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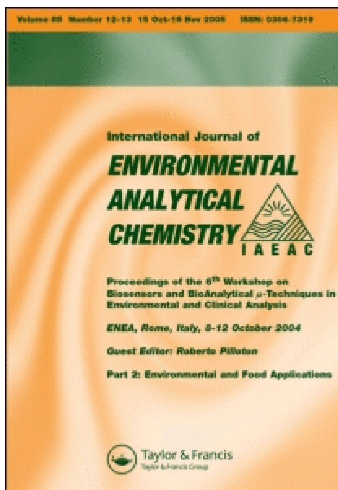

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Environmental Analysis of Cypermethrin and its Degradation Products After Forestry Applications

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To cite this Article Class, T. J.(1992) 'Environmental Analysis of Cypermethrin and its Degradation Products After Forestry Applications', International Journal of Environmental Analytical Chemistry, 49: 4, 189 — 205
To link to this Article: DOI: 10.1080/03067319208027571
URL: http://dx.doi.org/10.1080/03067319208027571

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Intern. J. Environ. Anal. Chem., Vol. 49, pp 189-205
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ENVIRONMENTAL ANALYSIS OF CYPERMETHRIN AND ITS DEGRADATION PRODUCTS AFTER FORESTRY APPLICATIONS

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(Received, 23 December 1991; in final form, 20 February 1992)

Cypermethrin and alphamethrin ([1*R*, cis, α*S*]- and [1*S*, cis, α*R*]-cypermethrin) are pyrethroid insecticides used in agriculture, forestry, and public health hygiene. After forestry applications of cypermethrin against bark beetles diastereo- and enantioselective analyses of pyrethroid residues are performed by gas chromatography (GC) with electron capture (ECD) and mass spectrometric (MS) detection, or by achiral and chiral high performance liquid chromatography (HPLC). The major soil metabolites of cypermethrin, i.e. 2,2-dimethyl-3-(1,1-dichlorovinyl)-cyclopropylic acid and 3-phenoxybenzoic acid, are analyzed as methyl esters by GC-MS with electron-impact (EI) ionization. On spruce bark cypermethrin persists several months as surface films of 1 $\mu g/cm^2$. In soil it is detected as long as seven months after treatment at levels of 0.15-0.7 μg/g. Sunlight-induced cis, trans isomerization on bark and leaves results in an excess of trans-cypermethrin isomers. In soil cis-cypermethrin isomers are more persistent than their corresponding trans-isomers. Enantioselective HPLC demonstrates chiral discrimination of a pair of trans-cypermethrin enantiomers. Analysis of soil metabolites is less meaningful since traces of the investigated products are formed from pyrethroid residues during the extraction and derivatization procedures.

KEY WORDS : Cypermethrin, gas chromatography, mass spectrometry, high performance liquid chromatography, chiral separations, environmental degradation.

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INTRODUCTION

The four pyrethroids permethrin, cypermethrin, deltamethrin (Figure 1a), and fenvalerate represent about one third of the world insecticide market and had an estimated volume of about 1.5 billion US $ in 1987 [1,2]. These insecticides are more potent than the natural pyrethrins, but are considered to be safe for mammals due to rapid biotransformation and excretion by the mammalian catabolic system[3-8]. Despite the fact that the modern pyrethroids are also more stable, they are still photo- and biodegraded considerably faster[7,9-12] than the persistent chlorinated insecticides such as DDT, hexachlorocyclohexane, chlordane, or toxaphene. Effects of the pyrethroids on non-target organisms in terrestrial and aquatic environments, including large-scale riverine and pine forest applications, have been reviewed lately[9,10].

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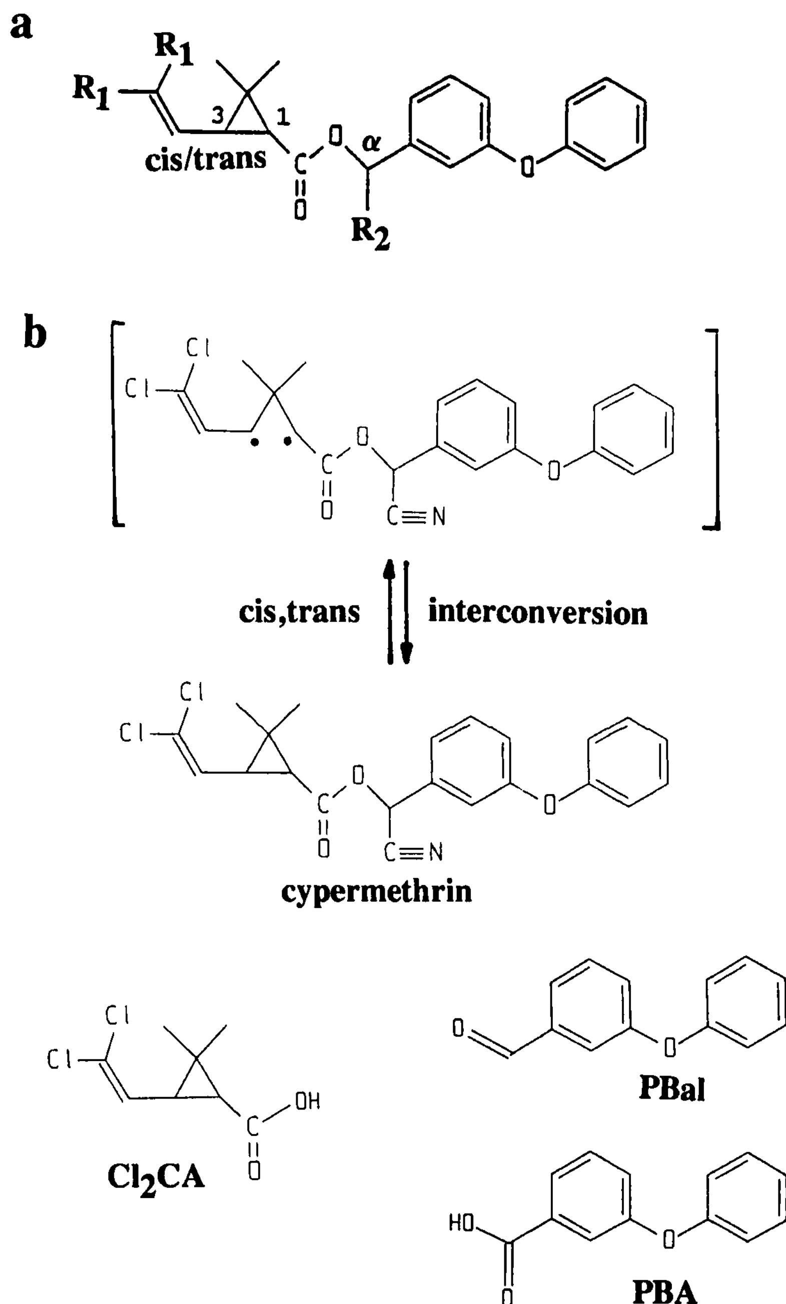

Figure 1 a and b Structures and names of the important pyrethroids (a) and of the major cypermethrin transformation products (b).

