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Decamethrin Metabolism in Rats

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On oral administration to male rats, the pyrethroid insecticide decamethrin [(*S*)-α-cyano-3-phenoxybenzyl (1*R*, 3*R*)-*cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate] and various metabolites derived from its acid and alcohol fragments are almost completely eliminated from the body within 2–4 days. Metabolites of the cyano substituent are eliminated more slowly, especially from the skin and stomach, due in the latter case to temporary retention of thiocyanate which is formed from released cyanide. The excreted metabolites include: esters monohydroxylated at the 2′, 4′, and 5 positions of the alcohol moiety; 2,2-dimethyl-3-(2,2-dibromovinyl)cyclopropanecarboxylic acid and its glucuronide and glycine conjugates and a hydroxylated derivative of this acid, with the hydroxymethyl group trans to the carboxyl, and its glucuronide; 3-phenoxybenzoic acid and its glucuronide and glycine conjugates, 3-(4′-hydroxyphenoxy)benzoic acid and its glucuronide and sulfate conjugates, and 3-(2′-hydroxyphenoxy)benzoic acid sulfate; thiocyanate and 2-iminothiazolidine-4-carboxylic acid. The trans isomer of decamethrin is also rapidly metabolized in rats.

More than 25 years of research on optimization of pyrethroids for high insecticidal activity culminated recently in discovery of the most potent pyrethroid, (*S*)-α-cyano-3-phenoxybenzyl (1*R*,3*R*)-*cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate (decamethrin) (Elliott, 1977; Elliott et al., 1974, 1975a,b; Owen, 1975), which is currently under development by Roussel-Uclaf-Procida (Paris, France) for control of insect pests of crops, livestock, and man. The (1*R*,3*R*,α*S*) configuration is essential for this remarkable potency (Figure 1).

The metabolic fate of the (1*R*,3*R*) and (1*R*,3*S*) isomers of the related pyrethroid permethrin (dichlorovinyl replacing dibromovinyl group, no cyano substituent) is well defined in rats (Elliott et al., 1976; Gaughan et al., 1977a) and other organisms (Gaughan et al., 1977b; Shono et al., 1978). On analogy with (1*R*,3*R*) permethrin metabolism in rats (Gaughan et al., 1977a), decamethrin is expected to undergo hydroxylation at the *trans*-methyl group of the acid moiety and the 2′ and 4′ positions of the alcohol moiety and to cleave at the ester linkage, resulting ultimately in a series of carboxylic acids in free and conjugated form. However, the permethrin isomers are rapidly metabolized by mouse liver microsomal esterases (1*R*,3*S*) and oxidases (1*R*,3*R* and 1*R*,3*S*) whereas each of the structural modifications introduced in decamethrin confers enhanced stability to microsomal metabolism (Soderlund and Casida, 1977a,b). On the other hand, the toxicity of decamethrin to mice is synergized by piperonyl butoxide and *S*,*S*,*S*-tributyl phosphorotrithioate, suggesting the importance of both oxidases and esterases in decamethrin detoxification (Ruzo et al., 1977; Soderlund et al., 1977b).

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An understanding of decamethrin metabolism is important in developing this new insecticide and more generally in expanding the knowledge of detoxication of novel pyrethroids. The present study considers the distribution and metabolic fate of decamethrin labeled with ^{14}C in three positions when administered orally to male rats at 0.64–1.6 mg/kg.

MATERIALS AND METHODS

Chromatography and Radiocarbon Analyses. Thin-layer chromatography (TLC) utilized silica gel 60 F-254 20 × 20 cm chromatoplates with 0.25-mm layer thickness (EM Laboratories, Inc., Elmsford, N.Y.) and the following solvent systems: A, butanol–acetic acid–water (6:1:1); B, benzene (saturated with formic acid)–ether (10:3), two developments; C, benzene–ethyl acetate (6:1); D, hexane–ether (4:1), three developments; E, hexane–ether (1:1), two developments; F, chloroform (saturated with formic acid)–ether (10:3); G, ethyl acetate–methanol–water (2:1:1); H, benzene–carbon tetrachloride (1:1), two developments. R_f values for decamethrin derivatives are given in Table I. In referring to solvent systems for two-dimensional development, (A, B) indicates development in the first direction with solvent system A and in the second direction with solvent system B. Unlabeled standard compounds were detected first with ultraviolet light (254 nm) and then by spraying with either $PdCl_2$ (0.5% w/v in 12 N HCl) or phosphomolybdic acid (20% w/v in ethanol) and heating at 110 °C for up to 30 min. Procedures for radioautography, ^{14}C quantitation, and cochromatography of ^{14}C metabolites or their derivatives with unlabeled standards are given by Ueda et al. (1975).

Chemicals. *^{14}C Compounds.* The following ^{14}C preparations (provided by Roussel-Uclaf-Procida; radiochemical purity > 99%) were used: decamethrin labeled in the dibromovinyl (^{14}Cv), benzylic carbon ($^{14}C\alpha$) and cyano (^{14}CN) substituents (Figure 1) with sp act. of 5.0, 60.0, and 51.5 mCi/mmol, respectively; the acid moiety (Br_2CA, Figure 2) labeled in the dibromovinyl substituent

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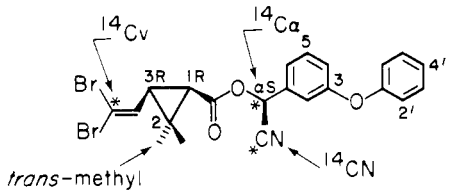

Figure 1. Structure of decamethrin showing stereochemical designations, numbering of rings, and ^{14}C labeling positions.

(^{14}Cv) (5.1 mCi/mmol). Immediately prior to each experiment, the purity of decamethrin preparations was confirmed by TLC (H, R_f 0.62 for decamethrin and 0.50 for its trans isomer) and if necessary they were purified by TLC with this solvent system.

(1*RS*)-*trans*-[^{14}Cv]Decamethrin was obtained by dissolving [^{14}Cv]decamethrin (20 μg) in 0.1 N isobutyrophenone in benzene (1 mL), irradiating this solution at 350 nm for 1 h and purifying by TLC (H) (Ruzo et al., 1977).

$K^{14}CN$ (9.0 mCi/mmol) was obtained from New England Nuclear (Boston, Mass.).

