Degradation of Decamethrin on Cotton Plants

Luis O. Ruzo* and John E. Casida

Decamethrin is degraded to the following compounds within 6 weeks after application to cotton leaves in the greenhouse and field: *trans*-decamethrin and the 4′-hydroxy, *trans*-hydroxymethyl, and 4′-hydroxy-*trans*-hydroxymethyl derivatives of decamethrin; 3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylic acid (Br_2CA) and three conjugates and the *trans*-hydroxymethyl derivative of this acid; 3-phenoxybenzaldehyde and the corresponding alcohol and acid, 4′-hydroxyphenoxybenzoic acid, and two or three conjugates of each of phenoxybenzyl alcohol, phenoxybenzoic acid, and α-cyanophenoxybenzyl alcohol. Decamethrin residues are dissipated not only by metabolism and photoisomerization but probably also by volatilization of the parent ester or its cleavage products. Bean but not cotton leaf disks convert decamethrin to glycosides of phenoxybenzyl alcohol and Br_2CA. Leaf disks from both species metabolize phenoxybenzaldehyde, from degradation of α-cyanophenoxybenzyl alcohol, to phenoxybenzoic acid and phenoxybenzyl alcohol, both of which form one to four glycosides. Br_2CA is also readily conjugated in these leaf disks.

Decamethrin is one of the most potent insecticides (Elliott, 1977; Elliott et al., 1974), controlling several important pests of cotton and other crops at dosages of 5–30g/ha (Roussel Uclaf/Procida, personal communication). It is therefore of interest to define its stability and degradation mechanisms under a variety of conditions. At sunlight wavelengths decamethrin undergoes several types of photochemical processes including cis–trans interconversion arising from the triplet excited state either by direct or sensitized pathways (Ruzo et al., 1977). It is metabolized in rats (Ruzo et al., 1978) and mice (Ruzo et al., 1979) by oxidative and hydrolytic reactions leading to a variety of metabolites most of which are excreted as conjugates. The present study examines the fate of decamethrin on cotton leaves under greenhouse and field conditions and its metabolism in cotton and bean leaf disks.

MATERIALS AND METHODS

Chemicals. The following ^{14}C preparations (provided by Roussel Uclaf/Procida, Paris, France; radiochemical purity >99%) were used: decamethrin labeled in the dibromovinyl (^{14}Cv), benzylic ($^{14}C\alpha$), and cyano (^{14}CN) carbons (Figure 1) with specific activities of 5.0, 60.0, and 51.5 mCi/mmol, respectively; 3-(2,2-[^{14}Cv]dibromovinyl)-2,2-dimethylcyclopropanecarboxylic acid (Br_2CA) (5.1 mCi/mmol) and 3-phenoxy[$^{14}C\alpha$]benzaldehyde (54 mCi/mmol). α-Cyano-3-phenoxy[$^{14}C\alpha$]benzyl alcohol (PBcy) and unlabeled standards for comparison with metabolites by cochromatography were available from previous syntheses or via synthesis procedures described by Ruzo et al. (1977, 1978, 1979) and Unai and Casida (1977). Compounds are designated as shown in Figure 1.

Chromatography. Six solvent systems were used for thin-layer chromatography (TLC) on silica gel F-254 chromatoplates (0.25-mm gel thickness) as follows: (A) butanol–glacial acetic acid–water (6:1:1); (B) benzene–carbon tetrachloride (1:1); (C) benzene–ethyl acetate (6:1); (D) benzene (saturated with formic acid)–ether (10:3), two developments; (E) carbon tetrachloride–ether (3:1); (F) ether–hexane (1:1), three developments. In referring to solvent systems for two-dimensional development, A × D indicates development in the first direction with A and in the second direction with D. Chromatographic properties in these systems of all compounds referred to below (other than the conjugates) and related materials are given by Ruzo et al. (1977, 1978).

Treatment and Analysis of Cotton. The Stoneville 7A variety was treated topically on the leaves with each of the [^{14}C]decamethrin samples in field studies near Davis, CA, and in greenhouse studies in Berkeley, CA, all during 1977. Conditions of plant growth, developmental stages, and treatment were identical with those of Gaughan and Casida (1978). The initial decamethrin deposits were 0.04–0.33 $\mu g/cm^2$ (3–15 ppm based on leaf fresh weight).

