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2-Haloacrylic Acids as Indicators of Mutagenic 2-Haloacrolein Intermediates in Mammalian Metabolism of Selected Promutagens and Carcinogens

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Rats treated orally with several promutagens and carcinogens containing haloallyl or halopropyl substituents excrete small amounts of the following urinary 2-haloacrylic acids, analyzed as their pentafluorobenzyl esters: 2-bromoacrylic acid from the nematocide 1,2-dibromo-3-chloropropane (DBCP) and the flame retardant tris(2,3-dibromopropyl) phosphate (tris-BP); 2-chloroacrylic acid from the herbicides sulfallate, diallate, and triallate; 2,3-dichloroacrylic acid from diallate; trichloroacrylic acid from triallate and the pyrethroid synergist octachlorodipropyl ether. Rabbit liver microsomal oxidases also yield 2-bromoacrylic acid from DBCP and tris-BP and 2-chloro- and trichloroacrylic acids from triallate. With the single exception of triallate forming 2-chloroacrylic acid, these conversions involve initial enzymatic sulfoxidation or hydroxylation at $-CH_2Br$, $-CH_2Cl$, $-CH_2O-$, or $-CH_2S-$ substituents and then facile nonenzymatic reactions to liberate 2-haloacroleins which are further oxidized to the 2-haloacrylic acids. 2-Haloacroleins as potent mutagens and intermediary metabolites may contribute to the adverse toxicological properties of DBCP, tris-BP, sulfallate, diallate, triallate, and octachlorodipropyl ether.

2-Chloroacrolein is of toxicological interest as a potent mutagen and microsomal oxidase metabolite of the *S*-chloroallyl dithiocarbamate sulfallate and the *S*-dichloroallyl thiocarbamate diallate (Rosen et al., 1980a,b; Schuphan and Casida, 1979a,b; Schuphan et al., 1979, 1981). These herbicides and their *S*-trichloroallyl thiocarbamate analogue triallate are promutagens (Carere et al., 1978; De Lorenzo et al., 1978; Rosen et al., 1980a; Schuphan et al., 1979, 1981; Sikka and Florczyk, 1978) or carcinogens (Innes et al., 1969; National Cancer Institute, 1978b). Other 2-haloacroleins (2-bromo, 2,3-dichloro, and 2,3,3-trichloro) are also potent mutagens (Rosen et al., 1980b) and possible metabolites of promutagens or carcinogens with haloallyl and halopropyl substituents (Figure 1). The nematocide and soil fumigant 1,2-dibromo-3-chloropropane (DBCP) is a carcinogen (Olson et al., 1973; Powers et al., 1975) and may cause male sterility (Lee and Suzuki, 1979). The flame retardant tris(2,3-dibromopropyl) phosphate (tris-BP) is a carcinogen (National Cancer Institute, 1978a; Reznik et al., 1979; Van Duuren et al., 1978), and the pyrethroid synergist octachlorodipropyl ether (OCPE) is a potential carcinogen (Van Duuren et al., 1969). The ultimate mutagen and possibly the carcinogen in each case might be the appropriate 2-haloacrolein, but these aldehydes are very reactive and therefore difficult to detect except indirectly in bioassays, e.g., Ames *Salmonella typhimurium* mutagenesis test (Rosen et al., 1980b; Schuphan et al., 1979).

2-Haloacrylic acids are probable urinary metabolites of compounds forming 2-haloacroleins as unstable intermediates. It might therefore be possible to evaluate possible precursors of mutagenic 2-haloacroleins by monitoring the urine for 2-haloacrylic acids, even though they are likely to be present in only small or trace amounts. This was accomplished by converting these acids to their pentafluorobenzyl (PFB) esters to provide enhanced sensitivity for analysis by gas chromatography (GC) with an electron capture detector (ECD). This report considers the identity and amount of 2-haloacrylic acids excreted by rats treated with DBCP, tris-BP, sulfallate, diallate, triallate, and OCPE.

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MATERIALS AND METHODS

Chromatography. GC utilized the Hewlett-Packard Model 5840 gas chromatograph with a Hewlett-Packard vitreous silica capillary column (25 m × 0.20 mm i.d.) coated with OV-101 deactivated with Carbowax 20. This computerized instrument recorded retention times and calculated peak areas. The operating conditions were 250 °C for the injector temperature, 125 °C for the column temperature, 300 °C for the ECD temperature, an injector split ratio of 100:1, helium carrier gas at a column flow rate of 0.30 mL/min, and a detector makeup gas of 95% argon–5% methane.