Metabolite Designations and Standards. The metabolites are designated as shown in Figure 2, e.g., Br_2CA is the acid moiety, a 4'-HO derivative is hydroxylated at the 4' position of the alcohol moiety, *t*-HO refers to the trans position for a hydroxymethyl substituent relative to the carboxyl group, PBacid is 3-phenoxybenzoic acid, and gluc and gly are glucuronide and glycine conjugates, respectively. Standard unlabeled compounds for tentative characterization of metabolites by cochromatography are described by Ruzo et al. (1977) and Unai and Casida (1977) except for those prepared specifically for the present study which are described below.

(*RS*)-α-Cyano-3-(hydroxyphenoxy)benzyl (1*R*,-3*R*)-*cis*-3-(2,2-Dibromovinyl)-2,2-dimethylcyclopropanecarboxylates (2'- and 4'-HO-dec). The reaction

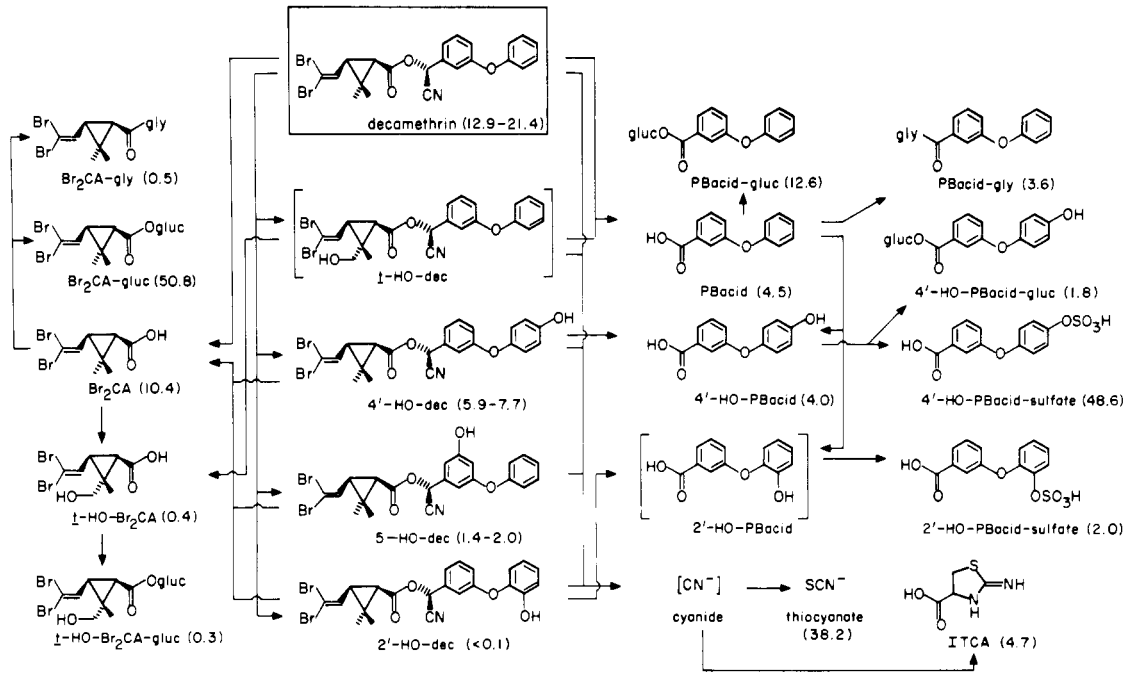

Figure 2. Metabolic pathways for decamethrin in rats indicating metabolite abbreviations and yields (percent of administered dose) in excreta after 8 days. Conjugates are designated by gluc for glucuronides and gly for glycine conjugates. Compounds shown in brackets although not detected are likely intermediates to identified metabolites. Aldehyde intermediates to benzoic acid derivatives are not shown. Glucuronides of dihydroxy compounds are arbitrarily shown with the conjugating moiety at the carboxylic acid position.

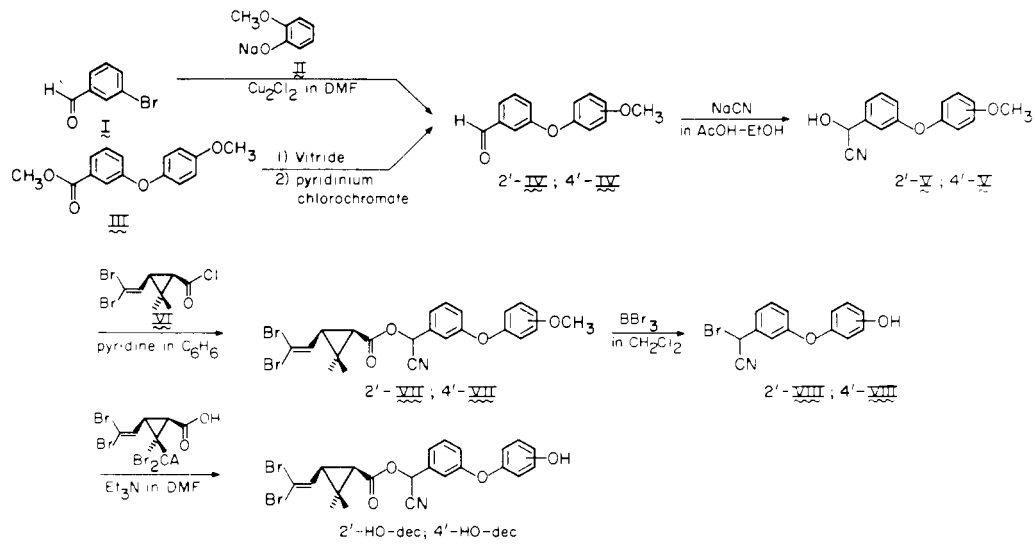

Figure 3. Synthesis of 2'- and 4'-hydroxydecamethrin (α*RS* isomer mixture).