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Cyphenothrin (two isomers)	$R_1 = CH_3$	$R_2 = CN$
Permethrin	$R_1 = Cl$	$R_2 = H$
Cypermethrin	$R_1 = Cl$	$R_2 = CN$
Alphamethrin	$R_1 = Cl$	$R_2 = CN$
[1*R*, cis, α *S*]- and [1*S*, cis, α *R*]-enantiomers		
Deltamethrin	$R_1 = Br$	$R_2 = CN$
[1*R*, cis, α *S*]-enantiomer		

Cypermethrin and alphamethrin contribute about one fifth of the world pyrethroid market and are used mainly on cotton. In Europe they are applied in the cultivation of wheat, corn, potato, rape, hops, vegetables and fruits. In forestry cypermethrin replaces lindane and is the active ingredient of insecticidal formulations (e.g. Ripcord) intended to prevent damage of stored timber and to avoid epidemic infestation of pine forests by bark beetles. Cypermethrin is also present in spray formulations for indoor and household usage against biting and stinging insects[13].

Similarly to the photochemical degradation of permethrin and deltamethrin, photochemistry of cypermethrin involves interconversion of cis- and trans-isomers leading to an excess of the thermodynamically more stable trans-isomers. Ester cleavage with successive oxidation of the α carbon atom forms the 3-phenoxybenzoic acid (PBA) (Figure 1b). Other possible pathways for photodegradation are the opening of the cyclopropyl ring, the replacement of one chlorine by hydrogen, and decarboxylation[11,12].

Major soil metabolites of cypermethrin are 2,2-dimethyl-3-(1,1-dichlorovinyl) cyclopropane carboxylic acid (Cl_2CA) and 3-phenoxybenzoic acid (PBA). Additional metabolites originate from hydroxylation of the 4'-position in the phenoxy moiety and at a methyl substituent of the cyclopropyl ring[11] (Figure 1b).

Analysis of pyrethroid residues[11,13-20] in various matrices usually involves extraction with polar solvents or solid phase extraction, clean-up by adsorption or gel permeation chromatography, gas chromatography (GC) with electron capture (ECD) or mass spectrometric (MS) detection, or high performance liquid chromatography (HPLC) with UV detection.

In this study GC and HPLC analyses of cypermethrin after forestry applications were performed. Our goal was to investigate persistence, to monitor modifications of the isomeric composition, and to investigate environmental degradation by analysis of soil metabolites. These were identified after methylation by GC-MS with electron impact (EI) ionization.

EXPERIMENTAL

Chemicals

Solvents were Nanograde from Promochem. Anhydrous sodium sulfate (p.a.), potassium chloride (p.a.), and Florisil (60 - 100 mesh, activated at 350 °C) were from Merck.

The pyrethroids cypermethrin (cyper), alphamethrin (the [1*R*, cis, α*S*] and [1*S*,cis, α*R*]

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enantiomers of cypermethrin), permethrin and deltamethrin were obtained from Riedel-de-Haën or Promochem, cyphenothrin (cyphen) was a gift from Thompson-Siegel and originated from Sumitomo. The Ripcord 40 formulation was from Shell Agrar and contained 400 g/l cypermethrin in xylene.

Chrysanthemic acid ethyl ester (CAEt), 3-phenoxybenzyl alcohol (PBol), 3-phenoxybenzaldehyde (PBal) and 3-phenoxybenzoic acid (PBA) were from Fluka. Chrysanthemic acid (CA) and 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (Cl_2CA) were obtained by hydrolysis (5 % NaOH in methanol, 1 h reflux) of CAEt and permethrin, respectively. Methyl esters of the acids (CAMe, Cl_2CAMe, and PBAMe) were prepared (20 min, 60°C) with 10 % BF_3 in methanol (Fluka). The ratios of the cis- and trans-isomers of CAMe and Cl_2CAMe were about 1:3. Hydrolysis of cypermethrin and subsequent methylation of the Cl_2CA resulted in a cis to trans ratio of 1:1. The trimethylsilyl (TMS) ether of PBol (PBOTMS) was formed by treatment (15 min, 60°C) with N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA, Macherey-Nagel). All products were characterized by GC flame ionization detection (FID) and by GC-MS using electron impact ionization (EI) and negativ chemical ionization (NCI).

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Chromatography

For GC-FID and GC-ECD analyses a Hewlett-Packard 5890 Series II gas chromatograph was equipped with a HP 7673B autosampler, programmable cool on-column and split/splitless injectors, FID and ECD (argon/methane, 300 °C) detectors, and a HP 3365 chemstation. A J&W DB 5 fused silica capillary column (25 m, 0.25 mm i.d., 0.1 µm film was used with H_2 (80 kPa, 60 cm/s) as carrier gas and the following temperature programs: from 70 °C (1 min) to 220 °C at a rate of 30 °C/min and then to 260 °C at 1 °C/min for the pyrethroids; or from 80 °C (2 min) to 280 °C at 5 °C/min for GC-FID analyses of their degradation products.

For GC-MS analyses a Hewlett-Packard 5890 GC was used, equipped with a split/splitless injector and a HP Ultra 5 fused silica capillary column (25 m, 0.32 mm i.d., 0.17 µm film) directly interfaced to a VG TS 250 mass spectrometer. With helium as carrier gas (40 kPa, 50 cm/s) the temperature program was from 80 °C (2 min) to 290 °C (3 min) at 5 °C/min. EI-MS was performed with a source temperature of 180 °C and an electron energy of 70 eV. Selected ion recording (SIR) technique was done with m/z 207 as lock mass, up to 15 ions were recorded with a dwell time of 50 ms. NCI-MS was carried out with methane or ammonia as reactant gases and a source temperature of 140 °C.

Normal-phase (NP) HPLC was performed using a Knauer Model 64 HPLC pump and a Gynkotech SP-4 variable wavelength spectrophotometer set at 220 nm. The flow rate was 1 ml/min. Achiral NP-HPLC separations were carried out on unmodified silica gel (Merck Lichrospher Si 100, 5 µm, 120 x 4 mm i.d. column) with 0.5 % tetrahydrofuran in cyclohexane as mobile phase. For chiral NP-HPLC we used silica gel modified with L-tartaric acid and L-dinitrobenzylphenylethylamine (Macherey-Nagel Chiral-2, 5 µm, 250 × 4 mm i.d. column) as a Pirkle-type column with 0.05 % trifluoroacetic acid and 0.05 % 2-propanol in hexane as mobile phase.