High-pressure liquid chromatography (HPLC) used a μPorasil column (25 × 0.5 cm) eluted with hexane–chloroform (4:1) and a 254-nm absorbance detector. Thin-layer chromatography (TLC) was accomplished on 0.25-mm silica gel chromatoplates.

Spectrometry. Mass spectrometry (MS) involved the Finnigan Model 3200 instrument operated in the chemical ionization (CI) mode with methane as the ionizing and carrier gas (0.7–0.9 torr). Pure samples were introduced by direct probe and metabolite mixtures through a Finnigan Model 9500 GC with a 5% OV-101 column (1 m × 32 mm i.d.). Nuclear magnetic resonance (NMR) spectra were obtained with a Perkin-Elmer R32B 90-MHz spectrometer.

Chemicals. Structures for the chemicals examined are given in Figure 1. Pure DBCP was a gift from Shell Development Co. (Houston, TX). Tris-BP (96%, Columbia Organic Chemicals Co., Inc., Columbia, SC) was obtained >99% pure by chromatography on a silica gel column with hexane–ethyl acetate (9:1). Sulfallate, diallate, and triallate from Chem Service (West Chester, PA) were purified on a silica gel column with a gradient of hexane to hexane–ether (9:1), monitoring the fractions by TLC [hexane–ether (9:1), UV visualization]. Pure triallate was obtained as slightly yellowish crystals (mp 30–32 °C) from hexane. OCPE was used as received from McLaughlin Gormley King Co. (Minneapolis, MN). GC examination of each of these compounds (20 μg) revealed no detectable haloacrylic acid impurities (<0.0002%; analyzed as PFB derivatives as described below). [*allyl*-^{14}C]Triallate was provided by Monsanto Agricultural Products Co. (St. Louis, MO).

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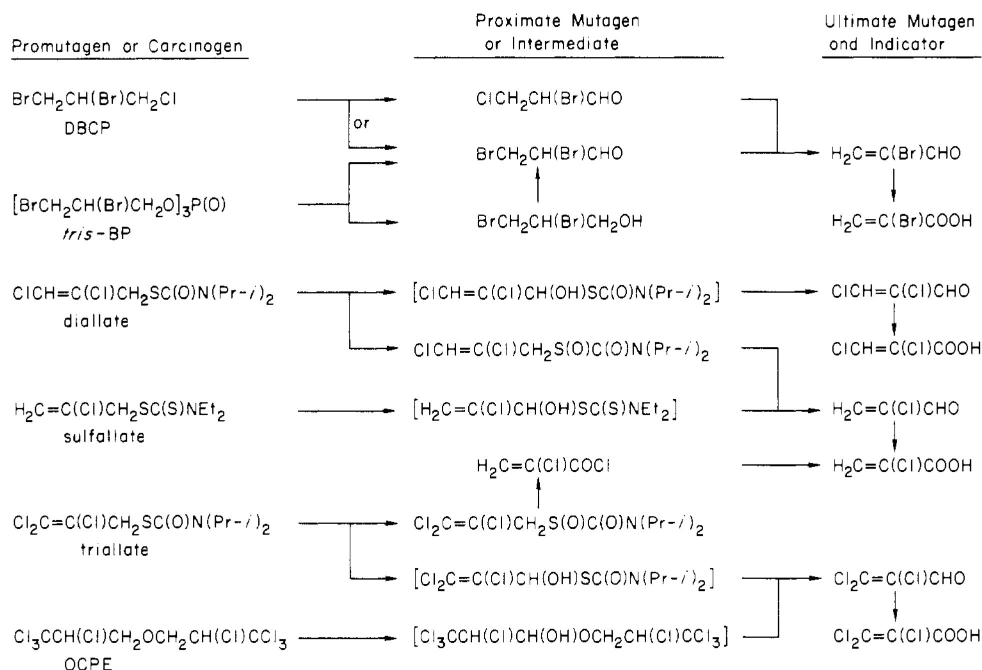

Figure 1. Partial metabolic pathways for several promutagens or carcinogens involving mutagenic haloacrolein intermediates. Abbreviations: Et = ethyl; Pr = propyl.