Table I. Thin-Layer Chromatographic Properties of Decamethrin and Various Metabolites, Related Compounds, and Their Derivatives

Compound[a]	R_f values with indicated solvent systems						
	A	B	C	D	E	F	G
Decamethrin and Hydroxydecamethrin Derivatives							
Decamethrin[b]	0.84	0.82	0.59	0.59 (0.63)[c]	0.83 (0.83)[c]	0.71	0.88
2′-HO-dec		0.74	0.50	0.18 (0.22)	0.59 (0.64)	0.61	
4′-HO-dec[b]		0.63	0.38	0.11	0.44 (0.46)	0.51	
5-HO-dec[d]		0.65	0.45		0.49 (0.51)		
4′-MeO-dec (4′-VII)		0.80	0.55	0.44 (0.49)	0.74 (0.76)	0.65	
Metabolites Derived from Acid Moiety and Their Derivatives							
Br_2CA[b]	0.81	0.64	0.28	0.23	0.52	0.51	0.71
Br_2CA-Me[e]		0.80	0.62	0.77	0.87	0.66	
t-HO-Br_2CA[d]	0.80	0.31	0.07		0.19	0.11	
Br_2CA-gluc[d]	0.40						0.24
Br_2CA-gly[d]	0.65						0.50
Br_2CA-gly-Me[e]		0.39	0.16			0.39	
t-HO-Br_2CA-gluc[d]	0.30						
Unknown gluc[d]	0.36						
Metabolites Derived from Alcohol Moiety and Related Compounds							
PBalc[f]		0.48	0.29	0.15	0.45	0.40	
4′-HO-PBalc[f]		0.16	0.09	0.01	0.14	0.16	
5-HO-PBalc[f]		0.20	0.09		0.13	0.16	
PBacid[g]	0.84	0.53	0.10	0.08	0.29	0.42	0.61
2′-HO-PBacid[g]	0.80	0.29	0.03	0.00	0.09	0.22	
4′-HO-PBacid[g]	0.84	0.23	0.03	0.01	0.09	0.19	0.59
5-HO-PBacid[g]		0.33	0.04		0.14	0.25	
6-HO-PBacid[g]	0.80	0.53	0.05	0.03	0.09	0.32	
PBacid-Me[e]		0.78	0.58	0.65	0.82	0.66	
4′-MeO-PBacid-Me[h]		0.78	0.55	0.48	0.73	0.63	
PBacid-gluc[d]	0.41						0.13
PBacid-gly	0.66						0.27
PBacid-gly-Me[e]	0.78	0.27	0.15			0.29	0.70
4′-HO-PBacid-gluc[d]	0.40						0.12
2′-HO-PBacid-sulfate[d]	0.50						0.20
4′-HO-PBacid-sulfate	0.59						0.22
Unknown 1[d]	0.29						
Unknown 2[d]	0.14						
Metabolites Derived from Cyanide Moiety and Their Derivatives							
SCN^-	0.38						0.31
IX		0.64	0.42	0.08	0.30	0.48	0.75
ITCA	0.12						0.07

[a] Structures of compounds not identified in subsequent footnotes are given in Figure 2. [b] Dichlorovinyl analogues give identical R_f values (B, C, E, and F). [c] αR enantiomer of decamethrin and its hydroxy and methoxy derivatives. [d] Detected as metabolite but not available as standard from synthesis. [e] Methyl ester. [f] 3-Phenoxybenzyl alcohol and its 4′- and 5-hydroxy derivatives. [g] 3-Phenoxybenzoic acid and its 2′-, 4′-, 5-, and 6-hydroxy derivatives. [h] Methyl 3-(4′-methoxyphenoxy)-benzoate.

routes and intermediates for preparing these esters are given in Figure 3. Aldehyde 2′-IV was synthesized in 19% yield using the general procedure of Unai and Casida (1977) by reacting equimolar amounts of I and II [prepared from the corresponding methoxyphenol with NaH in dimethylformamide (DMF) solution] and a catalytic amount of Cu_2Cl_2 in a N_2 atmosphere at 165–170 °C for 4.5 h. An ether extract of the reaction mixture was washed with 10% NaOH to remove unreacted phenol, concentrated, and purified on a Florisil column by eluting with benzene. 4′-IV was obtained by reducing III with excess Vitride [sodium bis(2-methoxyethoxy)aluminum hydride in benzene] at 25 °C for 1 h (~99% yield) (Unai and Casida, 1977), then oxidizing the benzyl alcohol with 1.5 molar equiv of pyridinium chlorochromate (Corey and Suggs, 1975) in methylene chloride at 25 °C for 1.5 h, followed by concentration of the methylene chloride and purification on a short Florisil column by eluting with benzene (94% yield).

Cyanohydrins 2′- and 4′-V were prepared from the corresponding aldehydes (2′- and 4′-IV) and HCN (Elliott et al., 1974). Acetic acid (1.2 g, 20 mmol) was added dropwise to a mixture of ethanol (9 mL) and NaCN (0.49 g, 10 mmol) over a period of 40 min at −10 °C, then 2′- or 4′-IV (1.5 g, 6.6 mmol) was slowly added to the solution (20 min) and stirring continued for 1 h at −10 °C. The reaction mixture was warmed to 50 °C for 2 h, poured into ice–water (70 mL), and extracted with ether (20 mL × 3). The ether extract was washed with water and dried over Na_2SO_4. The concentrate (solvent free) contained 2′- or 4′-V (90% yield) and minor amounts of 2′- or 4′-IV. The resulting methoxylated cyanohydrins were used for esterification without further purification.

Esters 2′- and 4′-VII were synthesized from the corresponding cyanohydrins (1.5 g, 5.9 mmol) by reacting with equimolar VI (prepared by refluxing Br_2CA in benzene with excess $SOCl_2$) and pyridine in dry benzene under ice cooling for 10 h. The reaction mixture was poured into saturated NaCl solution (150 mL), acidified (HCl), and extracted with ether (50 mL × 3). The organic layer was washed with water and dried (Na_2SO_4). The concentrate was purified by preparative TLC (H) to obtain an equal mixture of the αR and αS isomers of 2′- or 4′-VII (~110 mg each). Diastereomers of 4′-VII were separated by TLC [hexane–ether (4:1), four developments] (αR 51 mg at R_f 0.51 and αS 48 mg at R_f 0.45) and their structures assigned by NMR comparisons with decamethrin and its αR diastereomer.

