unknown. The fact that 1 and the 6-chloro and 6-bromo analogues 19 and 21 were either inactive or had only slight activity in the wheat cylinder, pea segment, and pea curvature tests (James and Wain, 1968) suggests that these compounds do not possess auxin activity. Additional studies will be required to elucidate the mode of action of 6-methylanthranilic acid and analogues.

ACKNOWLEDGMENT

I am grateful to W. H. de Silva, P. F. Bocion, and P. Eggenberg for carrying out biological evaluations and to A. Krohn for Hansch analysis.

Registry No. 1, 4389-50-8; 2, 2941-78-8; 3, 2305-36-4; 4, 4389-45-1; 5, 2840-04-2; 6, 2486-75-1; 7, 52130-17-3; 8, 2486-70-6; 9, 2458-12-0; 10, 118-92-3; 11, 66232-56-2; 12, 66232-47-1; 13, 66232-53-9; 14, 66232-54-0; 15, 53600-33-2; 16, 90321-28-1; 17, 90321-29-2; 18, 90321-30-5; 19, 2148-56-3; 20, 19407-42-2; 21, 20776-48-1; 22, 50573-74-5; 23, 6946-22-1; 24, 1885-31-0; 25, 56043-01-7; 26, 18595-13-6; 27, 65658-16-4; 28, 39967-87-8; 29, 90321-31-6; 30, 37777-66-5; 31, 66232-39-1; 32, 66232-55-1; 33, 90321-32-7; 34, 66232-45-9; 35, 66232-41-5; 36, 66232-48-2; 37, 66232-49-3; 38, 66232-50-6; 39, 66232-44-8; 40, 66232-60-8; 41, 567-61-3; 42, 17839-53-1; 43, 18239-19-5; 44, 21327-86-6; 45, 90259-31-7; 46, 54811-50-6; 47, 13506-76-8; 48, 15540-91-7; 49, 90321-33-8; 50, 5628-48-8; 51, 90321-34-9; 52, 90321-35-0; 53, 89977-14-0; 54, 89977-13-9; 55, 90321-36-1; 56, 90321-37-2.

LITERATURE CITED

Audus, L. J. "Plant Growth Substances"; Leonard Hill: London, 1972; Vol. 1, p 87.
Baker, B. R.; Schaub, R. E.; Joseph, J. P.; McEvoy, F. J.; Williams, J. H. *J. Org. Chem.* **1952a**, *17*, 164.
Baker, B. R.; Schaub, R. E.; Joseph, J. P.; McEvoy, F. J.; Williams, J. H. *J. Org. Chem.* **1952b**, *17*, 149.
Bullock, E.; Johnson, A. W. *J. Chem. Soc.* **1957**, 1602.
Courtin, A. *Chimia* **1975**, *29*, 260.
de Silva, W. H.; Bocion, P. F.; Eggenberg, P.; de Mur, A. *J. Plant Dis. Prot.* **1979**, *86*, 546.
Farkas, L.; Soti, F.; Incze, M.; Nogradi, M. *Chem. Ber.* **1974**, *107*, 3874.
Gabriel, S.; Thieme, A. *Chem. Ber.* **1919**, *52*, 1079.
James, C. S.; Wain, R. L. *Ann. Appl. Biol.* **1968**, *61*, 295.
Kovacic, P.; Levisky, J. A.; Goralski, C. T. *J. Am. Chem. Soc.* **1966**, *88*, 100.
Moll, H.; Musso, H.; Schroder, H. *Angew. Chem., Int. Ed. Engl.* **1963**, *2*, 212.
Piper, J. R.; Stevens, F. J. *J. Org. Chem.* **1962**, *27*, 3134.
Stom, D. I.; Khutoryanskii, V. A. *Fiziol. Biokhim. Kul't. Rast.* **1972**, *4*, 183; *Chem. Abstr.* **1972**, *77*, 97678c.
Theilacker, W.; Hopp, R. *Justus Liebigs Ann. Chem.* **1963**, *669*, 85.

Received for review October 12, 1983. Accepted March 21, 1984.

Metribuzin Metabolites in Mammals and Liver Microsomal Oxidase Systems: Identification, Synthesis, and Reactions

Marian Saeman Bleeke and John E. Casida*

Mercapturic acid derivatives are the major urinary metabolites of metribuzin [4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4*H*)-one] in intraperitoneally treated mice and orally treated rats, accounting for ~20% of the dose. These mercapturates of metribuzin and deaminometribuzin, in which the methylthio substituent is replaced by an *N*-acetylcysteinyl moiety, are also the major products on incubation of mouse and rat liver microsomes with metribuzin in the presence of *N*-acetylcysteine and NADPH. Other NADPH-dependent metabolites are deaminometribuzin and protein-bound material, the latter formed in large amounts only when *N*-acetylcysteine is not added. Deamination appears to be more important in rat than in mouse metabolism, both in vivo and in vitro. These findings suggest the formation of metribuzin sulfoxide and deaminometribuzin sulfoxide as activated intermediates. Oxidation of metribuzin and deaminometribuzin with *m*-chloroperbenzoic acid yields the corresponding sulfoxides, which react readily with *N*-acetylcysteine or protein in neutral aqueous solutions. The *N*-amino group is also cleaved on peracid oxidation, but *S*-methyl sulfoxidation occurs more rapidly.

Metribuzin or Sencor [4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4*H*)-one] is a potent photosystem II inhibitor used as a pre- and postemergence herbicide against a variety of broadleaf and grass weeds in potatoes, soybeans, and other tolerant crops (Draber et al., 1968; Eue, 1972). Acid hydrolyzes the SMe substituent from the metribuzin ring to give a diketo derivative (Frear et al., 1983a), and metal-catalyzed oxidation with *tert*-butyl hydroperoxide cleaves either the C–SMe or N–NH_2 group depending on the catalyst to form the diketo or deamino derivative, respectively (Nakayama et al., 1982). The metabolic fate of metribuzin is reported in several plant systems but not in animals. Soybean, sugarcane, and/or tomato form metribuzin *N*-glucoside and malonyl *N*-glucoside, the homoglutathione conjugate, and the deamino-, diketo-, and deaminodiketo derivatives (Hilton et al., 1974; Mangeot et al., 1979; Frear et al., 1983a,b). Metribuzin sulfoxide is a possible intermediate in metabolic formation of the homoglutathione conjugate (Frear et al., 1983b).

This investigation considers the metabolism of metribuzin in rats and mice and their liver microsomal oxidase systems. It also develops a chemical model for the observed reactions with emphasis on the importance of metribuzin sulfoxide as an activated intermediate.