Leaves (0.3–0.5 g) immediately after harvest were cut into ~5 mm^2 pieces and the ^{14}C products extracted by soaking in acetonitrile–chloroform (2:1) and decanting as follows: 10 mL for 3–4 h, 10 mL for 48 h, 5-mL rinse. The combined extracts and rinses were concentrated under N_2 for determination of the ^{14}C content by liquid scintillation counting (LSC) of an aliquot of the total extract and of the individual ^{14}C compounds separated by TLC (B and A × D). Radiocarbon in the unextractable residue was analyzed by combustion.

Metabolism in Cotton and Bean Leaf Disks. Leaves freshly removed from young greenhouse-grown cotton and bean plants were placed under water and punched with a sharp cork borer to obtain disks of 10-mm diameter (Kolattukudy and Walton, 1972). Each incubation mixture in a 25-mL Erlenmeyer flask consisted of 25 disks in 2 mL of distilled water. The ^{14}C substrate (decamethrin, Br_2CA, PBcy, or PBald; 1–5 μg) was introduced in 30 μL of ethanol and the mixture was incubated for 5 h at 30 °C under illumination with artificial light. The supernatant was decanted off, and the disks were washed twice with 5 mL of 50% ethanol. These solutions were combined, concentrated, quantitated (LSC), and found by TLC (A × D) to contain only the original substrate. The washed disks were extracted twice with methanol–chloroform (2:1) by homogenization and centrifugation and the ^{14}C content was quantitated for the combined supernatants. Each extract was concentrated and analyzed by TLC (A × D).

Tentative Identification of Metabolites and Degradation Products. Labeled compounds from the treated plants were tentatively identified by cochromatography with unlabeled standards using the following solvent systems: decamethrin and *trans*-decamethrin, B; 4′-HO-dec, *t*-HO-dec, and 4′-HO,*t*-HO-dec, A × D and C × E; Br_2CA, PBald, PBalc, PBacid, and PBcy, C, D, E, and

Pesticide Chemistry and Toxicology Laboratory, Department of Entomological Sciences, University of California, Berkeley, California 94720.

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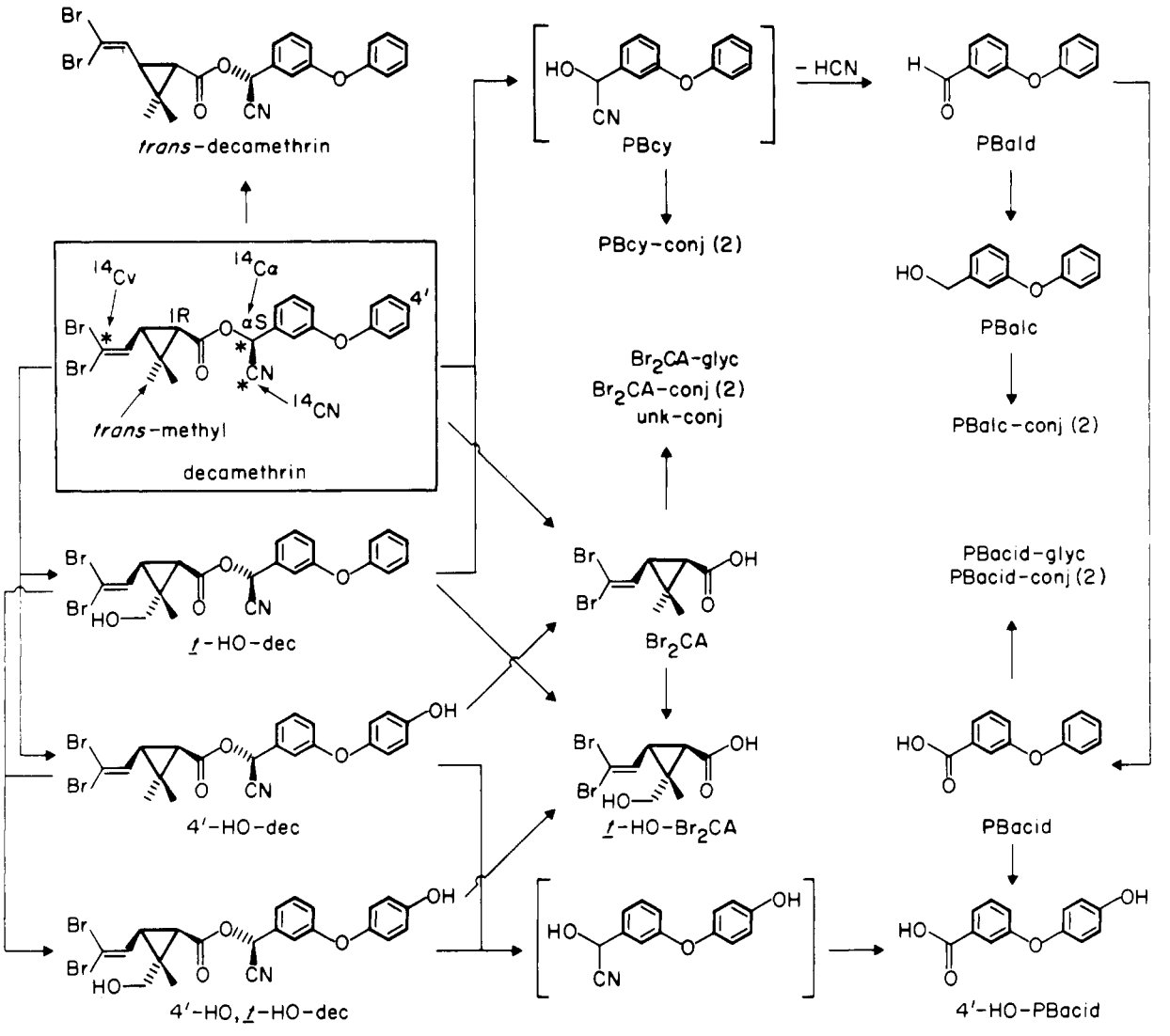