2′- or 4′-VII (300 mg, 0.56 mmol) in dichloromethane (2 mL) was treated with BBr_3 (450 mg, 1.79 mmol) in dichloromethane (1 mL) at −10 °C and stirred an additional 3 min at −10 °C, and the mixture was immediately concentrated to dryness in vacuo at 25 °C. Anhydrous methanol (1.5 mL) was slowly added to the reddish residue under ice cooling, and the clear solution was immediately subjected to preparative TLC (0.5-mm layer thickness, C). Compounds 2′- and 4′-VIII were isolated in 29 and 36% yields, respectively, by extraction of the silica gel with cold acetone, and they were used directly for esterification. Br_2CA (50 mg, 0.17 mmol) with freshly prepared VIII (35 mg, 0.16 mmol) and triethylamine (Et_3N) (250–300 mg) in DMF solution (1 mL) were heated in an ampule with N_2 gas at 80–90 °C for 4 h. Each reaction mixture was directly purified by preparative TLC (0.5 mm; first with C and then with E) to remove a small amount of DMF and impurities (20 and 35% yields for 2′- and 4′-HO-dec, respectively).

Methyl Ester of Br_2CA-gly. Br_2CA (0.1 g, 0.34 mmol) was refluxed in $SOCl_2$ (2 mL) for 18 h. Excess $SOCl_2$ was distilled off and the acid chloride was dissolved in 15 mL of benzene–tetrahydrofuran (4:1) and treated with glycine methyl ester–HCl (50 mg, 0.4 mmol) in pyridine (2 mL) at 0–5 °C for 30 min. The mixture was stirred at 25 °C for 3 h, poured into 50 mL of saturated NaCl solution, and acidified (pH 1–2, HCl). Extraction with ethyl acetate followed by concentration in vacuo and TLC (B) gave 95% yield based on starting acid. The purity was established by TLC and the identity by NMR and chemical ionization–mass spectrometry (CI–MS) using isobutane as the reagent gas [$(M + 1)^+$ m/e 368, 92% relative intensity].

***p*-Nitrobenzyl Thiocyanate (IX).** Reaction of *p*-nitrobenzyl bromide (2.2 g, 10 mmol) with KSCN (1.2 g, 12 mmol) in 90% ethanol at reflux for 1 h gave IX in essentially quantitative yield. Pale-yellow crystals [mp 82–84 °C; reported 85 °C (Lyman and Reid, 1917)] were obtained on recrystallization from ethanol. CI–MS (isobutane): $(M + 57)^+$ m/e 251, 59%; $(M + 1)^+$ m/e 195, 18%.

2-Iminothiazolidine-4-carboxylic Acid (ITCA). In accordance with the procedure of Schöberl et al. (1951), NaCN (120 mg, 2.4 mmol) was added slowly to a stirred suspension of L-cystine (100 mg, 0.4 mmol) in water (4 mL) at 27 °C and stirring was continued for 1.5 h. The mixture was acidified (pH 5–6) and evaporated, and the residue was recrystallized from ethanol–water (1:1) (mp 212 °C dec; lit. 212 °C dec; >80% yield).

Treatment of Rats, Collection and Analysis of Excreta, and Determination of Tissue Residues. The procedures of Gaughan et al. (1977a) were used except as follows: the male rats weighed 140–160 g and were held 8 days after oral treatments for collection of urine and feces during which time the $^{14}CO_2$ (if any) was collected for 2 days, and the residual ^{14}C in tissues was determined after 8 days. In a separate series of experiments, individual rats were dosed orally and intraperitoneally (IP) with [^{14}CN]decamethrin and K^{14}CN for analyses of the excreta, stomach, carcass, and expired $^{14}CO_2$ after 3 days.

The administered dose was considered to be the total ^{14}C recovered in excreta, tissues, and carcass; this ^{14}C recovery value was 97.3 ± 5.8% (average and standard error for all experiments) of the administered ^{14}C. Yields of fecal metabolites are corrected for cross contamination (0.4–14%) with urinary metabolites. The correction factor was determined from the proportion of polar metabolites in feces that reproduced exactly the TLC metabolite pattern of the corresponding urine sample. Tissue residues are given as parts per billion (ppb) equivalents of administered ^{14}C compound based on fresh tissue weights.

Data reported are average values for three rats with [^{14}Cv]- and [^{14}CN]decamethrin and two rats with [^{14}Cα]decamethrin and (1*RS*)-*trans*-[^{14}Cv]decamethrin; they are based on single animals in the other cases.

Tentative Characterization of Metabolites. Individual metabolites separated by one-dimensional (A) or two-dimensional TLC (A, B; D, E) were extracted with methanol from the appropriate gel regions (detected by radioautography). Esters were recognized by their identical chromatographic positions with the three labeled preparations. Appropriate unlabeled standards were added before concentration of the methanol extracts under N_2 to minimize metabolite decomposition (Shono et al., 1978). Compounds directly cochromatographed with authentic standards in the indicated solvent systems were: decamethrin (D, C); 2′-HO- and 4′-HO-dec (E, C); Br_2CA, PBacid and 4′-HO-PBacid (B, C); PBacid-gly (A); 4′-HO-PBacid-sulfate, thiocyanate, and ITCA (A and G). Metabolites converted to methyl esters or ethers (diazomethane; Ueda et al., 1975) prior to cochromatography were: Br_2CA, PBacid, and 4′-HO-PBacid (D, E); Br_2CA-gly and PBacid-gly (B, C); 4′-HO-dec (D, C).

Two ester metabolites were treated with $NaBH_4$ for identification of the alcohol fragment (5-HO-dec) or both the acid and alcohol fragments (4′-HO-dec). The individual metabolite in ethanol (0.5 mL) containing $NaBH_4$ (10 mg) was held 18 h at 25 °C, conditions that give high yields of PBalc and the alcohol derivative of Br_2CA from decamethrin. The cleavage products recovered by addition of water (0.5 mL) and extraction into ether (1.5 mL × 3) were subjected to cochromatography with Br_2CA (B, E) after oxidation with $KMnO_4$ and with 4′-HO-, 5-HO-, and other HO-PBalc derivatives (Unai and Casida, 1977) (B, E; B, F; C, E).

Several metabolites of decamethrin and its (1*RS*)-trans isomer are tentatively identified by cochromatography with the analogous dichlorovinyl esters (provided by D. H. Hutson, Shell Toxicology Laboratory, Sittingbourne, Kent, U.K.) or dichlorovinyl acids (Unai and Casida, 1977). This technique appears to be justified since replacing bromine with chlorine has no influence on the R_f values of three cis compounds (Table I) or of (1*RS*)-*trans*-decamethrin (R_f 0.84 in B, 0.59 in C, and 0.83 in E) or (1*RS*)-*trans*-Br_2CA (R_f 0.62 in B, 0.30 in C, and 0.52 in E) (for source of compounds, see Ruzo et al., 1977). Metabolites tentatively characterized in this way are: *t*-HO-Br_2CA (Table I) and the corresponding hydroxymethyl derivative of (1*RS*)-*trans*-Br_2CA (R_f 0.21 in B, 0.04 in C, and 0.11 in E); 4′-HO-(1*RS*)-*trans*-dec [R_f 0.62 in B, 0.38 in C, and 0.42 (αS) and 0.44 (αR) in E].