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

MATERIALS AND METHODS

Chromatography and Analysis. Thin-layer chromatography (TLC) utilized precoated silica gel 60 F-254 20

0021-8561/84/1432-0749$01.50/0

Table I. Thin-Layer Chromatography R_f Values for Metribuzin and Related Compounds

unconjugated triazinones[a]	TLC R_f in indicated solvent system[b] CE	DEH
I-SMe	0.54	0.46
I-S(O)Me	0.09	
II-SMe	0.42	0.29
II-S(O)Me	0.04	
III-NH_2	0.23	0.10
III-H	0.32	0.20
IV-NH_2	0.60	0.59
conjugated triazinones[a,c]	**TLC R_f in indicated solvent system[b] BAW**	**MBBW**
I-SMA	0.50	0.69
I-SG	0.26	
II-SMA	0.50	0.69
II-SG	0.26	

[a] For compound designations, see Figure 1. [b] Silica gel chromatoplates developed with the following solvent systems: CE, chloroform–ethyl acetate, 1:1; DEH, dichloromethane–ether–hexane, 3:2:2; BAW, 1-butanol–acetic acid–water, 4:1:1; MBBW, methanol–benzene–1-butanol–water, 2:1:1:1. [c] R_f = 0.00 in CE and DEH.

× 20 cm chromatoplates (EM Laboratories, Inc., Elmsford, NY) with 0.25 and a 0.5 mm layer thickness for analysis and product isolation, respectively. Solvent systems and R_f values are given in Table I. Nonradioactive products were detected by ultraviolet (UV) quench and ^{14}C-labeled compounds by autoradiography. High-performance liquid chromatography (HPLC) was carried out on a μBondapak CN column (Waters Associates, Milford, MA). Radiocarbon content was quantitated by liquid scintillation counting (LSC) of liquid samples, radioactive gel regions scraped from TLC plates, and combusted solid samples. Melting points (mp) are uncorrected.

Proton nuclear magnetic resonance (^1H NMR) spectra were obtained with either a Perkin-Elmer R32B 90-MHz or a Bruker WM 300-MHz spectrometer. ^{13}C NMR spectra were recorded with the Bruker WM 300 instrument (75.4 MHz for ^{13}C). Samples were dissolved in deuterated solvents containing 1% tetramethylsilane as the internal standard. IR spectra were taken with a Perkin-Elmer 457 grating spectrophotometer and UV spectra with a Perkin-Elmer 576 spectrophotometer. Chemical ionization mass spectra (CI-MS) were recorded with a Finnigan 3200 instrument with methane as the reagent gas.

Chemicals. Figure 1 gives the structures and designations for metribuzin (I-SMe) and its metabolites and reaction products and related compounds. The chemicals are *N*-aminotriazinones (I), triazinones (II), triazinediones (III), thioxotriazinones, (IV), or various N-2-Me (V), N-4-Me (VI), or O-Me (VII) derivatives. Abbreviations for the ring substituents are given in Figure 1.

I-SMe (99% chemical purity) and [*carbonyl*-^{14}C]I-SMe (4.4 mCi/mmol; >99% radiochemical purity) were provided by Mobay Chemical Corp. (Kansas City, MO). Spectral data for I-SMe: ^1H NMR ($CDCl_3$) δ 4.94 (br s, 2 H, NH_2), 2.61 (s, 3 H, SMe), 1.43 (s, 9 H, *t*-Bu); ^{13}C NMR ($CDCl_3$) δ 161.0, 160.7, 151.0, 37.6, 27.5, 14.2; UV (MeOH) λ_{max} 227 nm (log ε 3.9), 293 (3.9).

Metabolism of [^{14}C]Metribuzin in Rats and Mice. [^{14}C]Metribuzin was administered orally to male albino rats (180–200 g) at 200 mg/kg and intraperitoneally (ip) to male Swiss-Webster mice (20–25 g) at 75 mg/kg, in each case with methoxytriglycol (MTG) as the carrier vehicle. The animals were held for 5 days in all-glass metabolism cages with ground rat chow and water ad libitum. Expired

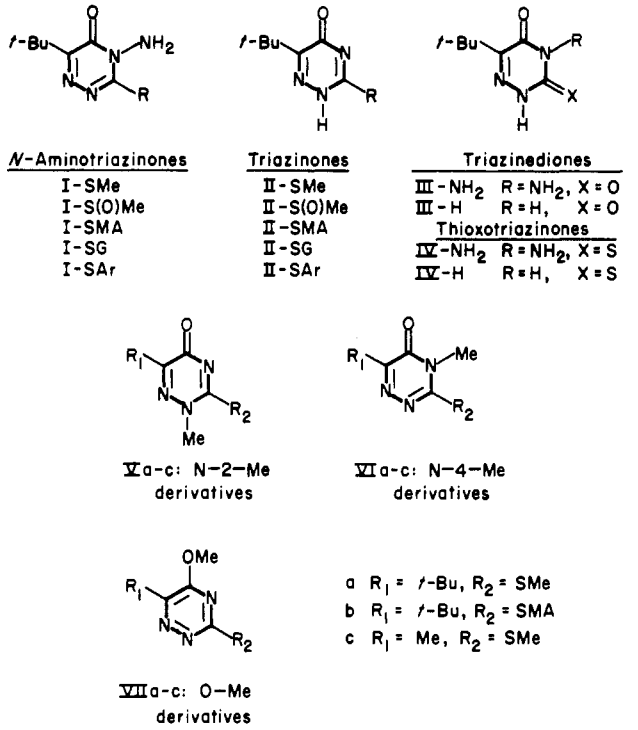


Figure 1. Structures and designations for metribuzin and related compounds. (Me = methyl; *t*-Bu = *tert*-butyl; Ar = 3,4-dichlorophenyl; SG = glutathionyl; SMA = *N*-acetylcysteinyl or mercapturic acid. The SMA derivatives Vb and VIIb are methylated).

$^{14}CO_2$ was monitored for the rats by collection in a monoethanolamine–methyl cellosolve, 2:1, mixture. The urine and a methanol extract of the feces were analyzed by LSC and TLC (CE and BAW). For isolation of the mercapturic acids, rat urine was diluted with an equal volume of saturated NaCl, acidified to pH 2 with 1 N HCl, and extracted with 3 volumes of ether–ethanol, 3:1, and the extract was subjected to preparative TLC (BAW) (mercapturic acids R_f = 0.50).