Dibromopropanal, chloroacrolein, trichloroacrolein, and chloroacrylyl chloride were prepared as previously described (Rosen et al., 1980b). Bromoacrylic acid was obtained by reacting 2,3-dibromopropionic acid (Aldrich Chemical Co., Milwaukee, WI) with excess sodium hydroxide in water (Owen and Babatunde Somade, 1947) and crystallization from petroleum ether (mp 69–70 °C; identity confirmed by CI-MS). Chloroacrylic acid from Polysciences, Inc. (Warrington, PA) was purified by sublimation. *cis*-Dichloroacrylic acid was prepared by refluxing 2,3-dichloromaleic acid in water for 4 days and sublimation of the chloroform-extractable products (mp 85–87°C; identity confirmed by CI-MS) (Badische Anilin- und Soda-Fabrik A.G., 1969). PFB *trans*-dichloroacrylate was obtained on photolysis of PFB *cis*-dichloroacrylate in benzene at 360 nm with isobutyrophenone as a photosensitizer. The identity of the trans ester was confirmed by GC–CI-MS and by NMR [(deuteriochloroform, ppm downfield from tetramethylsilane): 7.54 (1 H, s), 2.40 (2 H, s)] in comparison with that of the cis ester [7.61 (1 H, s), 2.40 (2 H, s)]. Trichloroacrylic acid was prepared by reacting hexachloropropylene (Aldrich) with sulfuric acid in aqueous aluminum sulfate (Bergmann and Haskelberg, 1941). Sources for other chemicals were as follows: 2,3-dibromopropanol, Aldrich; 2,3-dichloropropanol, Eastman Organic Chemicals, Rochester, NY; 2-bromo-3-chloro-1-propene, Columbia.

PFB haloacrylates were prepared by the procedure of Gyllenhaal and Ehrsson (1975). The acids in a two-phase mixture of aqueous 10% potassium carbonate (6 mL) and methylene chloride (2 mL) were reacted with PFB bromide (30 μL) (PCR Research Chemicals, Inc., Gainesville, FL) by using 3 mg of tetrabutylammonium hydrogen sulfate (Sigma Chemical Co., St. Louis, MO) as a phase transfer catalyst. After the mixture was stirred for 90 min at 25 °C, the recovered organic phase was reduced in volume under N_2 with addition of hexane to allow complete evaporation of methylene chloride without taking the mixture to dryness. Unreacted PFB bromide was removed on a 5% water-deactivated silica gel column (7.5 × 1 cm) eluted with 7 mL of hexane followed by 7 mL of hexane–benzene (9:1). The PFB haloacrylates were then eluted with 28 mL of hexane–benzene (3:1). Preparative samples of analytical standards were further purified by HPLC eluted with hexane–chloroform (4:1).

In Vivo Metabolism Studies. Male albino rats (180–200 g) were from Simonsen Laboratories (Gilroy, CA). Animals treated orally were starved for 8 h before 300 μL of a corn oil solution of the test compound was administered via a stomach tube. Intraperitoneal (ip) treatment involved methoxytriglycol (MTG) (200 μL) as the carrier vehicle. Glass metabolism cages were used for urine collection at room temperature into 5 mL of an aqueous solution 1 M in phosphoric acid, 0.05 M in *N*-ethylmaleimide (NEM), and saturated with boric acid. Phosphoric acid and NEM increased the recoveries of haloacrylic acids from urine and boric acid removed interfering peaks in the chromatograms. The 0–24-h urine was filtered through Celite Analytical Filter-Aid (Johns-Manville Products Corp., Lompoc, CA) that had been washed with 1 M phosphoric acid. The filtrate was adjusted to 30 mL with distilled water, saturated with sodium chloride, and extracted with ethyl acetate (20 mL × 3). The ethyl acetate extract was back-extracted with aqueous 5% potassium carbonate (2 mL × 3). This potassium carbonate extract was reacted with PFB bromide, additional potassium carbonate (to make to 10%), and tetrabutylammonium hydrogen sulfate in a two-phase system with methylene chloride as previously described. The methylene chloride fraction was subjected to cleanup as above to remove excess PFB bromide or in the case of diallate-treated rats to column chromatography and then HPLC to remove interferences with nearly the same retention times as the PFB *cis*- and *trans*-dichloroacrylates (Figure 2). The final volume of derivatized material was adjusted to 0.5–2 mL for GC-ECD analysis of a 2.0-μL aliquot. The amounts of recovered PFB haloacrylates obtained from standard calibration curves for the pure compounds were used to determine the equivalent amounts of haloacrylic acids in the urine.