Sampling, extraction, derivatization, and column chromatography

The sampling sites (Rübhau, a dry and sunny site, and Tränke, a site located in a dense and humid forest area) are located in a forest near Ulm/Danube in Southern Germany. The application of the diluted (recommendation: 1 g/l, 2-3 l per m^3 of stored timber) Ripcord formulation took place on March 15, 1991. Samples were collected from spring to fall 1991.

Sampling of thin (0.1-0.3 cm) bark slices from treated and untreated spruce trunks harvested in winter was done with a knife. Shedded needles, leaves, and soil samples were collected around the stored and treated timber. Soil samples were also taken several weeks after the removal of the trunks in August (Tränke) or September (Rübhau). All samples were kept in sealed glass bottles at 0 °C in the dark.

Small squares (1 cm^2, about 0.1 g/cm^2) were cut from different bark pieces randomly chosen from a sample and combined to give a representative 5 cm^2 subsample. Wet leaves (about 50 cm^2) or needles and soil samples (5 or 10 g) were homogenized and mixed with 5 g of sodium sulfate. Prior to the Soxhlet extraction (1 h with 70 ml of ethyl acetate or dichloromethane) cyphenothrin (1 to 10 μg in isooctane) was added as internal standard (IS).

The raw soil extracts analyzed for metabolites were concentrated to about 2 ml, transferred into conic centrifuge vials and further evaporated to yield an oily residue, which was partly redissolved by treatment with 10 % BF_3 in methanol (0.5 ml, 60 °C, 20 min). The methanolic extract was cooled and diethyl ether (3 ml) and saturated KCl solution (4 ml) were added. The two phases were vortexed and the organic phase was washed twice with saturated KCI solution, dried with sodium sulfate and evaporated to dryness. The residue was dissolved by 0.2-0.5 ml ethyl acetate. Addition of MSTFA allowed silylation (60 °C, 15 min) of hydroxy groups (such as in PBol) but decreased the yields of CAMe, CAEt, Cl_2CAMe, and PBal in recovery studies.

Column chromatography of the derivatized or underivatized extracts was performed by using 10 g of Florisil (1.5 % water) in glass columns (30 cm, 10 mm i.d.). The first fraction was eluted with 50 ml of hexane. The second fraction (50 ml hexane/ethyl acetate 9:1) contained all pyrethroids, the esters CAMe, CAEt, Cl_2CAMe and PBAMe, the aldehyde PBal, and the TMS-derivative PBOTMS. The underivatized carboxylic acids and PBol could be eluted in a third fraction with hexane/ethyl acetate (1:1) plus 0.5 % acetic acid. The second fraction was concentrated to 1 ml for GC-ECD analysis of the total cypermethrin residue when no derivatization steps were performed (injection volume 1 μl, i.e. 1/1000 of the sample); or to 0.2-0.5 ml for GC-MS analysis of the pyrethroids and their soil metabolites or the derivatives formed (injection volume 2-5 μl, i.e. 1/100 of the sample).

Extracts from bark or soil samples intended for NP-HPLC analysis were fractionated by a different procedure in order to obtain cleaner extracts for the less selective UV detection: 50 ml hexane, then 70 ml hexane/dichloromethane (9:1), 70 ml hexane/dichloromethane (7:3), 70 ml hexane/dichloromethane (3:2). The cypermethrin isomers were present in a fifth and sixth fraction using hexane/dichloromethane 1:1 (containing the first eluting cis-enantiomers), and 1:4 which eluted all other isomers. The two latter fractions were combined and concentrated to 0.2 ml for NP-HPLC analysis (injection volume 20 μl, i.e. 1/10 of the sample).

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Table 1 Structural designations of cypermethrin isomers following designations introduced for deltamethrin isomers[22-24].

isomer	*configuration*	*isomer*	*configuration*
1	1*R*,cis,αS[a,b]	3	1*R*,trans,α*S*[d]
1'	1*S*,cis,α*R*[b]	3'	1*S*,trans,αR[d]
2	1*R*,cis,α*R*[c]	4	1*R*,trans,α*R*
2'	1*S*,cis,αS[c]	4'	1*S*,trans,α*S*

a Biologically most active isomer.
b Enantiomers present in alphamethrin.
c Enantiomers obtained from alphamethrin in methanol, acetonitrile, and in methanol or acetonitrile/water mixtures but not with methanol/buffer (pH 2-6.5).
d Major interconversion products from alphamethrin on exposure to sunlight.

Discrimination and degradation experiments

With the intention to exclude discrimination of individual cypermethrin isomers and to study their degradation, experiments were performed with cypermethrin or alphamethrin on wet filter paper, on bark and on wet leaves at room temperature in the dark (one week); and as thin films (0.2 μg/cm^2) on glass plates in summer sunlight (about 70 h of sunlight). Controls were kept in the dark at 0 °C.

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RESULTS AND DISCUSSION

GC analysis

GC-FID and GC-MS analysis indicated equal amounts of all isomers in our cypermethrin standard whereas in the Ripcord formulation the first two GC peaks, assigned to two cis- and two trans-enantiomers of cypermethrin[21-24], are present with a higher abundance than the other two peaks. The ratio of the cis- to trans-isomers is about 1:1 in both cases.

On GC-ECD analysis the cypermethrin standard (4 ng/μl) is separated into four nearly resolved peaks with identical peak heights indicating equal detector response. This is confirmed by the equivalent detector signal obtained from an alphamethrin (1 ng/μl) standard. This excludes any discrimination of individual isomers by the electron capture detection mechanism.

The Ripcord formulation gave for cypermethrin the same isomeric GC-ECD pattern as expected from the GC-FID analysis. The relative abundance of the peaks did not change when the formulated cypermethrin was isolated by column chromatography (Florisil, hexane/ethyl acetate 9:1).

