water samples of turbidities 0−126 NTU, for 10 immunoassays with different antibody/enzyme conjugate combinations were between 8 and 15%, as follows: LHDEL-KLH with LHCYP-HRP, 9%, with LHDEL-HRP, 15%, and with DEL, 9%; CYH-KLH with LHCYP, 10%, with LH-DEL, 12%, and with CYP, 13%; DEL-OA with CYP, 8%; FULPYR-KLH with 4OHDEL-HRP, 10%; LHCYP-KLH with CYP, 12%; and CYP-OA with DEL, 10%.

Two immunoassays that exhibited high sensitivity for bifenthrin were selected for its analysis in water (without isomerization). These were LHCYP-KLH/CYP-HRP and CYH-KLH/CYP-HRP combinations. The purified water was spiked in duplicate at 2 and 10 μg/L, and samples were analyzed without isomerization. The mean recoveries for both assays were relatively low, probably due to adsorption of bifenthrin to the glass container: 70 ± 10% for the LHCYP-KLH/CYP-HRP combination and 85 ± 12% for CYH-KLH/CYP-HRP.

Relationship between ELISA and GC Data for Field Soil and Water Samples. The sensitivities of the gas−liquid chromatography/mass spectrometry (GC/MS) method for deltamethrin and bifenthrin are relatively low. This method identified deltamethrin and bifenthrin as single peaks, and confirmation by mass spectrometry at *m*/*z* 165, 166, and 181 for bifenthrin and at *m*/*z* 77, 253, and 181 for deltamethrin was performed. The limits of detection for bifenthrin and deltamethrin were 0.01 and 0.05 mg/L, respectively, somewhat higher than those of the immunoassay.

For GC/MS analysis, soil samples were extracted using 5 mL/g hexane/acetone (1:1) and then cleaned up by Florisil column chromatography. Water samples were extracted using dichloromethane and analyzed without cleanup, except for the turbid samples (Nolting et al., 1992). Although this method was reported to provide recoveries of 95−97 and 107−111% for deltamethrin and bifenthrin, respectively, between 1 and 1000 μg/L in water, we observed mean recoveries of only 73 ± 6% for 1 and 10 μg/L spikes of deltamethrin 90 ± 5% for 2 μg/L bifenthrin, and 72 ± 1% for 10 μg/L bifenthrin. The incomplete recoveries are probably due to inefficient partitioning of the pyrethroids between water and organic phases. Recoveries from soil samples were poorer. Deltamethrin standards in methanol were recovered quantitatively after Florisil chromatography, but only 15 ± 3% of the loaded bifenthrin was recovered using the same procedure, suggesting the elution system was inefficient for bifenthrin. The hexane/acetone extraction for soil gave recoveries of <10% of the initial spiked bifenthrin concentration, also much lower than those described by Nolting et al. (1992).

To avoid the use of correction factors for poor recoveries, which could lead to additional errors, correlations between GC/MS and immunoassay data for soil and water samples with incurred residues were established by analysis of the hexane fraction (prepared for GC/MS analysis). For immunoassay, this fraction was dried, then redissolved in methanol, then analyzed after treatment with NaOH for isomerization. The regression coefficients were >0.91, suggesting data obtained from immunoassays correlated well with the GC/MS analyses (Table 6). Since close relationships between spiked and recovered levels of the two pyrethroids were obtained, the assays were next applied to the analysis of pyrethroid residues arising from agricultural use. The relationships between residues of bifenthrin and deltamethrin in hexane extracts of 13 soil and water samples (containing up to ≈80 μg/L bifenthrin or ≈500 μg/L deltamethrin by GC/MS) determined by GC/MS and immunoassay using utilizing different antibody/enzyme conjugate combinations are shown in Table 6. The relationship between the data obtained using the two analytical methods was quite linear, with linear correlation coefficients typically in the range of 0.96−0.99. Most of the slopes were close to unity, indicating near-quantitative recoveries and the lack of a systematic bias for either of the methods. The intercept on the *y* (immunoassay data) axis varied from −8 to 23 μg/L, although this range is small compared with the residue range in the solvent extract (0−500 μg/L). The two assays that produced less accurate recoveries for deltamethrin spikes into soil (CYH-KLH/CYP-HRP and LHDEL-KLH/LHCYP-HRP) provided quite good recoveries for incurred residues of deltamethrin in soil (Table 6). The FULPYR-KLH/4OHDEL-HRP combination gave only a 77% recovery in soil spiking studies; similarly, the slope of the immunoassay versus GC/MS relationship was rather low (0.7). The other combination that gave poor accuracy (CYH-KLH/PBA-BULKY) in comparative GC/MS /immunoassay studies with incurred residues was not tested with deltamethrin spikes.