Compounds designated as glucuronides (Br_2CA-gluc, *t*-HO-Br_2CA-gluc, PBacid-gluc, and 4′-HO-PBacid-gluc) are almost quantitatively cleaved by β-glucuronidase but are not cleaved by β-glucuronidase plus saccharic acid 1,4-lactone or sulfatase (for methods, see Gaughan et al., 1977a). The released aglucons cochromatograph with appropriate standards as above.

Metabolites designated as sulfates (2′-HO- and 4′-HO-PBacid-sulfates) readily cleave with 3 N HCl or with sulfatase, partially cleave with glucuronidase/aryl sulfatase, and undergo no cleavage with β-glucuronidase alone (methods of Gaughan et al., 1977a). The sulfatase cleavage product of the metabolite designated as 4′-HO-PBacid-sulfate cochromatographs with 4′-HO-PBacid before methylation (B, C) and with its dimethyl derivative after methylation (B, E; D, E). The minor sulfate conjugate

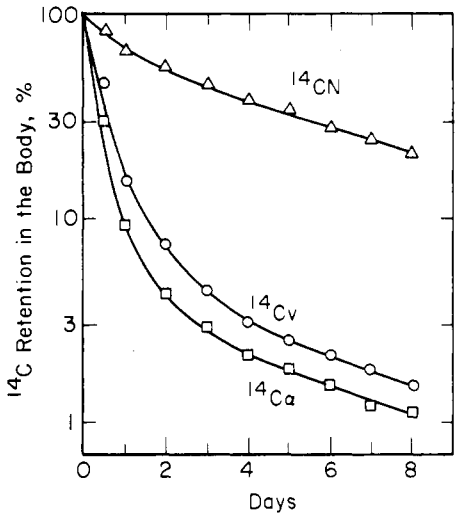

Figure 4. Radiocarbon retention in the body at up to 8 days after oral administration of [^{14}Cv]-, [^{14}Cα]- and [^{14}CN]decamethrin.

referred to as 2′-HO-PBacid-sulfate cochromatographs (A and G) with a permethrin metabolite (Gaughan et al., 1977a) and is cleaved as above to two products neither of which cochromatographs (B) with 2′-, 3′-, 4′-, 4-, 5-, or 6-HO-PBacids or the corresponding alcohols [for chromatographic properties, see Table I and Unai and Casida (1977) and Gaughan et al. (1977a)]. The instability of 2′-HO-PBacid is well documented (Gaughan et al., 1977a).

The presence of thiocyanate as a metabolite was confirmed by reacting an aliquot (200 μL) of urine or a methanol extract of stomach with *p*-nitrobenzyl bromide (0.10 mg) in ethanol (200 μL) to give quantitatively a labeled derivative cochromatographing (B, C; F, C) with IX. Isotope dilution experiments confirm this assignment.

Stomach Metabolism in Vitro. Stomachs were emptied of their contents and washed with saline. [^{14}Cv]- or [^{14}Cα]decamethrin (~10 μg) was incubated with ~1.0 g of stomach tissue in saline at either pH 2.2 or 7.2 (3 mL) at 37 °C for periods up to 24 h. In one experiment, stomach tissue was cut in strips, and following incubation the mixture was homogenized with product recovery and analysis by extraction with an equal volume of ether–ethanol (3:1) (3×) and TLC cochromatography (B, C). In another experiment, a stomach homogenate was centrifuged (8000*g*, 0.5 h) and the supernatant was used for incubation with subsequent analyses as above. In control experiments, decamethrin was almost quantitatively recovered following incubation in saline under the indicated conditions.

RESULTS

Radiocarbon Distribution. Radiocarbon from [^{14}Cv]- and [^{14}Cα]decamethrin is rapidly and almost completely eliminated from the body (Figure 4) and appears in the urine and feces with very little tissue retention after 8 days (Table II). The highest decamethrin equivalents from these labeled preparations appear in fat (Table II). These findings are in marked contrast to those with [^{14}CN]-decamethrin where the ^{14}C is excreted much slower (Figure 4) and tissue residues are generally higher, particularly in the stomach and skin (Table II). Rats receiving [^{14}CN]decamethrin or K^{14}CN either orally or IP also selectively retain the ^{14}C in the stomach 3 days after administration (Table III). Essentially no $^{14}CO_2$ is obtained with each ^{14}C preparation or compound.

Excretion of ^{14}C from [^{14}Cv]Br_2CA is essentially complete after 8 days, mostly in the urine, and tissue levels are very low (Table II). Radiocarbon from (1*RS*)-*trans*-[^{14}Cv]decamethrin is excreted more slowly than that

Table II. Radiocarbon in the Urine, Feces, Carbon Dioxide, and Tissues of Rats up to 8 Days after Oral Administration of [^{14}Cv]-, [^{14}Cα]- and [^{14}CN]Decamethrin

Sample analyzed	Labeling position		
	^{14}Cv[a,b]	^{14}Cα	^{14}CN
Administered Dose, mg/kg	0.90	1.60	0.64
% of Administered Dose			
Urine			
0-1 day	45.1	67.6	10.4
1-2 days	5.6	3.0	8.1
2-4 days	2.7	2.1	11.4
4-8 days	1.0	1.0	13.0
Feces			
Methanol extract			
0-1 day	35.6	22.7	12.1
1-2 days	2.0	1.8	2.3
2-4 days	1.7	0.3	3.3
4-8 days	0.4	0.0	3.3
Unextractable, 0-8 days	4.4	0.4	14.7
$^{14}CO_2$, 0-2 days	0.0	0.0	0.0
Carcass and tissues, 8 days	1.5	1.1	21.4
Tissue Residue at 8 Days, ppb of Decamethrin Equiv			
Blood	12	89	103
Bone	12	11	57
Brain	4	20	5
Fat	59	182	94
Heart	8	9	41
Intestine			
Large	9	5	77
Small	10	5	129
Kidney	8	10	66
Liver	12	38	66
Lung	4	5	73
Muscle	5	5	57
Skin	16	16	603
Spleen	2	5	49
Stomach	8	3	654
Testes	5	3	54