GC-ECD calibrations were performed using automatic on-column injections (1 and 3 μl isooctane) of solutions containing 0.01 to 10 ng/μl cyphenothrin, permethrin, cypermethrin and deltamethrin. The chemstation software was employed for automatic identification using the internal standard cyphenothrin as reference peak and for establishing the calibration functions based on area integration. For multi-level large-range calibration (e.g. 0.0075-7.5 ng for the individual cypermethrin peaks, 0.03-30 ng for cyphenothrin, per-

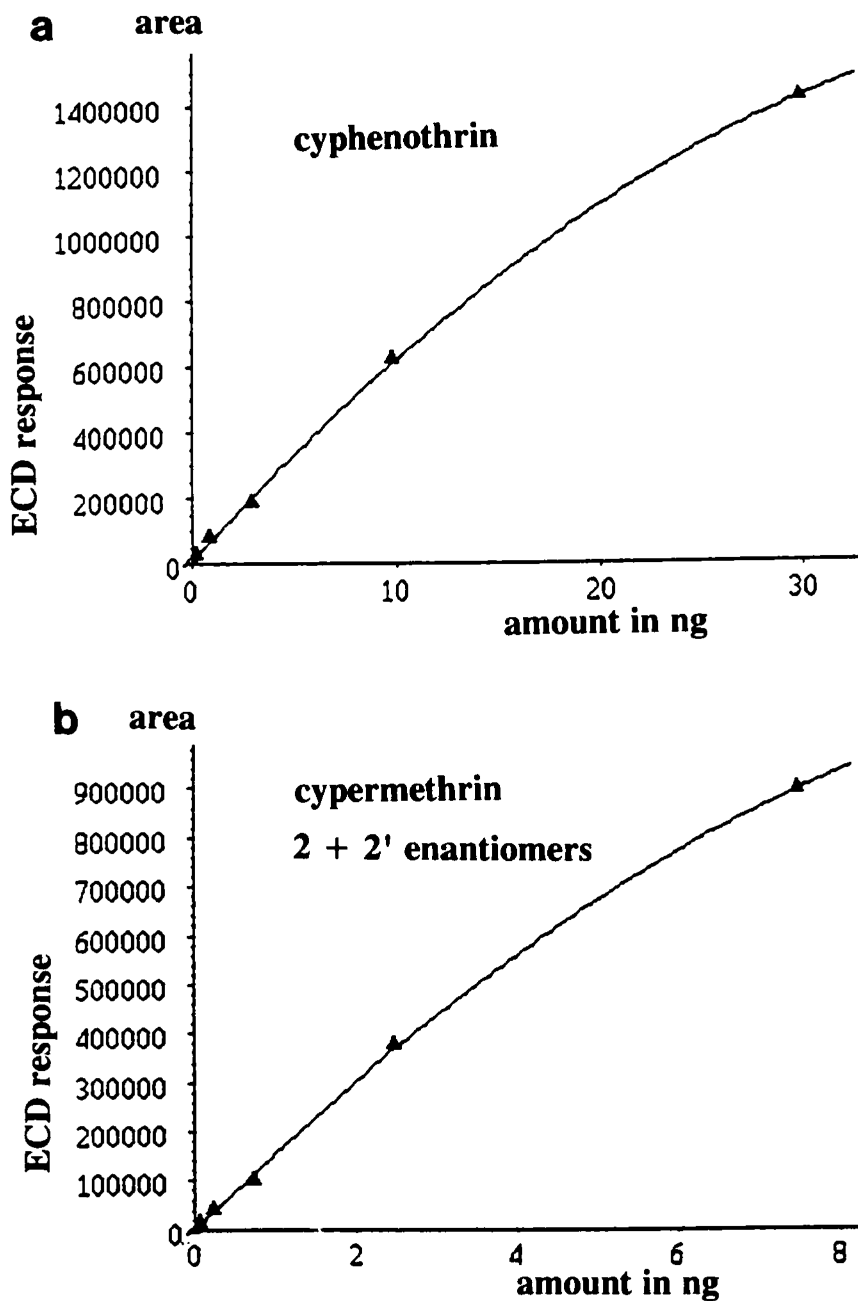

Figure 2 a and b Examples of GC-ECD multi-level large-range second order calibration functions for cyphenothrin (**a**) and a pair of enantiomers (2 + 2' see Table 1) of cypermethrin (**b**).

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Table 2 Ions selected for GC-MS(EI,SIR) analysis of cyphenothrin, chrysanthemic acid esters, and cypermethrin and some of its proposed soil metabolites or their derivatives.

Compound	M^+ *or fragment*	*ion trace m/z*	*relative intensity*[a] *%*
Cyphenothrin	$[C_9H_{15}]^+$	123	100
	$[C_6H_5OC_6H_4C]^+$	181	55
CAMe	$[C_9H_{15}]^+$	123	100
	M^+	182	65
CAEt	$[C_9H_{15}]^+$	123	100
	M^+	196	15
Cypermethrin	$[^{35}Cl_2C_7H_9]^+$	163	90
	$[^{35}Cl^{37}ClC_7H_9]^+$	165	70
	$[C_6H_5OC_6H_4C]^+$	181	100
Cl_2CAMe	$[^{35}Cl_2C_7H_9]^+$	163	50
	$[^{35}Cl^{37}ClC_7H_9]^+$	165	35
	$[M\text{-}Cl]^+$	187	100
	M^+	222	10
PBal	$[C_6H_5OC_6H_4]^+$	169	50
	$[C_6H_5OC_6H_4C]^+$	181	20
	M^+	198	100
PBAMe	$[C_6H_5OC_6H_4]^+$	169	25
	$[C_6H_5OC_6H_4CO]^+$	197	90
	M^+	228	100
PBOTMS	$[C_6H_5OC_6H_4CH_2]^+$	183	100
	M^+	272	50

[a]Relative intensities established by GC-MS(EI) under scanning conditions. These relative intensities can change with varying source and ionization conditions.

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methrin and deltamethrin) the ECD response (rsp in area units) as a function of the amount (amt in ng) injected was nearly linear and could best be described by second order calibration functions: $rsp = a * amt^2 + b * amt + c$ with the coefficients $-7 < a * 10^{-3} < 0$, $b * 10^{-3} = 69$, 49, 38, 162, and 188 for cyphenothrin, cis- and trans-permethrin, cypermethrin, and deltamethrin, respectively, and $-13 < c * 10^{-3} < 7$ (Figure 2).

Quantitative GC-ECD analysis of total cypermethrin in the samples was done by adding the individual amounts of the cypermethrin diastereomers. Recovery of the internal standard cyphenothrin was in the range of 80-110 %; reproducibility of samples analyzed in triplicate by GC-ECD was ± 15 % or better. Considering the extraction and clean-up procedure described above, the limit of detection for pyrethroid residue analysis is in the ng/g range.

GC-MS analysis

GC-MS with electron impact ionization of the pyrethroids yields only molecular ions with small (2-8 %) relative intensities. Major fragments originate from the substituted cyclopropanecarboxylic acid moiety after loss of the central carboxyl group, and from the alcohol moiety of the esters after loss of the α cyano group. GC-MS analyses of the available

or prepared metabolite standards and their derivatives gave EI mass spectra, which were used to select ions monitored in the SIR technique (Table 2).

The EI-MS(SIR) detection sensitivities of the pyrethroids and their derivatives lie in the range of 10 to 50 pg total injected amount depending on the stability of the molecular ions (e.g. for the phenoxybenzyl derivatives) and the identity and number of the fragment ions.