Figure 1. Degradation pathways for decamethrin on cotton foliage. Asterisks designate positions of ^{14}C in the preparations examined. Brackets indicate possible intermediates that were not isolated. Photoisomerization to (1*RS*)-*cis*,*trans*-decamethrin yields a mixture of isomers likely to undergo analogous metabolic reactions to those illustrated with decamethrin. For quantitation of products, see Table I.

F; *t*-HO-Br_2CA and 4'-HO-BBacid, D × E. Br_2CA and PBacid were further analyzed (C and D) following derivatization to their methyl esters (CH_2N_2). Conjugates separated in the A solvent system were subjected to β-glucosidase cleavage (pH 5, 24 h, 37 °C) or 6 N HCl cleavage (6 h, 70 °C) based on Gaughan and Casida (1978), followed by TLC analysis of the cleavage products in the solvent systems listed above.

RESULTS

Loss of Decamethrin and Its Derivatives from Cotton Leaves under Greenhouse Conditions. Under the greenhouse conditions examined, the half-life of decamethrin is 1.1 weeks and the time for 90% ^{14}C loss is 4.6 weeks (Figure 2A). The overall rate of ^{14}C loss and the amount of organosoluble or unextractable products are nearly the same with each ^{14}C-labeling position. There are significant amounts of photoproducts and metabolites (Figure 2A) including *trans*-decamethrin and ^{14}C compounds in the organosoluble and unextractable fractions (Figure 2B). The proportion of *trans*-decamethrin increases progressively from the time of application with trans/cis ratios of 0.06, 0.12, 0.19, 0.24, and 0.44 at 0.5, 1, 2, 3, and 6 weeks, respectively. In absolute amount,