Metabolism of [^{14}C]Metribuzin in Rat and Mouse Liver Microsomal Enzyme Systems. Rats and mice as above were treated ip with phenobarbital at 80 mg/kg on each of 3 consecutive days with sacrifice on the fourth day. Livers from these phenobarbital-induced animals were homogenized at 20% (fresh weight/volume) in 0.1 M phosphate, pH 7.4, buffer. Centrifigal fractionation gave the soluble fraction (100000*g* supernatant) and the microsomal pellet (13000*g* supernatant and 100000*g* pellet washed once by resuspension and recentrifigation). These fractions were stored at −80 °C until used. Incubation mixtures in 0.1 M phosphate, pH 7.4, buffer (2.5 mL) contained the microsomal preparation (0.75 mL at 20% fresh liver weight equivalent) with one or more of the following additions: soluble fraction (0.50 mL), reduced nicotinamide adenine dinucleotide phosphate (NADPH) (2.2 mg), *N*-acetylcysteine (NAcCys) or glutathione (GSH) (5 mg), and [^{14}C]I-SMe (0.05 μCi, 20 nmol) added last in ethanol (20 μL). After incubation in a 25-mL Erlenmeyer flask with shaking for 2 h at 37 °C, the mixture was extracted with chloroform (3 × 2.5 mL). The aqueous phase was lyophilized to dryness and the residue extracted with methanol (3 × 2.5 mL). The dried ($MgSO_4$) chloroform fraction and the methanol fractions were concentrated under a stream of nitrogen and separated by TLC (CE, BAW). Methanol fractions from large-scale incubations (500 μg of I-SMe/flask) were subjected to TLC (BAW) for isolation of mercapturic acids. Soluene (Packard Instru-

ment Co., Downers Grove, IL) was used to solubilize protein fractions for LSC. A comparative study employed enzyme from rats and mice that received no phenobarbital pretreatment.

Hydrolysis and Methylation of Mercapturic Acids. The TLC-purified mercapturic acid metabolites were subjected to acid hydrolysis (2 N HCl, 100 °C, 3 h), neutralized with NaOH, and extracted into chloroform for TLC analysis (CE). They were also methylated with diazomethane (a hazardous chemical; handle with caution) followed by separation by TLC (chloroform–ethyl acetate, 13:10; Vb R_f = 0.09, VIIb R_f = 0.21) and HPLC (hexane–ethyl acetyl acetate, 2:1; Vb elutes completely before VIIb) and analysis by CI-MS and UV.

Syntheses and Spectral Features of Triazinones, Triazinediones, Thioxotriazinones, and Related Compounds (Figure 1). The syntheses are based on procedures referenced in Neunhoeffer and Wiley (1978).

II-SMe. IV-H (3.5 g, 19 mmol, described below) was added to a solution of sodium (1.38 g, 60 mmol) in methanol (30 ml). Methyl iodide (1.3 mL, 21 mmol) was added, and the solution was heated at reflux for 15 min. Most of the methanol was then removed under vacuum, water (50 mL) was added, and the solution was neutralized with 2 N HCl. A precipitate formed, and the mixture was extracted with ethyl acetate. After drying ($MgSO_4$), the ethyl acetate was removed under vacuum. Recrystallization from chloroform–hexane yielded II-SMe (3.3 g, 87%, mp 199–200 °C): ^{1}H NMR (CD_3COCD_3) δ 2.55 (s, 3 H, Me), 1.33 (s, 9 H, *t*-Bu); ^{13}C NMR (CD_3COCD_3) δ 164.2, 160.0, 157.8, 37.1, 27.4, 12.4; IR (2% MeOH–$CHCl_3$) 1650 cm^{-1} (C=O); UV (MeOH) λ_{max} 236 (log ϵ 3.1); CI-MS *m/e* 200 (M + 1).

III-NH$_2$. I-SMe (200 mg, 0.93 mmol) was refluxed in 2 N HCl (50 mL) for 3 h. The solution was cooled, neutralized with 10% NaOH, and extracted with ether. Washing the ether layer with saturated NaCl, drying ($MgSO_4$), and solvent evaporation yielded III-NH_2 (145 mg, 79%, mp 167–168 °C): ^{1}H NMR ($CDCl_3$) δ 5.20 (br s, 2 H, NH_2), 1.33 (s, 9 H, *t*-Bu); ^{13}C NMR (CH_3COCH_3 + CD_3COCD_3) δ 152.8, 149.7, 148.1, 37.4, 27.8; UV (MeOH) λ_{max} 212 nm (log ϵ 3.7), 262 (3.7); CI-MS *m/e* 185 (M + 1).

III-H. II-SMe (200 mg, 1.0 mmol) was treated as above to obtain III-H (120 mg, 70%, sublimed 260–265 °C): ^{1}H NMR (CD_3COCD_3) δ 1.35 (s, *t*-Bu); ^{13}C NMR ($CDCl_3$ + CD_3OD) δ 155.9, 150.7, 149.9, 36.0, 26.7; UV (MeOH) λ_{max} 208 nm (log ϵ 3.7), 261 (3.7); CI-MS *m/e* 170 (M + 1).

IV-NH$_2$. Addition of trimethylpyruvic acid (from oxidation of pinacolone with alkaline $KMnO_4$; Saeman, 1984) (1.6 g, 12.3 mmol) to thiocarbohydrazide (1.3 g, 12.3 mmol; Aldrich) dissolved in refluxing water (20 mL) gave a fine white precipitate. The suspension was stirred for 1 h and filtered to yield IV-NH_2 (2.1 g, 84%, mp 219–220 °C): ^{1}H NMR (CD_3COCD_3) δ 6.43 (br s, 2 H, NH_2), 1.37 (s, 9 H, *t*-Bu); UV (MeOH) λ_{max} 204 nm (log ϵ 3.5), 270 (4.0); CI-MS *m/e* 201 (M + 1).

IV-H. Thiosemicarbazide (4.4 g, 48 mmol; Aldrich) in hot water (100 mL) was treated with trimethylpyruvic acid (6.3 g, 48 mmol) as above to obtain a white precipitate. NaOH pellets (3.8 g, 96 mmol) were added, the precipitate was dissolved, and the solution was heated for 30 min. After cooling and neutralization with 5 N HCl, the precipitate was filtered and recrystallized from ether to yield IV-H (3.9 g, 44%, sublimed 260–270 °C): CI-MS *m/e* 186 (M + 1).

Va, VIa, and VIIa. A solution of II-SMe (43 mg, 0.22 mmol) in methanol (5 mL) was treated dropwise with a diazomethane–ether solution until the bubbling stopped and the yellow color remained. The mixture was separated by preparative TLC (ether–hexane, 1:1) into three fractions with a combined yield of 32 mg (68%). Va: 8 mg (25% of products), R_f = 0.08; ^{1}H NMR ($CDCl_3$) δ 3.72 (s, 3 H, NMe), 2.55 (s, 3 H, SMe), 1.29 (s, 9 H, *t*-Bu); UV (MeOH) λ_{max} 236 nm (log ϵ 4.1); CI-MS *m/e* 214 (M + 1). VIa: 14 mg (44% of products), R_f = 0.22; ^{1}H NMR ($CDCl_3$) δ 3.45 (s, 3 H, NMe), 2.67 (s, 3 H, SMe), 1.37 (s, 9 H, *t*-Bu); UV (MeOH) λ_{max} 211 nm (log ϵ 4.0), 230 (3.8), 295 (3.9); CI-MS *m/e* 214 (M + 1). VIIa: 10 mg (31% of products), R_f = 0.47; ^{1}H NMR ($CDCl_3$) δ 4.03 (s, 3 H, OMe), 2.62 (s, 3 H, SMe), 1.37 (s, 9 H, *t*-Bu); UV (MeOH) λ_{max} 208 nm (log ϵ 3.9), 248 (4.1), 298 (3.6); CI-MS *m/e* 214 (M + 1).