Under NCI conditions[25] with methane as reactant gas the pyrethroids yielded intense carboxylic acid anions, i.e. at m/z 167 for cyphenothrin and other chrysanthemates, at m/z 207 and 209 for cypermethrin and its analogue permethrin, and at m/z 137 for deltamethrin after loss of two bromines. Thus NCI-MS is a very selective detection technique and results in excellent sensitivities. With ammonia as NCI reactant gas the mass spectra of the pyrethroids are dominated by signals originating from the alcohol moiety of the pyrethroid ester (i.e. m/z 183 from permethrin and m/z 208 from cyphenothrin, cypermethrin, and deltamethrin) with the carboxylic acid anions being only the second or third largest signal in the NCI mass spectra.

The esters of CA and Cl_2CA at 50 ng per compound were detected by NCI with methane as reactant gas only after background subtraction was performed. PBal, PBAMe, PBol, and PBOTMS gave more intense characteristic ions at m/z 198, m/z 227, m/z 198, and m/z 271, respectively, but their NCI signals were still much smaller than that of the parent pyrethroids.

As these results demonstrate, NCI-MS is preferable for mass spectrometric detection of parent pyrethroids in environmental samples[18], or for pyrethroid transformation products preserving the central ester moiety[20]. Positive chemical ionization (PCI) has been used successfully to study *in vitro* metabolism of the natural pyrethrins and allethrin[26]. For the investigation of the pyrethroid soil metabolites EI ionization proved to be more universal with limits of detection in the ng/g range and therefore became the technique of choice in the present study.

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HPLC analysis

NP-HPLC on silica gel separates the cypermethrin diastereomers with a cis, cis, trans, trans elution order, whereas the Pirkle-type phase gives a cis, trans, cis, trans sequence and resolves three of the four pairs of enantiomers. Assignment of the cypermethrin HPLC separations was done according to previous reports[24,27].

Preliminary studies showed that due to the lack of selectivity with UV detection, we had to replace ethyl acetate by dichloromethane in the extraction and clean-up procedure in order to obtain fractions with less interfering material.

In a different study[24] reversed-phase (RP) HPLC was performed on achiral C_{18} and chiral β-cyclodextrin silica gel based stationary phases. However, RP-HPLC with aqueous mobile phases showed to be not suitable for isomer selective analysis of cypermethrin due to isomerization at the α carbon atom and was therefore not employed in the course of the present investigation.

Cypermethrin residues on spruce bark, on leaves and in soil

After the application of Ripcord 40, bark samples from treated spruce were collected at different intervals. Starting from 4-5 $\mu g/cm^2$ on the third week after the application, the total cypermethrin on bark from the humid site Tränke is reduced to less than 1 $\mu g/cm^2$ after two months, whereas at the sunny and dry location of Rübhau about 1 $\mu g/cm^2$ remain even after four to five months (Figure 3).

Leaves and shedded needles, collected in the direct proximity of the Rübhau site and already present during the March treatment, showed cypermethrin residues of 0.44 and 0.23 $\mu g/cm^2$ three and six weeks after the application, respectively. In a sample of dry sun-exposed leaves collected four months after the application, 0.14 $\mu g/cm^2$ cypermethrin was recovered whereas on wet leaves from a shaded place only 0.02 $\mu g/cm^2$ cypermethrin could be detected.

Soil, collected at the Rübhau site directly under the log pile about five months after the treatment, gave a total cypermethrin residue of 0.8 $\mu g/g$. Seven months after the application of the Ripcord formulation and after the removal of the timber, soil samples had cypermethrin residues of 0.7 $\mu g/g$ at the Rübhau and 0.15 $\mu g/g$ at the Tränke site.

With the recommended application procedure the treatment of 50 m^3 of stored wood (e.g. 20 trunks with a length of 20 m and a diameter of 0.4 m) would require at least 100 g of cypermethrin applied to a bark surface of about 500 m^2 resulting in an initial cypermethrin deposition of 20 $\mu g/cm^2$. As this estimated amount is four times higher than our first value (4-5 $\mu g/cm^2$) obtained three weeks after the application, one could consider either a loss of 75 % of the active ingredient by spray drift, dripping, or evaporation during the process of drying, or, more likely, the use of less concentrated formulation of the insecticide due to economic or ecological reasons.

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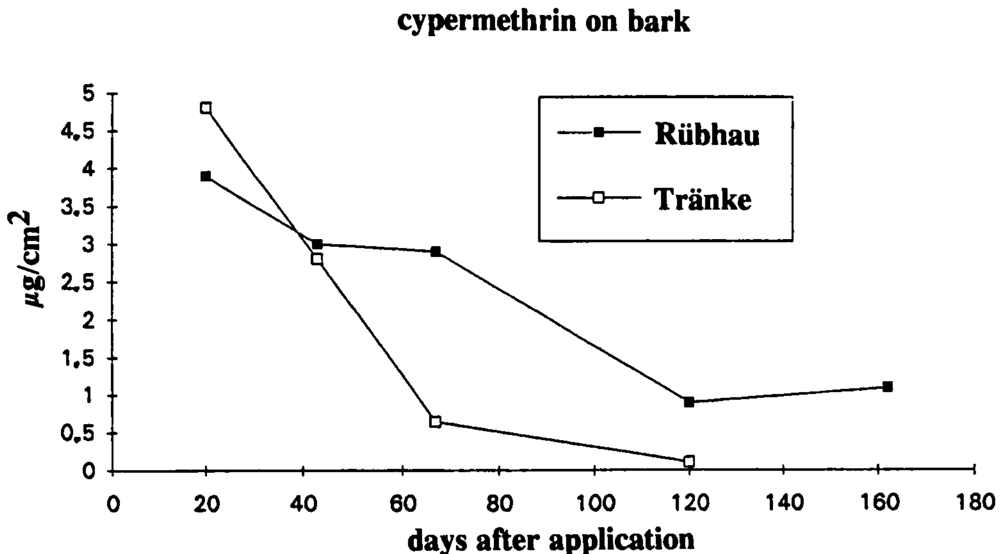

Figure 3 Total cypermethrin ($\mu g/cm^2$, mean of three determinations) on bark samples from two different locations of stored spruce logs treated with Ripcord 40 formulation March 15th, 1991.