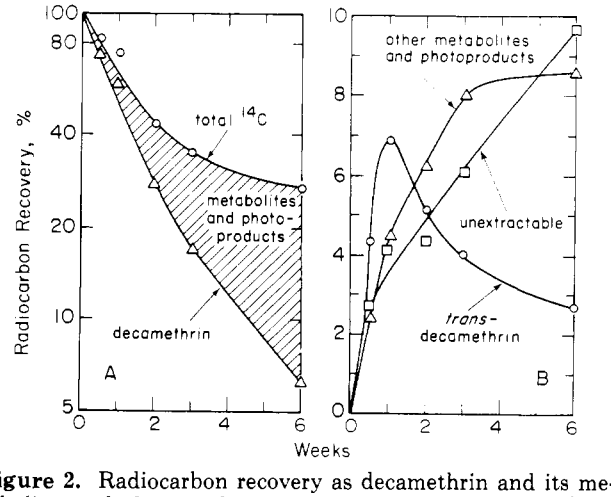

Figure 2. Radiocarbon recovery as decamethrin and its metabolites and photoproducts after topical application of [^{14}C]-decamethrin to cotton leaves under greenhouse conditions. Results are the averages of data with ^{14}Cv, $^{14}C\alpha$, ^{14}CN preparations since they generally gave similar values. Note that the curve for residue dissipation (A) is on a logarithmetic scale and the one for composition of metabolites and photoproducts (B) is on an arithmetic scale.

Table I. Decamethrin and Its Metabolites and Degradation Products in or on Cotton Leaves at 2 and 6 Weeks after Topical Treatment under Greenhouse and Field Conditions

compound	R_f, A	radiocarbon at indicated weeks after treatment as % of applied dose			
		greenhouse[a]		field[b]	
		2	6	2	6
Esters (Average of ^{14}Cv, ^{14}Cα, and ^{14}CN)					
decamethrin	0.89	27.4	6.1	11.1	1.7
trans-decamethrin	0.89	5.1	2.7	7.8	0.7
4′-HO-dec	0.85	0.2	0.3	0.6	0.1
t-HO-dec	0.85	0.5	0.3	0.8	0.1
4′-HO,*t*-HO-dec	0.84	<0.1	<0.1	<0.1	0.0
Acid Moiety (^{14}Cv)					
Br_2CA	0.78	4.1	3.0	4.0	0.3
t-HO-Br_2CA	0.73	0.0	0.2	0.0	0.0
Br_2CA-glyc	0.59	0.1	0.7	1.9	0.5
Br_2CA-conj	0.31, 0.38[c]	1.7	4.2	12.9	7.7
Unk-conj	0.38	<0.1	<0.1	0.4	0.2
decomposition[d]		0.7	0.5	1.2	0.1
unextractable		4.8	8.5	5.7	11.2
loss		55.4	73.5	53.6	77.4
Alcohol Moiety (^{14}Cα followed in Parentheses by ^{14}CN)					
PBald	0.89	1.3	1.1	1.2	1.2
PBalc	0.86	0.4	0.7	0.2	0.0
PBacid	0.76	1.1	2.0	2.0	0.0
4′-HO-PBacid	0.71	0.1	0.1	0.0	0.0
PBalc-conj	0.29, 0.38[c]	0.4	1.2	4.6	1.9
PBacid-glyc	0.60	0.5	1.7	1.8	0.9
PBacid-conj	0.29, 0.38[c]	0.8	1.5	11.4	5.9
PBcy-conj[e]	0.32, 0.42[c]	1.7 (1.4)	3.2 (1.4)	12.6 (23.8)	8.8 (8.3)
decomposition[d]		1.0 (1.1)	1.3 (1.1)	1.9 (9.0)	0.5 (0.0)
unextractable		3.9 (4.1)	11.9 (8.4)	13.4 (10.8)	23.9 (13.9)
loss		55.6 (60.2)	65.9 (79.7)	30.6 (36.1)	54.3 (75.2)

[a] Average of results with each ^{14}C preparation for two replicates in a May-June experiment and two replicates in an August-September experiment. [b] Average of results with each ^{14}C preparation for three replicates in a June-July experiment. [c] R_f values for two conjugates of the indicated type. [d] Decomposition on HCl hydrolysis of conjugates. [e] The cleavage product, PBcy, gives R_f values of 0.37, 0.54, 0.24, and 0.41 in C, D, E, and F, respectively.

trans-decamethrin reaches a peak level at 1 week. The unextractable products, not removed on soaking leaf segments in acetonitrile–chloroform, are of unknown composition. Identities of the extractable metabolites are considered below.

Metabolites and Degradation Products in or on Cotton Leaves under Greenhouse Field Conditions. Decamethrin degrades more rapidly under the field than under the greenhouse conditions examined, based on analyses at 2 and 6 weeks after treatment (Table I) with confirmation from analyses of field samples at 1 and 3 weeks after treatment. Thus, under field conditions there is more rapid loss of parent compound, a higher proportion of *trans*- to *cis*-decamethrin and larger amounts of unextractable products.