Syntheses, Spectral Features, and Reactions of Metribuzin and Deaminometribuzin Sulfoxides [I-S(O)Me and II-S(O)Me] (Figure 1). *Peracid Oxidation of I-SMe.* A solution of [^{14}C]I-SMe (6 mg) in chloroform (0.5 mL) was treated with appropriate amounts of *m*-chloroperbenzoic acid (MCPBA) with stirring at 0 °C. Analyses involved TLC (CE) and LSC.

I-S(O)Me. A solution of I-SMe (150 mg, 0.70 mmol) in chloroform (6 mL) at 0 °C was treated with MCPBA (65 mg, 0.35 mmol) dissolved in chloroform (6 mL) and stirred for 5 min at 0 °C. The chloroform was removed under vacuum and the residue taken up in ether. Solvent evaporation and cooling on partial removal of the ether under vacuum with no applied heat precipitated I-S(O)Me (30 mg, 37% based on MCPBA): ^{1}H NMR ($CDCl_3$) δ 5.92 (br s, 2 H, NH_2), 3.25 [s, 3 H, S(O)Me], 1.48 (s, 9 H, *t*-Bu); ^{13}C NMR ($CDCl_3$) δ 166.9, 155.9, 149.8, 38.5, 37.2, 27.2. IR ($CHCl_3$) 1675 (C=O), 1050 cm^{-1} (S=O); CI-MS *m/e* 231 (M + 1).

II-S(O)Me. A solution of II-SMe (500 mg, 2.5 mmol) and MCPBA (700 mg, 3.8 mmol) in acetone (50 mL) at 0 °C was stirred for 2 h at 0 °C, the acetone was removed under vacuum, and the residue was taken up in ether. Partial removal of the ether gave II-S(O)Me as a precipitate (380 mg, 70%): ^{1}H NMR (CD_3COCD_3) δ 3.03 [s,, 3 H, S(O)Me], 1.34 (s, 9 H, *t*-Bu); ^{13}C NMR ($CDCl_3$) δ 164.6, 161.7, 159.3, 39.9, 37.7, 27.0; IR ($CHCl_3$) 1672 (C=O), 1050 cm^{-1} (S=O); CI-MS *m/e* 216 (M + 1).

I-SAr. 3,4-Dichlorobenzenethiol (6 μL, 0.05 mmol; Aldrich) was added to a solution of I-S(O)Me (10 mg, 0.043 mmol) in chloroform (0.5 mL). After 30 min at 25 °C, the reaction mixture was separated by TLC (0.5 mm silica gel, developed twice in ether–hexane, 1:1), and the band at R_f = 0.32 was scraped and extracted with ether to give I-SAr (9 mg, 60%): ^{1}H NMR ($CDCl_3$) δ 7.82 (br s, 1 H, aromatic), 7.60 (br s, 2 H, aromatic), 4.98 (br s, 2 H, NH_2), 1.43 (s, 9 H, *t*-Bu); UV (MeOH) λ_{max} 213 nm (log ϵ 4.4), 294 (4.0); CI-MS *m/e* 345 (M + 1).

II-SAr. 3,4-Dichlorobenzenethiol (60 μL, 0.48 mmol) was added to an acetone solution (6 mL) of II-S(O)Me, prepared in situ from II-SMe (65 mg, 0.33 mmol) and MCPBA (80 mg, 0.43 mmol) as described above. The solution was stirred overnight. II-SAr (78 mg, 70%, mp 220–223 °C) was obtained both as a white precipitate on partial removal of the acetone under vacuum and by preparative TLC of the supernatant (0.5 mm silica gel, acetonitrile–chloroform, 1:7, R_f 0.67, recovered by chloroform extraction): ^{1}H NMR (CD_3COCD_3) δ 7.89 (br s, 1 H, aromatic), 7.65 (br, d, 2 H, aromatic), 1.30 (s, 9 H, *t*-Bu); UV (MeOH) λ_{max} 234 (log ϵ 4.2); CI-MS *m/e* 330 (M + 1).

I-SMA. MCPBA (150 mg, 0.90 mmol) in acetone (5 mL) at 0 °C was added to I-SMe (300 mg, 1.4 mmol) in acetone (10 mL) at 0 °C. After 10 min this cold solution was added dropwise to NAcCys (500 mg, 3 mmol) dissolved in 0.1 M

Table II. Metabolism of [^{14}C]Metribuzin by Phenobarbital-Induced Rat and Mouse Liver Microsomes in the Presence or Absence of *N*-Acetylcysteine and NADPH

^{14}C compd or ^{14}C fraction	radiocarbon recovery, %,[a] with indicated fortification[b]			
	none	NAcCys	NADPH	NAcCys + NADPH
Rat Liver Microsomes				
I-SMe	90	91	35	28
II-SMe	0.8	0.6	24	18
I-SMA + II-SMA[c]	0	3	0	45
unidentified[d]				
apolar	7	5	8	1
polar	1	0.4	19	7
bound	0.3	0.2	14	1
Mouse Liver Microsomes				
I-SMe	84	83	56	56
II-SMe	0.9	0.6	6	6
I-SMA + II-SMA[e]	0	2	0	28
unidentified[d]				
apolar	14	13	13	7
polar	0.6	0.8	8	3
bound	0.6	0.2	17	0.7

[a] Average of two independent studies with data for product yields varying by 0.8–1.2-fold between the experiments. [b] Mixtures of microsomes (150 mg fresh liver weight equivalent) with NAcCys (5 mg) and/or NADPH (2.2 mg) in 0.1 M phosphate, pH 7.4, buffer (2.5 mL) incubated for 2 h at 37 °C. [c] About equal amounts of I-SMA and II-SMA. [d] The unidentified metabolites are apolar (chloroform extractable), polar (methanol extractable), or protein bound. Apolar and polar unknowns include material at the origin or streaking on the plate. [e] >80% I-SMA with a small amount of II-SMA.

phosphate, pH 7.4, buffer (25 mL). After stirring 18 h at 25 °C, the solution was extracted with ether (2 × 20 mL), and the aqueous phase was then saturated with NaCl and extracted with ether–ethanol, 3:1 (3 × 40 mL), to recover crude I-SMA.