For confirmation of the identity of derivatized metabolites, separate groups of four rats were treated orally with each of DBCP (0.5 mmol/kg), tris-BP (0.5 mmol/kg), and triallate (1.5 mmol/kg). The pooled urine from each group

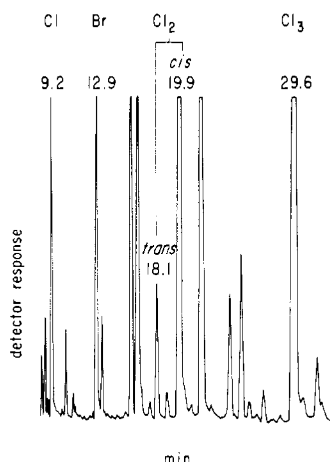

Figure 2. Gas chromatographic separation of haloacrylic acids in urine extract as their pentafluorobenzyl esters. Retention times are given in minutes for bromo (Br), chloro (Cl), *cis*- and *trans*-dichloro (Cl_2), and trichloro (Cl_3) PFB haloacrylate derivatives. Normal urinary products do not interfere with analysis of the Br, Cl, and Cl_3 compounds, but additional HPLC cleanup is required for analysis of the *cis*- and *trans*-dichloro derivatives.

was analyzed as above with final concentration of the derivatized material to 50 μL from which 10 μL was subjected to GC–CI-MS analysis for PFB haloacrylates.

In Vitro Metabolism Studies. Liver microsomes from rabbits were prepared in 50 mM pH 7.4 phosphate buffer. Ten microliters of an ethanolic solution of the substrate (10 μg of DBCP, tris-BP, or triallate) was added to 4 mL of this buffer containing the microsomal fraction (0.4 mg of protein) and reduced nicotinamide adenine dinucleotide phosphate (NADPH) (0 or 3.6 μmol). Following incubation for 10 min at 37 °C, nicotinamide adenine dinucleotide (NAD) (3.6 μmol) was added and the incubation was continued for 5 min to facilitate conversion of haloacroleins to haloacrylic acids. This mixture was treated with 0.5 mL of 1 M phosphoric acid, 0.1 g of NEM, and 0.1 g boric acid. It was then saturated with sodium chloride and extracted with ethyl acetate for workup as above. The derivatization and analysis procedures were modified from those previously described only in that 10 μL of PFB bromide was used, the potassium carbonate concentration was 5%, and the aliquot for GC-ECD was 2.0 μL from 200 μL of total volume.

Toxicity Studies. LD_{50} determinations involved ip treatment of male albino mice (18–22 g, Simonsen) using 200 μL of MTG as the carrier vehicle and recording the mortality at 48 h.

RESULTS

Haloacrylic Acid Recoveries from Fortified Rat Urine and from Urine of ip-Treated Rats. Recovery values from fortified rat urine (1 μg/mL) are relatively low (24% for trichloroacrylic acid, 6.1% for chloroacrylic acid, and 1.6% for bromoacrylic acid), probably due to facile reactions with thiols (Benisek, 1966) or other urine components and to instability under the alkaline conditions of conversion to PFB esters. Recovery values from 0–24-h urine following ip administration of haloacrylic acids to rats are also low (0.2–0.6%) (Table I) because of the aforementioned instability combined with loss due to in vivo detoxification systems.

Identification of Haloacrylic Acids as Rat Urinary Metabolites of Various Compounds with Haloallyl and Halopropyl Substituents. Haloacrylic acids in urine of rats treated orally with each of the compounds shown in Table II are identified by GC cochromatography of their

Table I. Haloacrylic Acids in Urine of Rats Intraperitoneally Administered Haloacroleins and Related Compounds at 10 μmol/kg

compound administered	haloacrylic acid recovery	
	substituent	%[a]
bromoacrylic acid	Br	0.35, 0.39
dibromopropanal[b,c]	Br	0.11, 0.084
dibromopropanol	Br	0.035, 0.035
bromochloropropene	Br	<0.0002, <0.0002
DBCP	Br	0.037, 0.037
chloroacrolein	Cl	0.25, 0.27
chloroacrylic acid	Cl	0.27, 0.16
chloroacrylyl chloride	Cl	0.28, 0.33
dichloropropanol	Cl	0.11
trichloroacrolein[b]	Cl_3	0.43, 0.43
trichloroacrylic acid	Cl_3	0.58, 0.61

[a] Each value is the average of duplicate analyses of urine from one rat. [b] 7 μmol/kg. [c] Bromoacrolein is formed on rapid dehydrobromination.