Chemical isomerization produces the DM2′ isomer with a half-life of <5 days in natural water (Perschke and Hussain, 1992). Photochemical isomerization pro-

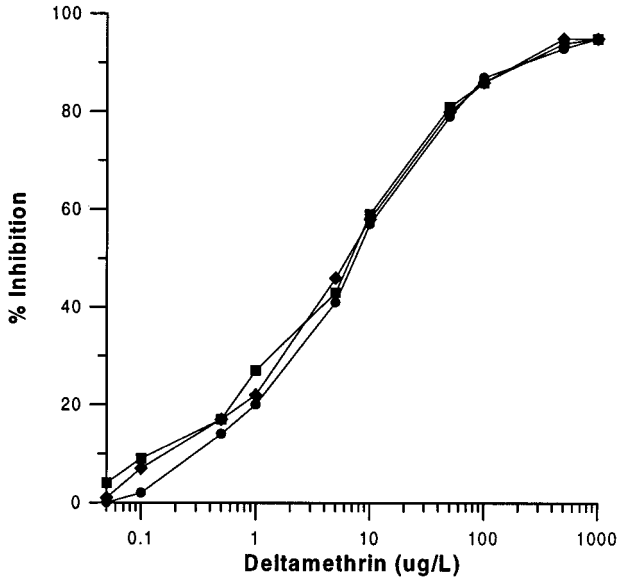

Figure 5. Absence of matrix interference in analysis of deltamethrin in extracts of wheat grain: methanol standards (▲), rapid (1 min) blending of grain in methanol (◆), methanol extraction (48 h) of whole grain (■).

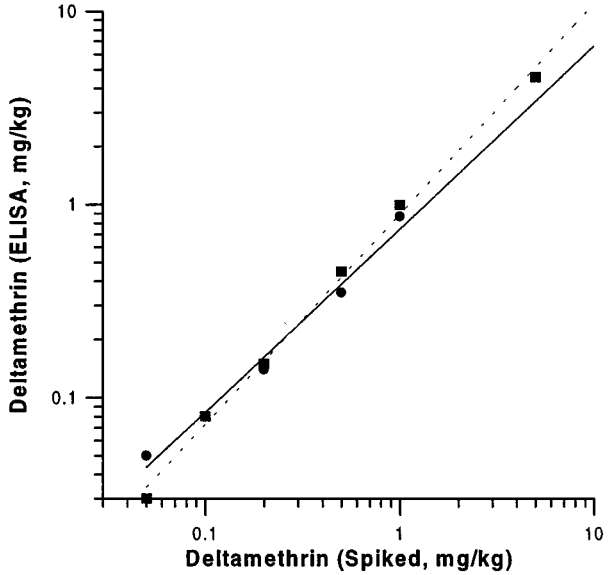

Figure 6. Relationship between deltamethrin levels spiked into wheat grain, determined using rapid (1 min) blending of grain in methanol (◆), and methanol extraction (48 h) of whole grain (■).

duces isomers, DM2′, DM3, and DM4′, and the half-lives of these isomers were also <5 days, indicating that deltamethrin was rapidly degraded in water to either less toxic isomers or oxidation products. In the preceding paper (Lee et al., 1998), we found that only two of the eight deltamethrin isomers, DM1 (the insecticidally active isomer of deltamethrin, α*S*, 1*R cis* isomer) and especially DM2 (the α*R*, 1*R cis* isomer), were sensitively detected by the immunoassays. Since the instrumental and immunoassay data for incurred residues correlated for each of the antibody combinations we studied, it is concluded that alkali treatment must convert both DM1 (found in deltamethrin formulations and analytical standards) and the isomers in "aged samples" into the more immunoreactive DM2 isomer.