II-SMA. II-S(O)Me (100 mg, 0.47 mmol) was dissolved in acetone (5 mL) and treated as above to obtain crude II-SMA.

Derivative with BSA. A solution of [^{14}C]I-SMe (10 μg) and MCPBA (30–45 μg) was held in acetone (35 μL) for 30 min at 25 °C. After analysis for I-S(O)Me content by TLC (CE), the solution was diluted with acetone to 0.2 mL, added to BSA (10 mg) dissolved in 0.1 M phosphate, pH 7.4, buffer (2 mL), and stirred for several hours at 37 °C. An equal volume of 10% aqueous trichloroacetic acid was added. The precipitate recovered by centrifugation was redissolved in water (2 mL) and reprecipitated as above, and the procedure was repeated a third time to ensure that no soluble radioactivity remained. The precipitate was dissolved in Soluene for LSC.

RESULTS

Metabolism of [^{14}C]Metribuzin in Rats and Mice. The major urinary metabolite in rats is II-SMA (identification discussed below) and in mice chromatographs (BAW) in the position of I-SMA + II-SMA, in each species accounting for ~20% of the administered dose within 5 days after treatment. Although other urinary products are not identified, they are polar compounds, such as conjugates, and no one of them accounts for more than 5% of the dose. Rat feces contain 0.1–1% of the administered radiocarbon as unmetabolized I-SMe. The other rat fecal products are not identified but do not include any of the nonconjugated compounds shown in Figure 1 based on TLC cochromatography (CE, BAW) (Table I). No radioactivity is expired by rats as [^{14}C]carbon dioxide.

Table III. Triazinediones from Acid Hydrolysis of [^{14}C]Metribuzin and the [^{14}C]Mercapturic Acids Formed on in Vivo and in Vitro Metabolism in Rats and Mice

material hydrolyzed[a]	triazinedione, %[b]	
	III-NH_2	III-H
I-SMe	89	11
II-SMe	0[c]	100[c]
I-SMA + II-SMA[d]		
rat urine	0.5	99
rat enzyme	51	49
mouse enzyme	84	16

[a] 2 N HCl, 3 h, 100 °C. [b] Yields of III-NH_2 and III-H normalized to 100%. Additional products were not recovered on chloroform extraction or not resolved by TLC, respectively, as follows: I-SMe, 3 and 2%; rat urine, 5 and 0.5%; rat enzyme, 11 and 11%; mouse enzyme, 5 and 5%. [c] Results with unlabeled II-SMe based on TLC and product visualization by UV quench. [d] The percent radiocarbon recovery as mercapturic acids (I-SMA plus II-SMA) was ~20% in rat urine (Bleeke et al., 1984) and 45 and 28% in rat and mouse enzyme systems, respectively (Table II).

Metabolism of [^{14}C]Metribuzin in Rat and Mouse Liver Microsomal Enzyme Systems. Comparative studies revealed that phenobarbital induction increases NADPH-dependent metabolism of I-SMe by 2–3-fold in rats and about 50% in mice. [^{14}C]I-SMe undergoes relatively little metabolism or decomposition on incubation with phenobarbital-induced rat or mouse liver microsomes alone or fortified with NAcCys (Table II). Metabolism is greatly facilitated and new metabolites are formed on fortification with NADPH or a combination of NADPH and NAcCys. Deamination to II-SMe is more prominent with rat than with mouse liver preparations. Fortification with both NADPH and NAcCys gives a mixture of I-SMA and II-SMA, as discussed later. Protein-bound ^{14}C derivatives are major NADPH-dependent products in the absence of NAcCys but not in its presence, suggesting that the activated intermediates forming the I-SMA plus II-SMA mixture with NAcCys are also trapped by reaction with protein. TLC analysis (CE and BAW, Table I) revealed that the apolar and polar unknowns do not include I-S(O)Me, I-SG, II-S(O)Me, II-SG, and IV-NH_2 and that little (<1%) III-H is present. III-NH_2 is occasionally detected as a minor (1–2%) NADPH-dependent metabolite formed in the absence of added thiols.

A GSH conjugate appears to form as a microsomal metabolite under suitable conditions. Thus, a product of appropriate TLC characteristics (BAW) is obtained with suitable mouse liver enzyme systems in yields as follows: microsome plus NADPH <1%; soluble fraction plus NADPH 2%; microsome plus soluble fraction plus NADPH 16%; microsome GSH plus NADPH 13%.

Identification of Mercapturic Acids in Urine and Enzyme Systems. I-SMA and II-SMA are not separated in the TLC systems examined (Table I). They were therefore identified as mercapturates by TLC cochromatography and as I-SMA or II-SMA by degradation and spectroscopic methods described below.

Acid hydrolysis of I-SMe and II-SMe gives primarily III-NH_2 and III-H, respectively (Table III). Analogous reactions of the mercapturic acids serve to distinguish I-SMA from II-SMA. The rat urinary mercapturic acid fraction yields almost only III-H, strongly indicating that it is almost entirely II-SMA (Table III). The mouse mercapturic acids were not isolated for identification, but direct acid hydrolysis of the urine yields 35% III-NH_2, 5% III-H, and 60% polar products. The isolated mercapturic acids from the mouse enzyme give III-NH_2 and III-H in a 5:1 ratio, approximating the 8:1 ratio for hydrolysis of [^{14}C]I-SMe, but in marked contrast to the 1:1 ratio for the

Table IV. Comparison of Dimethylated Mercapturic Acids (Vb and VIIb) with Related *S*-Methyl Derivatives

methylated deriv[a]			NMR, δ				UV, λ_{max},
compd	R_1	R_2	S-Me	N-2-Me	N-4-Me	O-Me	nm
			N-2-Me Derivatives				
Va	*t*-Bu	SMe	2.55	3.72			236
Vb	*t*-Bu	SMA	*b*	*b*			239
Vc	Me	SMe	2.48	3.66			236
			N-4-Me Derivatives				
VIa	*t*-Bu	SMe	2.67		3.45		211, 230, 295
VIc	Me	SMe	2.60		3.35		231, 284, 295
			O-Me Derivatives				
VIIa	*t*-Bu	SMe	2.62			4.03	208, 248, 298
VIIb	*t*-Bu	SMA	*b*			*b*	211, 246, 294
VIIc	Me	SMe	2.58			4.02	247, 305

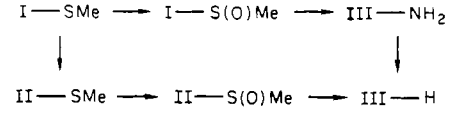
[a] Series a from synthesis, b from methylation of a rat urinary metabolite, and c from the literature (Daunis et al., 1971). [b] Not determined.

rat enzyme products (Table III). These findings suggest that the mercapturic acid fraction is >80% I-SMA with a small amount of II-SMA for the mouse but about equal amounts of I-SMA and II-SMA for the rat. Thus, the ratio of III-H to III-NH_2 both in vivo and in vitro suggests that deamination of I-SMe is more important in rats than in mice.