[a] With (1*RS*)-*trans*-[^{14}Cv]decamethrin administered orally at 0.94 mg/kg, the ^{14}C balance sheet at 8 days as percent of dose is 72.6% in urine (48.6% at 1 day, 62.3% at 2 days, and 71.2% at 4 days), 23.2% in the methanol extract of feces (12.3% at 1 day, 18.6% at 2 days, and 21.7% at 4 days), 3.0% in the unextractable portion of feces (0-8 days), and 1.2% in carcass and tissues (all individual tissues as above <10 ppb decamethrin equivalents). [b] With [^{14}Cv]Br_2CA administered orally at 3.74 mg/kg, the ^{14}C balance sheet at 8 days as percent of dose is 94.0% in urine (70% at 1 day, 82% at 2 days, and 91% at 3 days), 5.8% in feces (5.4% in methanol extract), 0.0% $^{14}CO_2$, and 0.2% in carcass and tissues. The tissue residues are <3 ppb decamethrin equiv except for liver which is 27 ppb.

Table III. Radiocarbon in the Urine, Feces, Stomach, and Carcass of Rats 3 Days after Oral and Intraperitoneal Administration of [^{14}CN]Decamethrin and K^{14}CN at 0.1 mg/kg

Sample analyzed	% of administered ^{14}C			
	[^{14}C]Decamethrin		K^{14}CN	
	Ip	Oral[a]	Ip	Oral[a]
Urine	13.7[b]	19.8[b]	42.0[c]	38.5[c]
Feces				
Methanol extract	8.4	10.0	3.5	3.4
Unextractable	6.3	9.1	8.9	13.4
Stomach	3.1	3.3[b]	1.5	3.3
Carcass	68.5	57.8	44.1	41.4

[a] 0.0% $^{14}CO_2$. [b] Consists only of SCN^-. [c] Consists of >95% SCN^- and <5% ITCA or other products of low R_f.

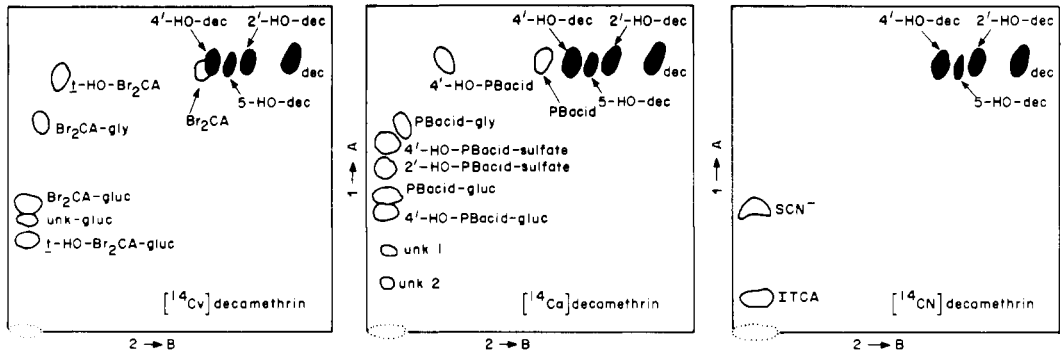

Figure 5. ^{14}C-labeled compounds in the urine and the methanol extract of feces of rats after oral administration of three labeled preparations of decamethrin as resolved by two-dimensional TLC. The solvent fronts are the appropriate outlines of the figure. The origin, which contains no ^{14}C compounds, is indicated at the lower left of each figure. Decamethrin and ester metabolites detected with all labeled preparations are indicated by solid circles. Open circles indicate metabolites detected with only one of the ^{14}C preparations. Metabolites designated as "unk" are unidentified. Figure 2 gives the structures of the compounds and Table IV gives the quantitative data for the indicated ^{14}C metabolites in urine and feces.

from [^{14}Cv]decamethrin; in contrast to decamethrin, the trans isomer yields more ^{14}C in urine than in feces (Table II).

Excreted Metabolites. TLC chromatographic patterns of the excreted decamethrin metabolites are shown in Figure 5 and their amounts are given in Figure 2 and Table IV.

The major product in feces is unmetabolized decamethrin but the feces also contain 4'-HO- and 5-HO-dec and a trace level of 2'-HO-dec. The major fecal α-cyano esters (decamethrin and 4'-HO-dec) appear not only as the administered αS isomer but also in part as the αR epimer (Table IV); this epimerization is probably not a biological reaction but rather is an artifact resulting from racemization on exchange of the α proton on holding the samples in methanol (Ruzo et al., 1977).

Decamethrin metabolites from the acid moiety are mostly Br_2CA and its glucuronide with trace levels of Br_2CA-gly and a HO-Br_2CA derivative both free and as a glucuronide. The latter metabolite is probably hydroxylated on the methyl group trans to the carboxyl (i.e., *t*-HO-Br_2CA) based on its chromatographic properties compared to the *trans*- and *cis*-hydroxymethyl derivatives of the dichlorovinyl acid, the failure to undergo a lactonization reaction on acidification, and on analogy with the metabolism of (1*R*,3*R*) permethrin (Gaughan et al., 1977a). The same metabolites appear in almost the same proportions on direct administration of [^{14}Cv]Br_2CA (Table IV). A single unknown, appearing only in urine, is detected from Br_2CA and the acid moiety of decamethrin and in each case it chromatographs (A) between Br_2CA-gluc and *t*-HO-Br_2CA-gluc (Figure 5). This metabolite of Br_2CA is cleaved by β-glucuronidase to two products each chromatographing (B) between Br_2CA and *t*-HO-Br_2CA.

Metabolites of the alcohol moiety are similar to those previously encountered with permethrin, i.e., predominantly 4'-HO-PBacid-sulfate and PBacid-gluc. Lower yields are obtained for PBacid and its glycine conjugate, 4'-HO-PBacid and its glucuronide, and a sulfate conjugate, probably 2'-HO-PBacid-sulfate. The finding of 2'-HO-dec in trace amounts in feces supports the suggestion that this sulfate is that of 2'-HO-PBacid. There are two minor unknowns, each chromatographing (A) below 4'-HO-PBacid-gluc. Compounds not present in the excreta either free or conjugated are other HO-PBacid and HO-PBalc derivatives listed in Table I or reported by Unai and Casida (1977).