Pyrethroids Spiked in Grain. The combination of LHDEL-KLH antibody/LHDEL-HRP was evaluated since it was the most specific to deltamethrin. Cross-reaction with cypermethrin was ∼15% at their IC_{50} concentrations but only 6% at the corresponding IC_{15} concentrations. Cross-reaction of other pyrethroids was <1%. Residue-specific identification of deltamethrin was important, since other compounds such as permethrin, phenothrin, bioresmethrin, and cyfluthrin can find use as grain protectants (Snelson, 1987). Initially the extraction procedures were evaluated for potential matrix interferences, by comparing standards prepared in methanol, blended methanol extract of grain, and a methanol extract of whole grain that had been shaken rather than blended. After dilution of the extracts 1:10 in 1% BSA in PBS, the standard curves from both grain extracts were superimposable with the methanol standard curves. The IC_{50} and IC_{15} (limit of detection) values for the standards in diluted grain extract were 6−7 and 0.5 μg/mL, respectively, corresponding to limits of detection of 0.025 mg/kg (rapid extraction in 5 mL/g grain) and 0.013 mg/kg, respectively (Figure 5). Analysis of the recovery by ELISA revealed good recoveries: 83 ± 14% for the standard extraction and 81 ± 13% for the rapid extraction (Figure 6). Recoveries were lower for the higher deltamethrin concentrations. This is believed to be due to incomplete dissolution of the methanolic deltamethrin extracts after their dilution in water.

GENERAL DISCUSSION

Through selection of antisera and conjugates derived from haptens differing in structure, spacer arm, and points of attachment, a series of immunoassays of differing pyrethroid specificities has been developed. Some antibody/conjugate combinations reacted with other pyrethroids and were applied to the detection of deltamethrin and bifenthrin in water and soil and of deltamethrin in wheat grain. With further validation, the assay of deltamethrin in wheat grain will provide a useful complement to the assays for other pyrethroids in grain, including phenothrin and permethrin (Skerritt et al., 1992) and bioresmethrin (Hill et al., 1993). Other groups have applied pyrethroid immunoassays to the analysis of environmental matrices. Bonwick et al. (1994) also synthesized haptens based on permethrin hydrolysis products, but in this case using either 6-aminohexanoic acid or 4-aminobutanoic acid spacer arms to link the permethric and phenoxybenzoic acids to protein carriers. Their best assay, using the latter hapten, was not particularly sensitive (IC_{50} of 1 mg/L, limit of detection of 2 μg/L), but a good correlation was obtained between levels of permethrin measured in sediment samples using immunoassay and GC/MS (Bonwick et al., 1995). The assays described in this paper are up to 2 orders of magnitude more sensitive and enabled deltamethrin and bifenthrin residues to be quantitated in water, without preconcentration.

Isomerization of deltamethrin increased its sensitivity in soil, grain, and water matrices as well as in assay standards. Detection of bifenthrin, a type I pyrethroid, did not require isomerization and had a detection limit of 2 μg/L in water. These immunoassays provided analyses of water and soil samples. The spike and recovery studies for both water and soil indicated that most of the selected immunoassays perform with high precision, and analyses of residues arising from agricultural application demonstrated that the assays can

be applied for accurate quantitation of deltamethrin and bifenthrin in these matrices. For example, any of the immunoassays except the CYP-OA/DEL-HRP combination would be useful for quantitation of deltamethrin in water, while combinations such as LHDEL-KLH/LHDEL-HRP, CYH-KLH/CYP-HRP, DEL-OA/CYP, and CYP-DEL would provide better quantitation of deltamethrin in soil. The CYH-KLH/CYP-HRP combination may be useful for detection of bifenthrin in both water and soil. This combination also detected unisomerized deltamethrin at a similar sensitively, so that the identity of the detected residues would require validation if the history of chemical use had not been established. Data obtained from immunoassays for various spiked samples and field samples correlated relatively well with those analyzed by GC/MS. The cleanup procedure and the limits of detection for the instrumental method require improvement. For both the immunoassays and GC/MS, detection of trace residues (i.e. $<0.5\ \mu$g/L) in water samples may require a preconcentration step. Solid-phase extraction, with column elution using water-miscible solvents (Hadfield et al., 1992) in which isomerization through addition of NaOH prior to immunoassay is possible, should be an attractive approach.

ABBREVIATIONS USED

DDE, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene, DDT, 1,1,1-trichloro-2,2-bis(*p*-chloroethane), GLC, gas−liquid chromatography; GC/MS, gas chromatography/mass spectrometry; NTU, nephelometric turbidity units; PEG, poly(ethylene glycol) [see also Lee et al. (1998) preceding paper].

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