The UV spectra of triazinones give absorbances characteristic of the substitution pattern on the ring, allowing comparison of the mercapturic acid metabolites with I-SMe and II-SMe. The TLC-isolated mouse microsomal product is primarily I-SMA since the spectrum in methanol has two main absorbances [λ_{max} as nm (log ε)], i.e., 223 (4.0) and 296 (3.6), very similar to those of I-SMe with 227 (3.9) and 293 (3.9). The rat urinary mercapturic acid fraction is almost only II-SMA, giving 238 (4.2), much like that of II-SMe with 236 (3.1).

Methylation of the rat urinary mercapturic acid fraction gives two dimethyl derivatives with spectral features further supporting the identification of this metabolite as primarily II-SMA. Thus, treatment with diazomethane, followed by HPLC and TLC, results in the isolation of two products. The CI-MS for each compound gives a $(M + 1)^+$ signal of 343, the expected mass of II-SMA after methylation of both the ring and the carboxylic acid. Table IV compares the spectral data of the two methylated derivatives of the mercapturic acid (Vb and VIIb) with the three methylated products from II-SMe, V–VIIa, and the related compounds V–VIIc. The UV λ_{max} values of the methylated mercapturic acid derivatives correspond to those of the N-2-Me (239 nm) and the O-Me (246 nm) compounds. Clearly, the mercapturic acid metabolite is methylated at the N-2 and O positions as anticipated for II-SMA.

Peracid Oxidation of Metribuzin and Deaminometribuzin. The reaction of I-SMe with MCPBA in chloroform at 0 °C proceeds as follows (Figure 2):



I-S(O)Me is the principal product formed with 1 part of MCPBA to 2 parts of I-SMe (30–40% yield based on MCPBA). With increasing oxidant (1:1, 2:1, and 4:1

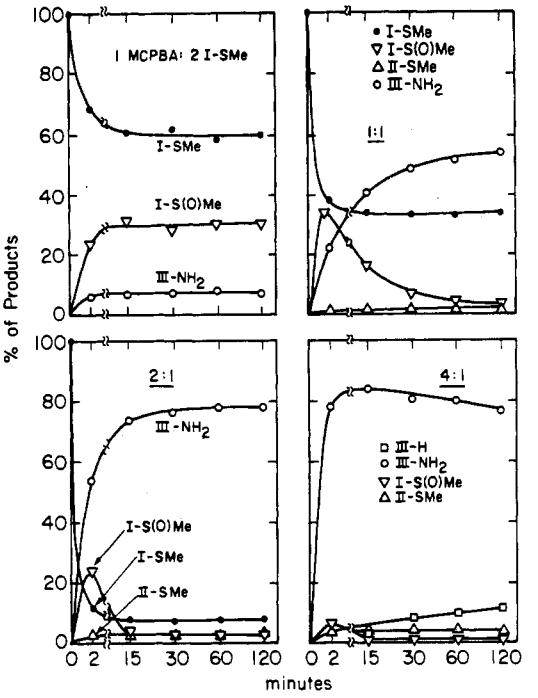


Figure 2. Reaction rates and product profiles for varying ratios of metribuzin to *m*-chloroperbenzoic acid in chloroform at 0 °C.

MCPBA–I-SMe), I-S(O)Me is rapidly converted to III-NH_2, which becomes the major final product. At the 4:1 ratio very little I-S(O)Me is detected, even at short reaction times. The product profile indicates that I-S(O)Me serves as the intermediate in forming III-NH_2, a proposal confirmed by finding that MCPBA directly converts I-S(O)Me to III-NH_2.

The deamino derivatives II-SMe and III-H are minor products. I-SMe is oxidatively deaminated to II-SMe but at a very slow rate compared with sulfoxidation. III-H is the final product resulting from oxidation at both the SCH_3 and NH_2 groups and becomes significant only with a large excess of MCPBA. Studies comparable to those shown in Figure 2 but with II-SMe establish a much slower conversion rate for II-SMe to II-S(O)Me and in turn to III-H than for the analogous reactions in the I-SMe series. II-S(O)Me is formed almost quantitatively on oxidation of II-SMe with a slight excess of MCPBA, showing that in this case sulfoxidation is much faster than subsequent cleavage to the diketo compound. II-S(O)Me is not detected as a product on treatment of I-SMe with MCPBA because little II-SMe is formed and its subsequent oxidation is slow.

Preparation, Properties, and Reactions of Metribuzin and Deaminometribuzin Sulfoxides. I-S(O)Me and II-S(O)Me are formed in 70–80% yields (NMR) based on MCPBA and can be isolated in 37 and 70% yields, respectively, on MCPBA oxidation of I-SMe in chloroform and of II-SMe in acetone at 0 °C. I-S(O)Me is rarely obtained in >80% purity due to decomposition to III-NH_2; partial decomposition occurs within 1 day either neat or in chloroform at 0 °C and complete breakdown is evident within 8 h at 25 °C. II-S(O)Me, obtained in >95% purity, is stable for weeks as a crystalline solid at 0 °C and for at least a few days at 25 °C.

Sulfoxides I-S(O)Me and II-S(O)Me are identified on the basis of their reactions, described below, and of their spectral features that are characteristic of sulfoxides (Silverstein et al., 1981). They each give CI-MS base peaks appropriate for monooxygenated derivatives, a strong IR band at 1050 cm^{-1} associated with the S=O absorption, a ^1H NMR spectrum with a signal for the S(O)Me protons