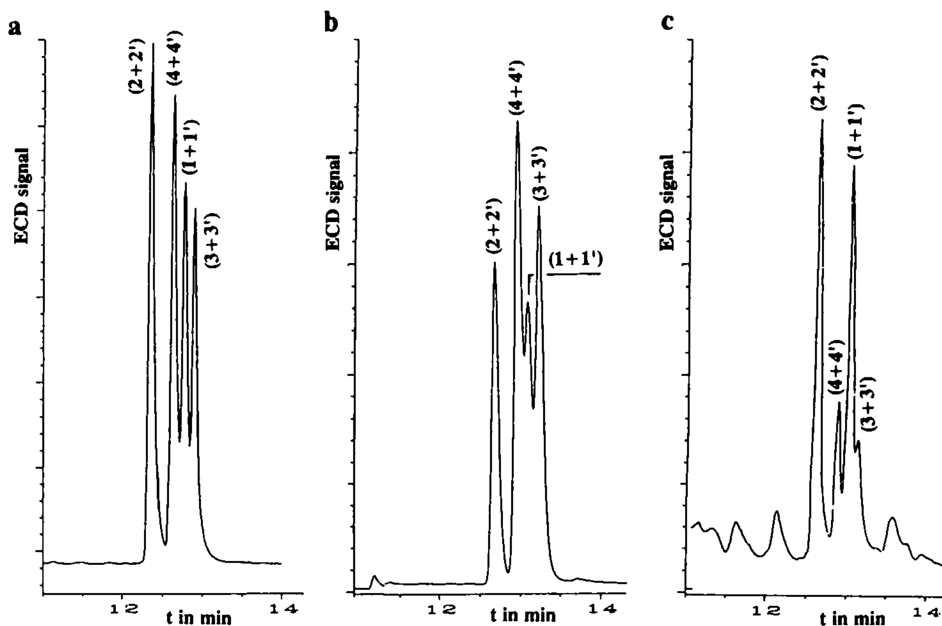

Figure 4 a-c GC-ECD chromatograms of cypermethrin isomers in the Ripcord formulation (**a**) and in a bark sample (1 $\mu g/cm^2$) (**b**) collected 120 days after the application at the Rübhau sampling site. All bark samples from both locations showed the same relative abundances of the resolved diastereomers. Soil samples from the Rübhau site (0.7 $\mu g/g$) and from the Tränke site (0.15 $\mu g/g$) (**c**) collected seven months after the cypermethrin application. For assignment see Table 1.

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Isomeric pattern of cypermethrin residues

Residues of cypermethrin from bark and leave samples collected at both sampling locations showed a modified isomeric pattern. In three weeks after the application the ratio of the cis- to trans-cypermethrin peaks decreased from 1:1 in the Ripcord formulation (Figure 4a) to about 0.7:1 and remained at this ratio even after four months (Figure 4b). On leaves sampled underneath the treated logs the isomeric pattern from the original formulation is retained for the first three weeks, but after four months of environmental degradation the composition of the residual cypermethrin changed with a remarkable loss of the cis-enantiomers (1 + 1') also present in alphamethrin.

Soil collected five months after the treatment of the log pile gave an isomeric pattern with reduced amounts of the trans-cypermethrin isomers. They were further diminished in the soil samples collected after seven months when the timber was removed. While at the dry Rübhau site the trans-isomers were still present the wet soil from the Tränke site contained mostly cis-cypermethrin (Figure 4c).

Extracts from bark samples were fractionated according to the modified florisil/hexane/dichloromethane procedure and analyzed by NP-HPLC on silica gel. They also showed reduction of the two pairs of cis-enantiomers, which elute in this case as the first and the second peak (Figure 5a and b).

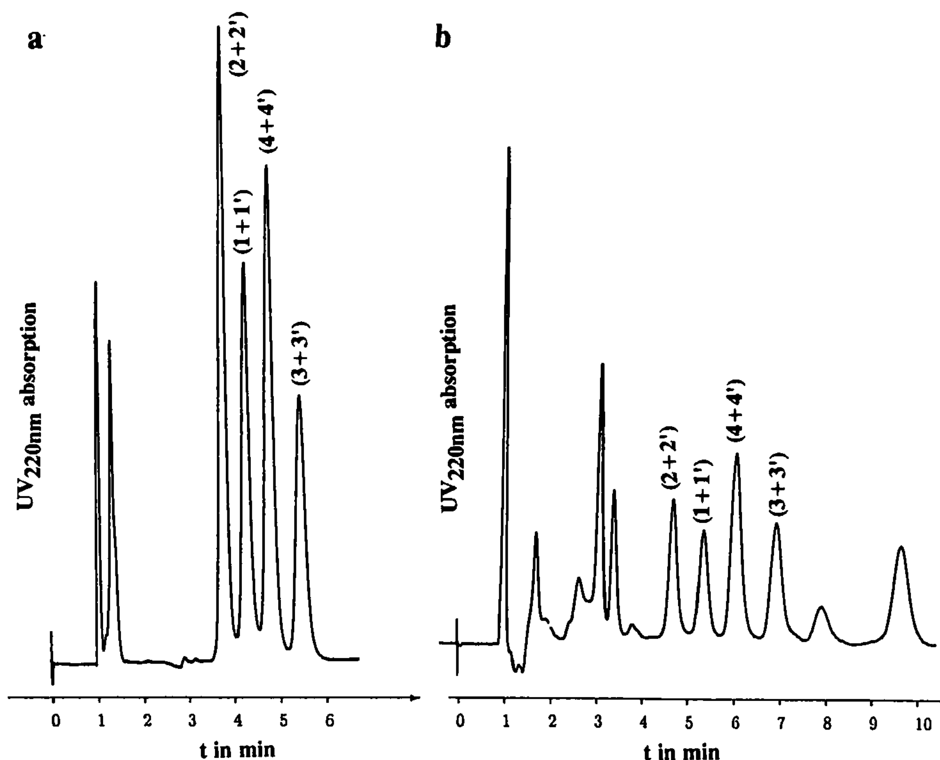

Figure 5 a and b NP-HPLC chromatograms (silica gel) of cypermethrin in the Ripcord formulation (**a**) and of residual cypermethrin isomers (3 $\mu g/cm^2$) on bark from the Rübhau site collected 21 days after the application (**b**). For assignment[24] see Table 1.

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Chiral NP-HPLC with the Pirkle-type phase separated the eight cypermethrin stereoisomers into seven peaks (Figure 6a). Soil extracts obtained by the more selective florisil/hexane/dichloromethane procedure (Figure 6b and c) showed no significant chiral discrimination of the persistent cis-cypermethrin enantiomers. The resolved pair of trans-enantiomers in the Rübhau soil sample, however, indicates a more rapid disappearance of the first eluting enantiomer assigned to the [1*R*, trans, α*R*] isomer. This stands in contrast to a study by Chapman[27], who found by NP-HPLC on a similar Pirkle-type phase, that the later eluting enantiomer, there assigned as [1*S*, trans, α*S*], is less persistent in moist soil spiked initially with 10 μg/g of cypermethrin. This discrepancy can be explained either by reversed chiral separation (and hence reversed assignment in our HPLC studies[24]), or by differences in enantio-selective microbial degradation.