Table II. Haloacrylic Acids in Urine of Rats Orally Administered DBCP, Tris(dibromopropyl) Phosphate, *S*-Chloroallyl Herbicides, and Octachlorodipropyl Ether at 100 μmol/kg

compound administered	haloacrylic acid recovery	
	substituent	%[a]
DBCP	Br	0.038, 0.036
tris-BP	Br	0.027, 0.027
sulfallate	Cl	0.0064, 0.0081
diallate	Cl[b]	0.021, 0.021
triallate	Cl	0.016, 0.020
triallate	Cl_3	0.065, 0.072
OCPE	Cl_3	0.014, 0.018

[a] Each value is the average of duplicate analyses of urine from one rat. [b] *cis*-Dichloroacrylic acid and *trans*-dichloroacrylic acid are also detected but they are not quantitated because of the additional HPLC cleanup step required for their PFB esters prior to GC.

PFB esters with authentic standards (Figure 2). The products from DBCP, tris-BP, and triallate give identical GC–CI-MS with those of the appropriate standards (*m/e*, relative abundance): for PFB bromoacrylate, 331 (0.7) (M + 1), 181 (100) (PFB ion), 133 (11) (bromoacrylium ion); for PFB trichloroacrylate 355 (0.6) (M + 1), 181 (100) (PFB ion), 157 (14) (trichloroacrylium ion). Although not detailed here, we find that the urine of rats treated orally with [*allyl*-^{14}C]triallate (>99% radiochemical purity) contains both [^{14}C]chloroacrylic acid and [^{14}C]trichloroacrylic acid analyzed as their PFB derivatives with identification by two-dimensional TLC cochromatography.

Haloacrylic Acids in Rat Urine following ip Administration of Haloacroleins and Related Compounds. Haloacrylic acid recoveries are similar for rats treated with haloacroleins (chloro and trichloro) (0.3–0.4%) and those treated directly with haloacrylic acids (bromo, chloro, and trichloro) or with chloroacrylyl chloride (0.2–0.6%) (Table I), suggesting the ease of in vivo conversion of haloacroleins to haloacrylic acids and their further degradation.

Dibromopropanol and dichloropropanol undergo oxidation and dehydrohalogenation in an undetermined sequence to give the corresponding urinary haloacrylic acids in amounts of 10 and 50%, respectively, of those resulting on direct haloacrylic acid administration (Table I). Bromoacrylic acid is formed more efficiently from dibromopropanal than from dibromopropanol or DBCP whereas none is detected from 2-bromo-3-chloro-1-propene.

Haloacrylic Acids in Rat Urine following Oral Administration of DBCP, Tris-BP, *S*-Chloroallyl

Table III. Metabolic Conversion of DBCP, Tris-BP, and Triallate to Haloacrylic Acids in the Rabbit Liver Microsome-NADPH System with or without NADPH

		haloacrylic acid recovery	
		%[a]	
substrate	substituent	−NADPH	+NADPH
DBCP	Br	<0.002	0.11, 0.12
tris-BP	Br	<0.002	0.54, 0.40
triallate	Cl	<0.002	0.26, 0.10
triallate	Cl_3	<0.002	0.46, 0.28

[a] Each value is the average of duplicate analyses of different incubation mixtures.

Herbicides, and OCPE. Urinary bromoacrylic acid recoveries are the same on a percentage basis from DBCP administered ip or orally (Tables I and II) and are 10% of that resulting on direct bromoacrylic acid administration (Table I). Orally administered tris-BP gives 73% as much urinary bromoacrylic acid as orally administered DBCP (Table II). Chloroacrylic acid is detected in much larger amount with diallate and triallate than with sulfallate. *cis*- and *trans*-dichloroacrylic acids are also excreted on administration of diallate and trichloroacrylic acid on treatment with triallate and OCPE.