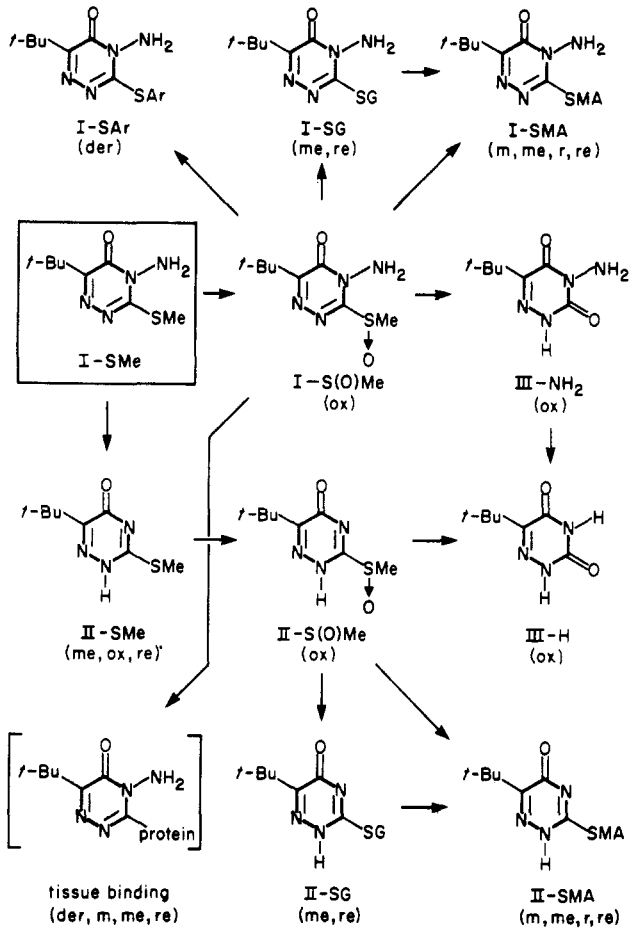


Figure 3. Reactions of metribuzin. Abbreviations: der, derivatization; m, mouse; me, mouse enzyme; ox, MCPBA; r, rat; re, rat enzyme.

shifted 0.5–0.6 ppm downfield from the SMe protons of the parent compound, and a ^{13}C signal for the S(O)Me substituent shifted 24–27 ppm downfield with respect to the parent SMe group.

The thiol 3,4-dichlorobenzenethiol reacts quickly with I-S(O)Me and II-S(O)Me, displacing the S(O)Me group to form thioethers I-SAr and II-SAr, respectively, which are crystalline or solid products; an analogous reaction was used to characterize cyanatryn sulfoxide (Bedford et al., 1975). I-S(O)Me and II-S(O)Me also react with NAcCys to give products with chromatographic properties identical with those of the mercapturate metabolites of I-SMe discussed above.

The protein BSA also reacts readily with I-S(O)Me at physiological pH. Thus, addition of the crude reaction mixture of [^{14}C]I-SMe and MCPBA [containing ~25% I-S(O)Me] to BSA leads to binding of about 5% of the total radioactivity compared to only 0.1% binding for an equivalent reaction of BSA and [^{14}C]I-SMe with no oxidant.

DISCUSSION

Several chemical and metabolic reactions of I-SMe are given in Figure 3. Sulfoxides I-S(O)Me and II-S(O)Me, from MCPBA oxidations of I-SMe and II-SMe, respectively, are reactive, electrophilic compounds. I-S(O)Me is harder to isolate and less stable than II-S(O)Me. Further oxidation of I-S(O)Me with MCPBA gives diketo derivative III-NH_2. The mechanism of this conversion (formally a replacement of S(O)Me with OH followed by tautomerization) is not known. Although a possible short-lived intermediate, no sulfone has been isolated or identified by TLC or NMR of the reaction mixture. A side reaction in MCPBA oxidation of I-SMe is the loss of NH_2, resulting in II-SMe. Reactions at both sites to give III-H is important only with a large excess of MCPBA. No intermediates are detected in the deamination reaction.

In vitro metabolism of I-SMe with rat or mouse liver microsomal enzymes occurs at both the N-NH_2 and SMe substituents. The enzymes involved require NADPH and are induced by phenobarbital, suggesting an oxidative mechanism for both processes. In contrast, deamination of I-SMe in plants appears to be a reductive cleavage (Fedtke and Schmidt, 1983). I-S(O)Me is the proposed intermediate in yielding thiol conjugates and protein derivatives. Analogous pathways are known in mammalian metabolism of other sulfur-containing pesticides (Casida et al., 1975; Bedford et al., 1975; Hubbell and Casida, 1977; Crawford et al., 1980; Hutson, 1981). Formation of I-SG requires oxidative activation by microsomes and is not dependent on the soluble fraction, indicating that conjugate formation is mediated by a microsomal GSH *S*-transferase or is due to direct chemical reaction between I-S(O)Me and GSH; the latter proposal is consistent with the demonstrated reactivity of chemically formed I-S(O)Me. Systems lacking thiols generally give more unidentified products and streaking on TLC, possibly due to decomposition of I-S(O)Me to III-NH_2 and other compounds. No IV-NH_2 is detected in the microsomal oxidations, suggesting that I-SMe does not undergo S-demethylation. No attempt was made to look for products resulting from oxidation of the *tert*-butyl group.

The findings on in vivo mammalian metabolism parallel the in vitro results. Deamination appears more important in rats than in mice, as is also the case for microsomal metabolism. The lack of [^{14}C]carbon dioxide formation by rats treated with [*carbonyl*-^{14}C]I-SMe suggests that ring opening followed by decarboxylation does not occur. Mercapturic acid formation is a major metabolic pathway, consistent with initial formation of a sulfoxide followed by conjugation with GSH. Sulfoxidation in mice appears to activate I-SMe for conjugation with GSH until the thiol is depleted and then for reaction with tissue proteins and associated hepatotoxicity (Bleeke et al., 1984).

ACKNOWLEDGMENT

Luis Ruzo and Ian Holden of this laboratory assisted in the MS and NMR analyses, respectively.

Registry No. I-SMe, 21087-64-9; I-S(O)Me, 90269-30-0; I-SAr, 90269-27-5; I-SMA, 90269-25-3; II-SAr, 90269-28-6; II-SMe, 35045-02-4; II-S(O)Me, 90269-26-4; II-SMA, 90269-24-2; III-H, 52236-30-3; III-NH_2, 56507-37-0; IV-H, 66392-60-7; IV-NH_2, 33509-43-2; Va, 79988-50-4; VIa, 62036-60-6; VIIa, 90269-29-7; trimethylpyruvic acid, 815-17-8; thiocarbohydrazide, 2231-57-4; thiosemicarbazide, 79-19-6; 3,4-dichlorobenzenethiol, 5858-17-3.

LITERATURE CITED

Bedford, C. T.; Crawford, M. J.; Hutson, D. H. *Chemosphere* **1975**, *4*, 311.

Bleeke, M. S.; Smith, M. T.; Casida, J. E. *Pestic. Biochem. Physiol.* **1984**, in press.

Casida, J. E.; Kimmel, E. C.; Ohkawa, H.; Ohkawa, R. *Pestic. Biochem. Physiol.* **1975**, *5*, 1.

Crawford, M. J.; Hutson, D. H.; Stoydin, G. *Xenobiotica* **1980**, *10*, 169.

Daunis, J.; Jacquier, R.; Viallefont, P. *Bull. Soc. Chim. Fr.* **1971**, 3658.