Metabolite analysis (Table I) reveals small amounts (<1%) of three hydroxy esters (4′-HO-dec, *t*-HO-dec, 4′-HO,*t*-HO-dec) detected with all three ^{14}C preparations, of *t*-HO-Br_2CA from [^{14}Cv]decamethrin, and of PBalc and 4′-HO-PBacid from [^{14}Cα]decamethrin. There are larger amounts (1–4%) of Br_2CA detected with [^{14}Cv]decamethrin and of PBacid and PBald from [^{14}Cα]decamethrin.

Conjugates appear in three TLC regions (R_f values in A of 0.29–0.32, 0.38–0.42, and 0.59–0.60) (Table I) but they are only partially characterized. Metabolites in the upper TLC region are detected with [^{14}Cv]- and [^{14}Cα]- but not [^{14}CN]decamethrin, so they are formed by ester cleavage and then conjugation. These relatively minor metabolites (up to 1.9%) cleave readily with glucosidase or HCl, yielding Br_2CA and PBacid. They are therefore likely to be the glucosides of these acids.

The two lower R_f regions (0.29–0.32 and 0.38–0.42) detected with all three labeled preparations contain conjugates which cleave readily with HCl but not glucosidase. Both conjugate regions cleave to the same products, i.e., Br_2CA from [^{14}Cv]decamethrin; PBalc, PBacid, and PBcy from [^{14}Cα]decamethrin; and PBcy from [^{14}CN]-decamethrin. It appears that each of these decamethrin fragments is conjugated with two different types of moieties. The PBalc and PBacid conjugates reach levels of 5–11% of the dose. It is interesting to note that the conjugates designated Br_2CA-conj and PBcy-conj are present in similar amounts as detected with [^{14}Cv]decamethrin and either [^{14}Cα]- or [^{14}CN]decamethrin, respectively. These conjugates hydrolyze in HCl but not with glucosidase to yield [^{14}Cv]Br_2CA from the [^{14}Cv]-decamethrin metabolites and [^{14}C]PBcy from the [^{14}Cα]- and [^{14}CN]decamethrin metabolites; the products from HCl treatment do not include decamethrin or other esters. While it is conceivable that these TLC regions contain conjugates of decamethrin, such as may be formed by reaction at the benzylic position with a tissue component, it is more likely that the relevant products are formed by decamethrin hydrolysis, followed by rapid conjugation of the cleavage products.

Metabolism in Cotton and Bean Leaf Disks. Incorporation of ^{14}C into the disks proved to be relatively efficient with uptake values of 21–64% (Table II). Bean but not cotton leaf disks convert the absorbed [^{14}Cv]- and [^{14}Cα]decamethrin in small yield (~6%) to [^{14}C]Br_2CA-glyc and [^{14}C]PBalc-glyc, respectively (Table II). The cleavage products used as substrates undergo more extensive metabolism than decamethrin, largely because of their ease of conjugation or conversion to readily conjugatd products. Two or three types of glycosides are formed from Br_2CA and four from PBalc in both cotton and bean.

Table II. Metabolism of Decamethrin and Its Hydrolysis Products by Cotton and Bean Leaf Disks

product	R_f, A cotton	R_f, A bean	radiocarbon as % of initial dose cotton	radiocarbon as % of initial dose bean
[^{14}Cv]Decamethrin as Substrate				
Br_2CA-glyc		0.39	0.0 (24.1)[a]	6.2 (52.2)
[^{14}Cv]Br_2CA as Substrate				
Br_2CA-glyc-1	0.56	0.56	6.6	12.0
Br_2CA-glyc-2	0.39	0.44	0.4	49.2
Br_2CA-glyc-3	0.29		1.2	
total			8.2 (21.3)	61.2 (64.3)
[^{14}Cα]Decamethrin as Substrate				
PBalc-glyc		0.41	0.0 (46.6)	6.0 (53.5)
[^{14}Cα]PBcy as Substrate[b]				
PBalc	0.86	0.86	11.9	1.0
PBald	0.89	0.89	5.0	2.2
PBacid	0.76		1.7	0.0
PBcy-glyc		0.41	0.0	0.2
PBalc-glyc-1	0.54	0.58	22.6	6.0
PBalc-glyc-2	0.41	0.41	3.0	40.0
PBalc-glyc-3	0.36	0.35	1.4	2.5
PBalc-glyc-4	0.26	0.32	4.4	2.2
PBacid-glyc	0.54	0.41	3.0	2.1
total			53.0 (53.0)	56.2 (56.2)