Metabolic Conversion of DBCP, Tris-BP, and Triallate to Haloacrylic Acids in Rabbit Liver Microsomal Oxidase Systems. DBCP and tris-BP are converted to bromoacrylic acid and triallate to chloro- and trichloroacrylic acids, each in an NADPH-dependent reaction (Table III). These yields for haloacrylic acids (0.1–0.5%) are similar to those obtained for [^{14}C]chloroacrolein from [*allyl*-^{14}C]sulfallate (0.6%) in a rat liver microsomal oxidase system (Rosen et al., 1980a) and from [*allyl*-^{14}C]diallate (1.6%) in a mouse liver microsomal oxidase system (Schuphan and Casida, 1979b).

Acute Toxicity of Haloacroleins to Mice. Mouse ip 48-h LD_{50} values (milligrams per kilogram) are 4–8 for chloroacrolein and trichloroacrolein and 5–10 for dibromopropanal. The latter compound rapidly dehydrobrominates to bromoacrolein.

DISCUSSION

This study is the first demonstration of in vivo formation of haloacrylic acids from halopropyl and haloallyl derivatives. These conversions involve haloacrolein intermediates except for formation of 2-chloroacrylic acid from triallate (Figure 1). The low yields are attributable to the reactive nature of the haloacroleins (Rosen et al., 1980b) and haloacrylic acids (Benisek, 1966) as well as the variety of biological reactions they undergo. Although the observed levels of haloacrylic acids underestimate the amounts of haloacrolein intermediates, the PFB derivatization technique provides a convenient monitoring procedure since it does not require ^{14}C-labeled test compounds and detects submicrogram amounts of excreted haloacrylic acids.

The carcinogenic and mutagenic activities of DBCP and tris-BP may be associated in part with metabolic activation to form bromoacrolein. The most likely precursor of this aldehyde is dibromopropanal or bromochloropropanal (Figure 1), which under physiological conditions would undergo rapid spontaneous dehydrohalogenation to bromoacrolein (Rosen et al., 1980b). Dihalopropanal formation may be most efficiently accomplished by hydroxylation at the $-CH_2Cl$, $-CH_2Br$, or $-CH_2OP(<)=$ substituent resulting in direct aldehyde release. Dibromopropanol and bromoallyl alcohol from hydrolysis of tris-BP (Blum et al., 1978; St. John et al., 1976) and DBCP (Worthing, 1979), respectively, may also serve as intermediates in haloacrolein formation. The pathway of DBCP to bromoacrylic acid apparently does not involve initial dehydrobromination to 2-bromo-3-chloro-1-propene. DBCP undergoes extensive fragmentation and conjugate formation in rats (Kato et al., 1979).

A portion of the adverse toxicological properties of sulfallate, diallate, triallate, and OCPE may be due to mutagenic chloroacrolein metabolites. *S*- and *O*-methylene hydroxylation provides direct access to chloroacrolein from sulfallate, dichloroacrolein from diallate, and trichloroacrolein from triallate and OCPE (Figure 1) (Rosen et al., 1980a; Schuphan et al., 1981). Alternative metabolic routes initiated by *S*-oxygenation yield chloroacrolein from diallate and chloroacrylyl chloride from triallate (Figure 1). Additional but less likely pathways to the haloacroleins involve chloroallyl mercaptan intermediates (most of which are converted to the chloroallylsulfonic acids; Schuphan and Casida, 1979b).

Microsomal oxidases convert DBCP, tris-BP, and triallate to haloacrylic acids, presumably via bromo- and trichloroacroleins (Rosen et al., 1980a; Schuphan and Casida, 1979b), confirming in vivo findings. Larger haloacrylic acid yields in vitro than in vivo are probably due in part to the absence of soluble enzymes and glutathione for decomposition and conjugation reactions.

Compounds known or suspected to be haloacrolein precursors can be considered as candidate mutagens and carcinogens.