Discrimination and degradation experiments

When untreated bark, wet filter papers, or glass plates were treated with cypermethrin or alphamethrin and kept in the dark at 0 °C or at room temperature for one week, the isomeric

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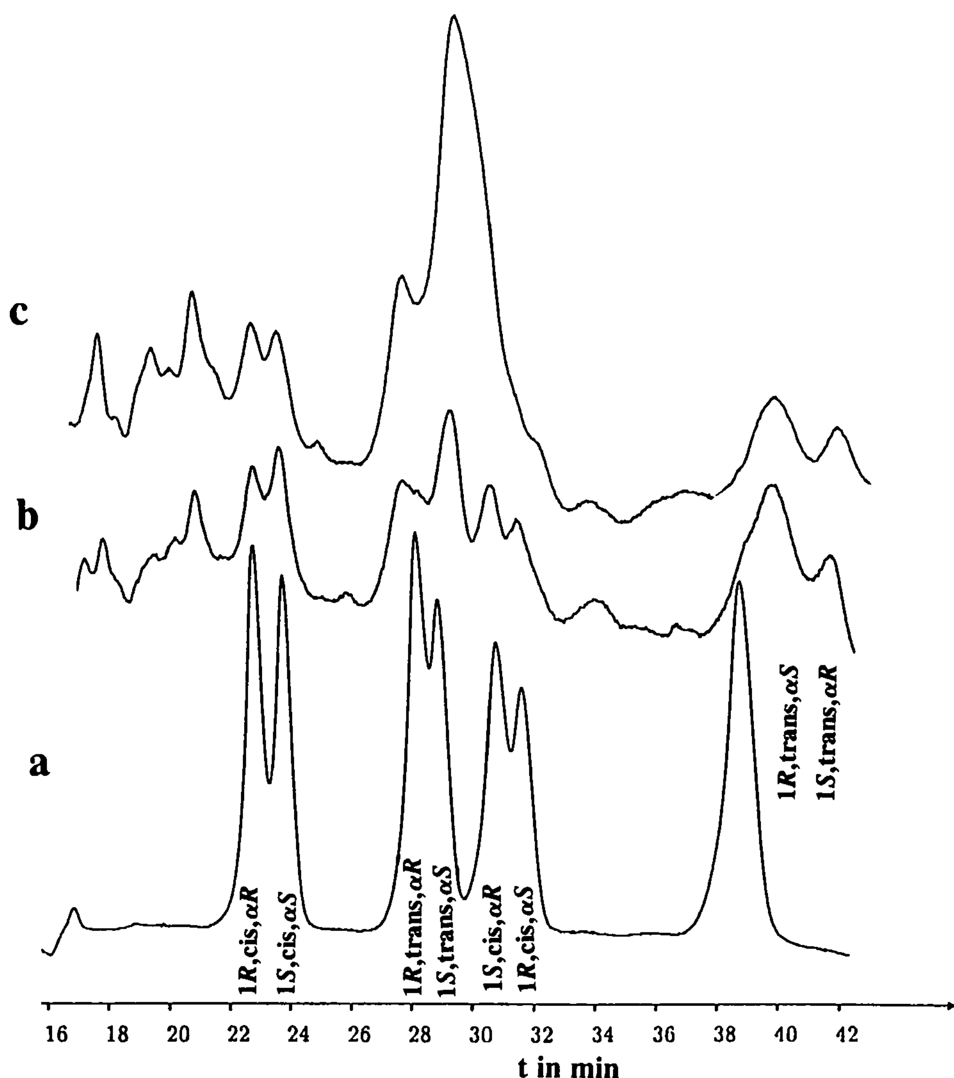

Figure 6 a-c NP-HPLC chromatograms (chiral Pirkle-type phase) of cypermethrin standard (**a**) and residual cypermethrin in soil extracts (fractionated by the modified florisil/hexane/dichloromethane procedure) from the Rübhau (**b**) and the Tränke (**c**) site. Assignment according to[24] and[27].

pattern of cypermethrin did not change and alphamethrin did not isomerize. Cypermethrin exposed to sunlight (70 h) as a thin film on a glass plate, however, gave an isomeric pattern with reduced amounts of cis-isomers (Figure 7a) similar to the one found on treated bark, leaves and needles from the forest (Figure 4b).

Alphamethrin, consisting of a mixture of the [1*R*,cis,α*S*] and [1*S*,cis,α*R*] enantiomers (1 and 1') of cypermethrin, was exposed to intense sunlight for 70 h. It underwent isomerization at the 1,3-cyclopropyl bond, resulting in the corresponding [1*R*,trans,α*S*] and [1*S*,trans,α*R*]

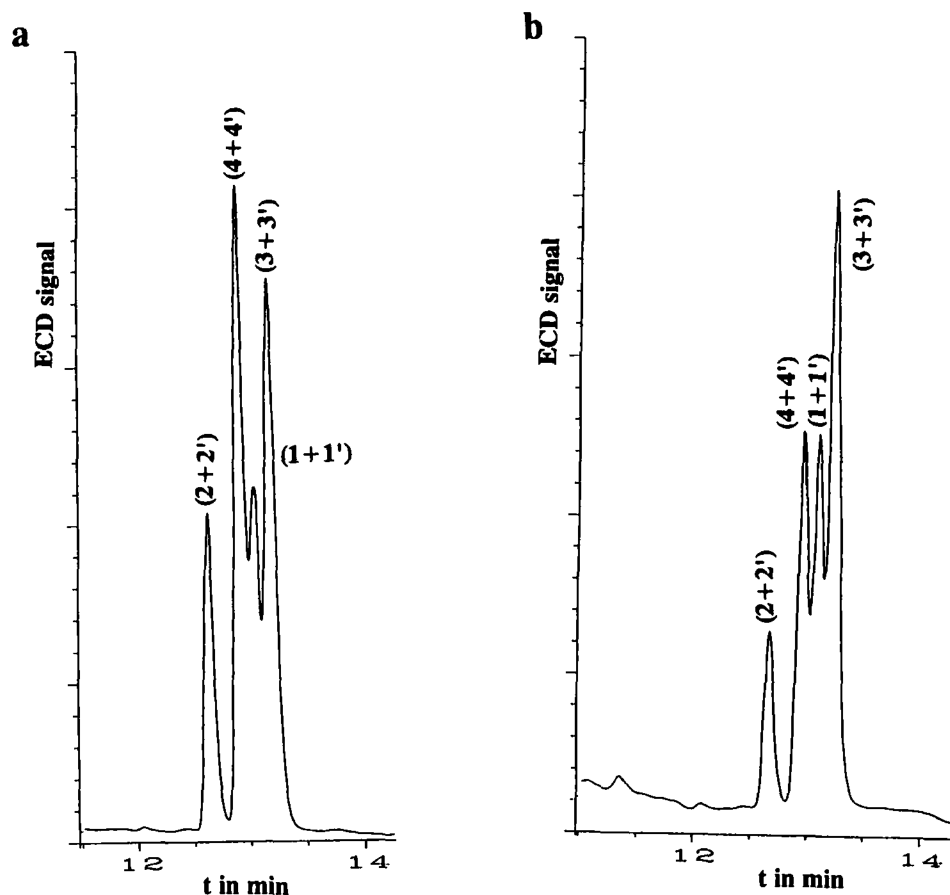

Figure 7 a and b GC-ECD chromatograms of cypermethrin (**a**) and alphamethrin (**b**) exposed to intense sunlight for about 70 h. For assignment see Table 1.