[a] Numbers in parentheses are total ^{14}C incorporation into the disks as percent of initial dose. That portion of these values not indicated as products is unmetabolized substrate. [b] Essentially the same products and yields are obtained with PBald as substrate.

Conjugated PBacid and PBcy are also formed. All of these leaf disk conjugates are readily cleaved by either glucosidase or HCl. PBald, administered directly or as the cyanohydrin, is largely reduced to PBalc although a portion is oxidized to PBacid. Some of the conjugates are likely to be the same in leaf disks of cotton and bean and on in vivo formation in cotton. However, the conjugates are identified only as to R_f value and glucosidase cleavage and not as to the structure of the conjugating moiety.

DISCUSSION

Decamethrin is degraded on cotton leaves by photodecomposition and metabolism in accordance with pathways shown in Figure 1. Decamethrin and its cleavage products are also lost from the foliage by volatilization or other types of weathering. Photochemical reactions probably account for all of the *trans*-decamethrin and a portion of the ester cleavage products (Ruzo et al., 1977). Metabolic reactions are undoubtedly the major source of the phenolic and hydroxymethyl derivatives and of the intracellular ester cleavage products. It appears likely that PBcy-conj and Br_2CA-conj originate in large part from hydrolysis of absorbed decamethrin and conjugation of the hydrolysis products. PBcy formed on the leaf surface by photochemical ester cleavage is more likely to further photodegrade to PBald and HCN (Ruzo et al., 1977) than to undergo extensive absorption and conjugation. PBald is both oxidized to PBacid and reduced to PBalc.

Some compounds found as decamethrin photoproducts at 290–320 nm or in sunlight (Ruzo et al., 1977) are notable by their absence from plant foliage. Under TLC conditions suitable for separate analysis of decamethrin and its 1*R*,cis,α*R* isomer (Ruzo et al., 1977), little if any of the isomerized product is detected, indicating that epimerization at the benzylic carbon is not a significant reaction in decamethrin degradation on cotton foliage. Other compounds not detected as photoproducts in the present study include α-cyano-3-phenoxybenzyl 3,3-dimethylacrylate and the monodebrominated and decarboxylated derivatives of decamethrin (Ruzo et al., 1977).

Several products are only partially identified. Thus, no attempt is made to separately analyze photoproducts containing the four isomers of the acid moiety. Several types of conjugates are present, varying in the fragment conjugated and in the conjugating moiety. They differ in chromatographic properties and ease of glucosidase cleavage. Some of the conjugates are probably glucosides while others may involve polysaccharides or other unidentified fragments. The proposed conjugates of PBcy are of particular interest since they may be similar to naturally occurring cyanogenic glycosides of many plant species (Eyjólfsson, 1970). The PBcy conjugates in cotton chromatograph in the general region of amygdalin (A, R_f ~0.34), a disaccharide which requires three distinct enzymes for cleavage to glucose, HCN and benzaldehyde (Haisman et al., 1967). In contrast to cotton plant, the cotton leaf disks give very little PBcy-conj and in fact metabolize PBcy in very much the same manner as PBald. PBcy apparently degrades in these disks prior to reaching a favorable site for conjugation.

The identified products of decamethrin metabolism in plants are analogous to those in mammals (Ruzo et al., 1978, 1979) but differ in the conjugating moieties involved. In addition, PBcy is conjugated in cotton, whereas it quickly degrades to PBald and HCN in mammals. Photoisomerization products are also formed on plants, but decamethrin is present in larger amounts and is more toxic to mammals than its seven possible photoisomers (Ruzo et al., 1977). Photodecomposed decamethrin and several of its individual metabolites and photoproducts (Br_2CA, PBald, PBalc, and PBacid) are of much lower toxicity than decamethrin itself to intraperitoneally treated mice (Ruzo et al., 1977).

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