The major excreted ^{14}C metabolite of [^{14}CN]decamethrin administered orally or intraperitoneally is [^{14}C]thiocyanate (Tables III and IV), as it is also following K^{14}CN administration (Table III). There is large variability (0–8%) with different animals in the yield of a minor metabolite, ITCA. All of the ITCA that is excreted appears within 24 h and only thiocyanate appears in urine thereafter. The cyanohydrin that would result from direct ester cleavage is not detected as a metabolite nor is 3-phenoxybenzaldehyde which would be formed on its decomposition during analysis.

The identified metabolites excreted within 8 days account for 93.5 and 97.9%, respectively, of the administered [^{14}Cv]- and [^{14}Cα]decamethrin. However, the identified metabolites are only 63.9% with [^{14}CN]decamethrin due to two factors: a large portion (14.7%) of the ^{14}C is not extracted from feces by the procedure employed (Table II); [^{14}CN]metabolites are still being excreted at the termination of the experiment at 8 days (Figure 4).

In comparison with decamethrin, the trans isomer gives less parent compound and more 4'-HO derivative and undergoes more extensive ester cleavage. [^{14}Cv]Br_2CA gives essentially the same pattern and proportion of excreted metabolites as [^{14}Cv]decamethrin (Table IV).

Metabolites in the Stomach. Essentially all of the ^{14}C in the stomach at 3 days after oral administration of [^{14}CN]decamethrin is [^{14}C]thiocyanate (Table III) analyzed as derivative IX. At 24 h after treatment with this labeled preparation, thiocyanate accounts for ~60% of the stomach ^{14}C content.

Incubation of decamethrin with stomach strips in pH 7.2 medium for 6–24 h yields ~60% metabolism, the major products being PBacid (^{14}Cα) and Br_2CA (^{14}Cv) with smaller amounts of 4'-HO-PBacid and PBalc (^{14}Cα). The homogenate supernatant at pH 2.2 or 7.2 gives less metabolism but the same products.

DISCUSSION

Figure 2 gives the decamethrin metabolic pathways based on identified metabolites with support by analogy with permethrin metabolism in rats (Gaughan et al., 1977a). A portion of an oral dose is excreted in the feces without metabolism. Since there is no significant production of $^{14}CO_2$ with any ^{14}C preparation, the pathways do not include extensive fragmentation of the acid and alcohol moieties. The principal mechanisms of metabolism are ester cleavage and oxidation at the 4' position of the alcohol moiety. Minor oxidation sites are the 5 and 2' positions of the alcohol moiety and the methyl group trans to the carboxyl. The ester metabolites are not conjugated but the corresponding acids undergo extensive conjugation at both the phenolic hydroxyl and carboxylic acid groups.

Table IV. ^{14}C-Labeled Compounds in the Urine and in the Methanol Extract of Feces of Rats 8 Days after Oral Administration of [^{14}Cv]-, [^{14}Cα]- and [^{14}CN]Decamethrin

^{14}Cv label				^{14}Cα label		^{14}CN label	
Compd[a]		% of admin. ^{14}C		Compd[a]	% of admin. ^{14}C	Compd[a]	% of admin. ^{14}C
Decamethrin and Hydroxydecamethrin Derivatives							
Dec[b]	21.4	(6.4)[c]		Dec[b]	13.5	Dec[b]	12.9
4'-HO-dec[b]	7.7	(14.9)		4'-HO-dec[b]	5.9	4'-HO-dec[b]	6.4
5-HO-dec	2.0	(0.0)		5-HO-dec	1.4	5-HO-dec	1.7
Total	31.1	(21.3)		Total	20.8	Total	21.0
Metabolites Derived from Ester-Cleavage Fragments							
Br_2CA	10.4	(11.7)[c]	[9.9][d]	PBacid	4.5	SCN^-	38.2
t-HO-Br_2CA	0.4	(2.2)	[0.2]	4'-HO-PBacid	4.0	ITCA	4.7
Br_2CA-gluc	50.8	(60.6)	[84.9]	PBacid-gluc	12.6	Total	42.9
Br_2CA-gly	0.5	(0.0)	[1.3]	PBacid-gly	3.6		
t-HO-Br_2CA-gluc	0.3	(0.0)	[0.6]	4'-HO-PBacid-gluc	1.8		
Unknown gluc	0.6	(0.0)	[2.5]	4'-HO-PBacid-sulfate	48.6		
				2'-HO-PBacid-sulfate	2.0		
Total	63.0	(74.5)	[99.4]	Unknowns (2)	0.6		
				Total	77.7		

[a] Decamethrin, the hydroxydecamethrin derivatives, and *t*-HO-Br_2CA appear in feces only. Three carboxylic acids appear in both urine and feces as follows (percent of administered ^{14}C): Br_2CA, 8.2% feces, 2.2% urine; PBacid, 1.6% feces, 2.9% urine; 4'-HO-PBacid, 2.5% feces, 1.5% urine. All other metabolites appear in urine only. [b] About one-third of the designated amount is detected as the αR isomer of decamethrin or its (1*RS*)-*trans* isomer. [c] Values in parentheses are ^{14}C recoveries as (1*RS*)-*trans*-decamethrin and its metabolites in excreta 8 days after oral administration of (1*RS*)-*trans*-[^{14}Cv]dec. This decamethrin isomer and its 4'-hydroxy derivative appear in feces only, *t*-Br_2CA-gluc appears in urine only, and the carboxylic acids appear in both urine and feces as follows: *t*-Br_2CA, 0.6% feces, 11.1% urine; *t*-HO,*t*-Br_2CA, 1.3% feces, 0.9% urine. [d] Values in brackets are ^{14}C recoveries as Br_2CA and its metabolites in excreta 8 days after oral administration of [^{14}Cv]Br_2CA. This acid appears in both urine (4.7%) and feces (5.2%) while *t*-HO-Br_2CA appears in feces only and the conjugates appear in urine only.

The acid moiety is rapidly excreted as the glucuronide with smaller amounts free and as the glycine conjugate. The *trans*-hydroxymethyl derivative is also excreted both free and as the glucuronide.