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enantiomers present in the largest peak (3 + 3') of the isomeric pattern in Figure 7b. Isomerization at the α carbon atom is also observed and yields the corresponding isomers with the [1*R*,cis,α*R*] and [1*S*,cis,α*S*] configuration represented by the first and smallest (2 + 2') peak, and, in combination with the cis, trans interconversion, the [1*R*,trans,α*R*] and [1*S*,trans,α*S*] isomers present in the second (4 + 4') peak of the cypermethrin chromatogram.

Analysis of soil metabolites

Soil metabolites of cypermethrin were analyzed by a combination of derivatizations, column chromatography, high resolution gas chromatography and selective detection by mass spectrometry in the EI-MS SIR technique. When untreated soil was spiked with cyphenothrin and cypermethrin (0.1 μg/g) traces of Cl_2CAMe, PBal and PBAMe were

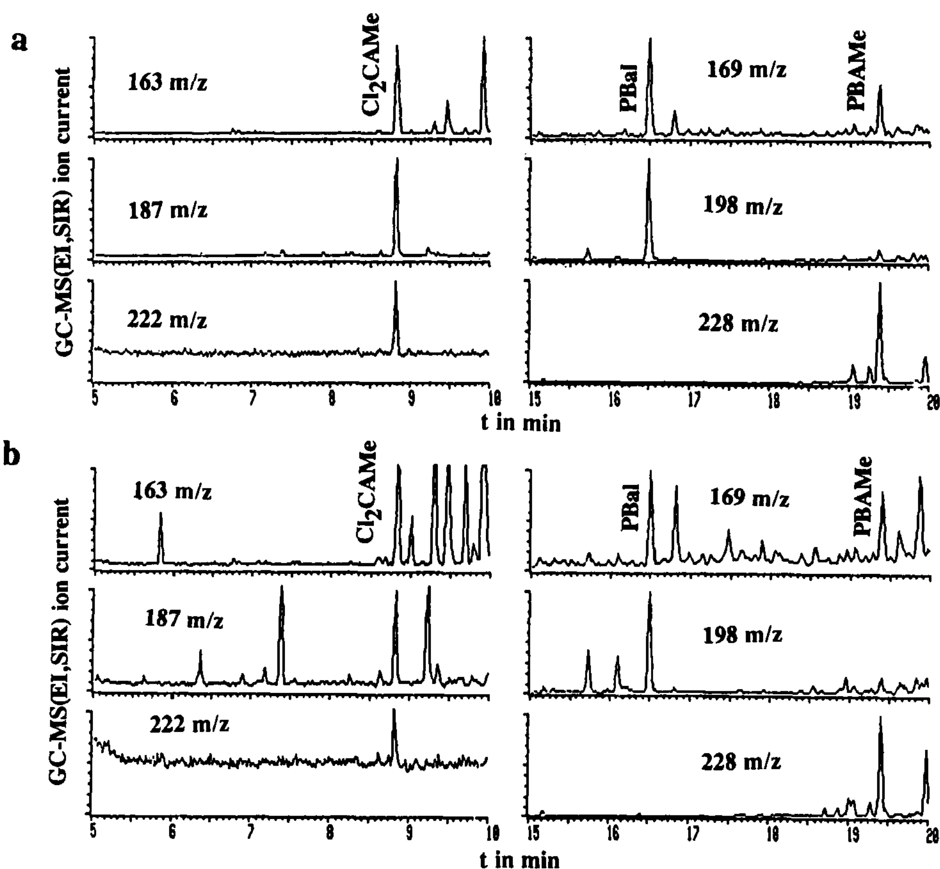

Figure 8 a and b GC-MS(EI) selected ion chromatograms of soil extracts: soil (5g) from the Rübhau sampling site (**a**); and soil (10g) from the Tränke site (**b**). Samples were collected seven months after the Ripcord application.

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observed in yields of 0.2-0.4 %. These amounts, however, were 2-10 times smaller than the recoveries of the derivatized metabolites in the soil samples from the forest application sites.

Figures 8a and b give the selected ion chromatograms for Cl_2CAMe, PBal, and PBAMe in soil extracts from the Rübhau and the Tränke site, respectively. Only trans-Cl_2CAMe is detected in both soil samples and the amount of the three metabolites is in the range of 1-10 ng/g of soil or about 1-10 % of the residual cypermethrin.

CONCLUSIONS

Environmental analysis of cypermethrin residues after forestry applications is performed by Soxhlet extraction, florisil column chromatography and GC-ECD or GC-MS with limits of detection in the ng/g range.

Diastereoselective separations on apolar GC phases and with NP-HPLC on silica gel reveal sunlight induced cis, trans interconversion about the 1,3-cyclopropane bond, resulting in reduced portions of insecticidal cis-cypermethrin isomers. In soil, however, the cis-isomers are more persistent against microbial attack than the trans-isomers. Thus, environmental degradation is mainly a combination of cis-to-trans photoisomerization and microbial degradation of the less persistent trans-isomers. Chiral discrimination by biodegradation is observed for one pair of trans-enantiomers when residual cypermethrin from soil is analyzed by chiral NP-HPLC on a Pirkle-type stationary phase.

Soil metabolites can be analyzed after derivatization by GC-MS with EI ionization. However, formation of potential metabolites in the course of the extraction and derivatization procedure is observed. This makes detection of the cypermethrin metabolites less meaningful.

Acknowledgment

The continued support of Professor Dr. K. Ballschmiter is gratefully acknowledged. Forester Wittlinger assisted in choosing the sampling sites. S. Reusch, W. Steiner, and P. Pfuhl provided assistance in sample preparation, and J.P. Kutter performed the HPLC analyses. The University of Ulm and the Fonds der chemischen Industrie (FCI) granted financial support.

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