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Pyrethroid Metabolism: Comparative Fate in Rats of Tralomethrin, Tralocythrin, Deltamethrin, and (1*R*,α*S*)-*cis*-Cypermethrin

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The insecticides tralomethrin and tralocythrin, (*S*)-α-cyano-3-phenoxybenzyl *cis*-(1*R*,3*R*,1′*R* or *S*)-3-(1,2-dibromo-2,2-dihaloethyl)-2,2-dimethylcyclopropanecarboxylates, were compared with deltamethrin and (1*R*,α*S*)-*cis*-cypermethrin relative to their distribution, excretion, metabolic fate, and tissue residues following oral administration to male rats. Tralomethrin and tralocythrin are not normally detected in treated animals or their excreta since they undergo rapid and essentially complete debromination to form deltamethrin and (1*R*,α*S*)-*cis*-cypermethrin, respectively. Deltamethrin and cypermethrin are then hydroxylated at the 2′, 4′, and 5 positions of the alcohol moiety and the methyl group trans to the carboxylate linkage. Extensive ester cleavage reactions for deltamethrin and cypermethrin and further metabolism of the cleavage products yield the expected series of alcohols and carboxylic acids and their glucuronide, glycine, and sulfate conjugates. The cyano fragment is retained several days in the stomach and skin. Toxicity studies with mice provide evidence that intracerebrally administered tralomethrin and tralocythrin may be activated by debromination in the brain.

Tralomethrin and tralocythrin (proposed common names) are potent pyrethroids (Roussel-Uclaf, 1978) that differ from the established deltamethrin and cypermethrin in having 3-tetrahaloethyl substituents instead of 3-dihalovinyl groups (Figure 1). The 1*R*,α*S*-cis configuration confers the highest insecticidal potency, and esters with the *R* and *S* configurations at the 1′ center of the 3 side chain are comparable in activity (Ackermann et al., 1980). Tralomethrin and tralocythrin are rapidly converted to deltamethrin and cypermethrin, respectively, in insects (Ruzo et al., 1981) and on irradiation with light (Ruzo and Casida, 1981).

The present study compares the distribution and metabolism of tralomethrin and tralocythrin in rats with those known for deltamethrin and cypermethrin in rats and mice (Crawford et al., 1981; Hutson et al., 1981; Ruzo et al., 1978, 1979).

MATERIALS AND METHODS

Chemicals. Tralomethrin, tralocythrin, deltamethrin, and (1*R*,α*S*)-*cis*-cypermethrin were supplied by Roussel-Uclaf (Paris, France) as unlabeled samples and as ^{14}C-labeled compounds of 40–60 mCi/mmol labeled in separate preparations in each of the benzylic methine (alcohol-^{14}C), geminal dimethyl (acid-^{14}C), and cyano (^{14}CN) positions. Thin-layer chromatography (TLC) on silica gel (see below) with toluene–carbon tetrachloride (6:1) (two developments) was used to obtain 1′*R* (lower R_f component), 1′*S* (higher R_f component), and 1′*RS* samples of >99% radiochemical purity at the time of use (Ackermann et al., 1980; Ruzo and Casida, 1981).

Metabolites are designated as shown in Figure 1, e.g., 4′-hydroxy and *trans*-hydroxy derivatives are hydroxylated at the 4′ position of the phenoxybenzyl moiety and the methyl trans to the carboxyl group, respectively, and ITCA is 2-iminothiazolidine-4-carboxylic acid. Standard unlabeled compounds for tentative metabolite identification are previously described (Ruzo and Casida, 1981; Ruzo et al., 1978; Unai and Casida, 1977). Although not shown in Figure 1, X_4CA refers to the free acid moieties of tralomethrin and tralocythrin.

Treatment of Rats and Determination of Radiocarbon in Excreta and Tissues. Male albino Sprague-Dawley rats (160–170 g, Simonsen Laboratories, Gilroy, CA) fasted for 18 h were individually treated by stomach tube with each labeled compound dissolved in a mixture of diethyl ether (75 μL) and partially hydrogenated soybean oil (Crisco oil) (150 μL); the stomach tube was then rinsed with soybean oil (100 μL). The treated rats were held in all-glass metabolism cages (Gaughan et al., 1977) for collection of urine and feces for 7 days (all compounds) and of expired $^{14}CO_2$ for 48 h (^{14}CN-labeled compounds only) in sequential traps of 10% KOH and a monoethanolamine–methyl-Cellosolve mixture (1:2). Procedures for radiocarbon quantitation by liquid scintillation counting (LSC) are given by Ueda et al. (1975).

Urine was analyzed directly by LSC. Feces (0–24-h wet or fresh samples) were extracted with cold ether (10 mL/g)

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