Draber, W.; Dickore, K.; Büchel, K. H.; Trebst, A.; Pistorius, E. *Naturwissenschaften* **1968**, *55*, 446.

Eue, L. *Pflanzenschutz-Nachr.* **1972**, *25*, 175.

Fedtke, C.; Schmidt, R. R. In "Pesticide Chemistry: Human Welfare and the Environment"; Miyamoto, J.; Kearney, P. C., Eds.; Pergamon Press: Oxford 1983; pp 177–181.

Frear, D. S.; Mansager, E. R.; Swanson, H. R.; Tanaka, F. S. *Pestic. Biochem. Physiol.* **1983a**, *19*, 270.

Frear, D. S.; Swanson, H. R.; Mansager, E. R. "Abstracts of Papers", 185th National Meeting of the American Chemical Society, Seattle, WA, March 1983; American Chemical Society: Washington, DC, 1983b; PEST 103.
Hilton, H. W.; Nomura, N. S.; Yauger, W. L., Jr.; Kameda, S. S. *J. Agric. Food Chem.* **1974**, *22*, 578.
Hubbell, J. P.; Casida, J. E. *J. Agric. Food Chem.* **1977**, *25*, 404.
Hutson, D. H. In "Sulfur in Pesticide Action and Metabolism"; Rosen, J. D.; Magee, P. S.; Casida,, J. E.; American Chemical Society: Washington, DC, 1981; ACS Symp. Ser. No. 158, p 53.
Mangeot, B. L.; Slife, F. E.; Rieck, C. E. *Weed Sci.* **1979**, *27*, 267.
Nakayama, Y.; Sanemitsu, Y.; Yoshioka, H.; Nishinaga, A. *Tetrahedron Lett.* **1982**, *23*, 2499.
Neunhoeffer, H.; Wiley, P. F. *Heterocycl. Compd.* **1978**, *33*, 194–574.
Saeman, M. C. Ph.D. Dissertation, University of California, Berkeley, CA, 1984.
Silverstein, R. M.; Bassler, G. C.; Morrill, T. C. "Spectrometric Identification of Organic Compounds", 4th ed.; Wiley: New York 1981.

Received for review January 11, 1984. Accepted March 19, 1984. Presented in part as paper 38, Division of Pesticide Chemistry, at the 184th American Chemical Society National Meeting, Kansas City, MO, Sept 1982. This study was supported in part by the National Institute of Environmental Health Sciences (Grant PO1 ES00049).

Interaction between γ-Hexachlorocyclohexane and the Gastrointestinal Microflora and Their Effect on the Absorption, Biotransformation, and Excretion of Parathion by the Rat

Robert W. Chadwick,* M. Frank Copeland, Ritchie Froehlich, Nathaniel Cooke, and Douglas A. Whitehouse

Pretreatment of rats with the organochlorine insecticide lindane reduced the estimated absorption rate of parathion from the gastrointestinal tract. Lindane pretreatment also significantly reduced the metabolism of parathion to *p*-nitrophenol in vivo. Lindane pretreatment altered the gastrointestinal (GI) microflora by increasing the ratio of anaerobes to aerobes. Consistent with this alteration was a significantly greater retention of unaltered parathion and the microbial metabolite aminoparathion in the GI tract of the lindane-pretreated rats 1 h after the administration of parathion. Enhanced conversion of parathion to aminoparathion together with a slower absorption rate may play a role in the antagonism parathion toxicity by lindane.

In the past it has been widely reported that pretreatment of animals with halogenated chemicals antagonizes the toxicity of organophosphate pesticides (Ball et al., 1954; Triolo and Coon, 1966; Townsend and Carlson, 1981; Iverson, 1976; Mendoza and Shields, 1976; Welch and Coon, 1964; Triolo et al., 1970). Moreover, this antagonism has generally been attributed to induction of either esterases or hepatic mixed function oxidases. While examining the dynamics of the absorption, biotransformation, and excretion of parathion [*O,O*-diethyl *O*-(*p*-nitrophenyl) phosphorothioate], we observed that pretreatment of rats with lindane (γ-hexachlorocyclohexane) impaired the estimated absorption rate of parathion. Corresponding to this impaired absorption, significantly lower excretion rates during the initial absorption were also observed in the lindane-pretreated rats. A series of experiments designed to determine the mechanism by which lindane elicited these effects produced data that indicate that altered gastrointestinal microflora may contribute to the protective effect of lindane and possibly other halogenated chemicals against the toxicity of ingested organophosphate pesticides.

MATERIALS AND METHODS

Apparatus. Gas–liquid chromatographic analysis was performed on a Tracor Model MT-220 gas chromatograph equipped with a ^{63}Ni electron capture detector (ECD) and a flame photometric detector (FPD). Urinary *p*-nitrophenol was determined by ECD on 1% SP1240-DA on 100–120-mesh Supelcoport at 170 °C with 60 cm^3/min of 95:5 methane–argon carrier gas. Derivatized diethyl phosphorothioic acid (DETP), diethylphosphoric acid (DEP), and paraoxon [*O,O*-diethyl *O*-(*p*-nitrophenyl) phosphate] were determined by FPD with 3% OV-1 on 80–100-mesh Chromosorb W at 210 °C. Aminoparathion and parathion were analyzed by FPD with 3% QF-1 on 80–100-mesh Gas-Chrom Q at 185 °C. Air, H_2, and N_2 carrier gas flows were regulated at 100, 50, and 60 cm^3/min, respectively.

Reagents. [*ring*-2,6-^{14}C]Parathion (specific activity 12.2 mCi/mmol and 98% purity) was obtained from Amersham Corp., Arlington Heights, IL. Parathion, aminoparathion, DETP, DEP, paraoxon, and lindane were obtained from the EPA, Health Effects Research Laboratory Analytical Reference Standards Repository, Research Triangle Park, NC. Pentafluorobenzyl bromide (PFB-Br) was obtained from Aldrich Chemical Co., Milwaukee, WI. Tetrahexylammonium hydrogen sulfate was obtained from Regis Chemical Co., Morton Grove, IL; 3% QF-1 on 80–100-mesh Gas-Chrom Q and 3% OV-1 on 80–100-mesh Chromosorb W were obtained from Applied Science Laboratories, Inc., State College, PA. 1% SP-1240 DA on 100–120-mesh Supelcoport was obtained from Supelco Inc., Bellefonte, PA. Thioglycollate medium was obtained from Becton, Dickinson and Co., Cockeysville, MD. Bacto nutrient broth was obtained from Difco Laboratories, Detroit, MI.

Procedures. Separate experiments were conducted to determine (1) the effects of pretreatment with lindane on

U.S. Environmental Protection Agency, Health Effects Research Laboratory, Developmental Biology Division (MD 67), Perinatal Toxicology Branch, Research Triangle Park, North Carolina 27711.