All major metabolites of the aromatic portion of the alcohol moiety are rapidly excreted and probably arise from ester cleavage of decamethrin or its ester metabolites, conversion of the released cyanohydrins to the aldehydes which rapidly give the corresponding acids, and conjugation of these acids. PBacid is excreted either without conjugation or as glucuronide and glycine conjugates. The major metabolite, 4'-HO-PBacid-sulfate, is probably formed by hydroxylation of the phenoxy group and ester cleavage, in an undetermined sequence, followed by oxidation to the benzoic acid and sulfate conjugation.

Cleavage of the decamethrin ester group leads to release of cyanide which is converted mainly to thiocyanate and a small amount of ITCA, the latter via reaction with cystine or other tissue thiol (Wood and Cooley, 1956). The slow release of thiocyanate from the body is due in part to selective tissue retention. That portion in the stomach could arise from decamethrin cleavage and localization of the thiocyanate formed metabolically at this site. However, [^{14}CN]decamethrin and K^{14}CN give similar patterns of ^{14}C retention in the stomach following either oral or IP administration. The localization pattern of ^{14}C from [^{14}CN]decamethrin is probably a characteristic of thiocyanate localization rather than of the site of decamethrin metabolism.

The potency of decamethrin indicates use levels of only a few grams per hectare for efficient pest control. Its photodecomposition on crops (Ruzo and Casida, 1978) yields several products including (1*RS*)-*trans*-decamethrin which is also readily metabolized in rats. The pathways involved in rat metabolism of the decamethrin isomers are similar to those utilized for other pyrethroids in many segments of the ecosystem (Elliott, 1977; Gaughan et al., 1977a,b; Miyamoto, 1976).

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Supplementary Material Available: A listing of spectral (IR, NMR) and TLC chromatographic characteristics in five different solvent systems (C–E, H, and benzene) for the hydroxydecamethrin isomers and each of the intermediates in their syntheses (1 page). Ordering information is given on any current masthead page.

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Metabolism of Phosphorothioic Acid, *O,O*-Dimethyl-*O*-(6-ethoxy-2-ethyl-4-pyrimidinyl) Ester (Etrimfos), in Bean and Corn Plants

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The organothionophosphate etrimfos [phosphorothioic acid, *O,O*-dimethyl-*O*-(6-ethoxy-2-ethyl-4-pyrimidinyl) ester] volatilized rapidly from treated primary leaves of bean and corn seedlings. Twenty-one days after treatment with [^{14}C]etrimfos, 36.4 and 29.5% of the total applied radioactivity was recovered from bean and corn seedlings, respectively. Intact etrimfos was by far the principal compound recovered, followed by small amounts of EEHP (6-ethoxy-2-ethyl-4-pyrimidinol) and EDHP (2-ethyl-4,6-pyrimidinediol). Small amounts of the P:O analogue [phosphoric acid, dimethyl *O*-(6-ethoxy-2-ethyl-4-pyrimidinyl) ester] were also present in corn but not in bean leaves. Four additional products were also detected in small quantities in both plants but were not identified.

Etrimfos [phosphorothioic acid, *O,O*-dimethyl *O*-(6-ethoxy-2-ethyl-4-pyrimidinyl) ester] is a new nonsystemic contact and stomach insecticide, effective against pest species of lepidoptera, coleoptera, diptera, and to a variable extent hemiptera (Knutti and Reisser, 1975). Residue field trials on a variety of crops have shown that residues generally ranged from nondetectable amounts to 0.4 ppm at time of harvest (Karapally, 1977). Etrimfos, therefore, is a promising material for the management of pest problems of crops such as vegetables, fruits, and corn.

Knowledge concerning the absorption and the extent and types of biotransformation of etrimfos in plants is lacking; therefore, the metabolic fate and associated residues of etrimfos must be evaluated before the compound can be used for extensive field trials against insect pests. This paper describes the results of our investigations of the fate of [^{14}C]etrimfos in bean and corn seedlings.

MATERIALS AND METHODS

Compounds. Etrimfos labeled with ^{14}C at carbons 4 and 6 of the pyrimidinyl ring was provided by Dr. James Karapally of Sandoz Inc., East Hanover, N.J. The specific activity of the compound was 11 μCi/mg and radiochemical purity as determined by TLC was greater than 99%. The specific activity was diluted to 1.6 μCi/mg with purified nonradioactive etrimfos. The following nonlabeled compounds were also provided by Sandoz Inc.: etrimfos; P:O analogue of etrimfos [phosphoric acid, dimethyl *O*-(6-ethoxy-2-ethyl-4-pyrimidinyl) ester]; EEHP (6-ethoxy-2-ethyl-4-pyrimidinol); and EDHP (2-ethyl-4,6-pyrimidinediol).

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Treatment of Seedlings. *Sieva* variety lima bean seeds (Asgro Seed Company, Kalamazoo, Mich.) were sown in a mixture of vermiculite and sterilized soil (1:1) in plastic trays. Two weeks after germination, the seedlings were transplanted individually in plastic-coated paper cups (8.9 cm diameter × 5.1 cm high). Merit variety corn (Ferris Seed Co., East Brunswick, N.J.) was planted directly in plastic cups (8.9 cm diameter × 10.2 cm high), in a mixture of vermiculite and soil (1:1). Germination took place in 6 days. Plants were subirrigated and held in a greenhouse equipped with plastic lexan windows which did not exclude UV light.

The primary leaves of bean (21 days after germination) and corn plants (11 days after germination) were treated with 10 μL of [^{14}C]etrimfos solution in acetone. The amount of radioactivity applied per leaf was ca. 40000 dpm (11 μg of etrimfos) and was distributed on the leaf surface as evenly as possible.

Extraction and Analysis. At posttreatment intervals of 0, 3, 7, 14, and 21 days, the treated leaves from three plants of corn or bean were cut at the petiole, and the combined leaves were analyzed as described below. The experiment was repeated so that the data are averages of duplicate analyses.

For the leaf rinse, the leaves were placed in a beaker containing 10 mL of 1% v/v Tween 80 in distilled water, and the beaker was shaken for 3 min. This procedure was repeated three times, and the combined rinses were shaken vigorously with 80 mL of chloroform in a separatory funnel. The chloroform was removed and the extraction was repeated two more times. The combined chloroform extracts were dried over anhydrous sodium sulfate, filtered, and then concentrated to 2 mL at 40 °C under reduced pressure. One-half milliliter of the chloroform concentrate was added to scintillation vials, the solvent was removed with the aid of a stream of dry air at room temperature,

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