Metabolic Fate of the N,N-Dialkylcarbamoyl Moiety of Thiocarbamate Herbicides in Rats and Corn

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A major metabolic pathway for EPTC and butylate in rats, accounting for at least 27–45% of the administered dose, is as follows: thiocarbamate → thiocarbamate sulfoxide → S-(N,N-dialkyl-carbamoyl)glutathione → S-(N,N-dialkylcarbamoyl)cysteine → S-(N,N-dialkylcarbamoyl)mercapturic acid, S-(N,N-dialkylcarbamoyl)mercaptoacetic acid, and N-[S-(N,N-dialkylcarbamoyl)mercapto-acetyl]glycine (from butylate only). S-(N-Isobutylcarbamoyl)mercapturic acid is also formed from butylate. These findings are based on identification and quantitation of the urinary metabolites from ^{14}CO-labeled preparations of the first four compounds in the metabolic sequence, in both the EPTC and butylate series. About 40% of the administered [^{14}CO]EPTC and [^{14}CO]butylate dose is metabolized by ester cleavage and $^{14}CO_2$ liberation without going through the thiocarbamate sulfoxide as an intermediate. Benthiocarb, cycloate, molinate, and pebulate also give the appropriate mercapturic acid derivatives in the urine. The roots and leaves of corn seedlings convert [^{14}CO]EPTC or its sulfoxide to the glutathione and cysteine conjugates and many other metabolites that are the same compounds in control plants and those treated with the antidote, R-25788.

Thiocarbamate herbicides are rapidly degraded in mammals and plants and some metabolites from the S-alkyl or S-benzyl fragments are identified (Ashton and Crafts, 1973; Casida et al., 1974, 1975a,b; Fang, 1975; Ishikawa et al., 1973, 1976; Ong and Fang, 1970). Thio-carbamates also undergo N-dealkylation and sulfoxidation in mammals or liver microsomal mixed-function oxidase (mfo) systems (Casida et al., 1974, 1975a,b; Ishikawa et al., 1973). The sulfoxide metabolites are carbamoylating agents for tissue thiols [e.g., glutathione (GSH), cysteine, and coenzyme A (CoASH)] and are generally more potent than the parent thiocarbamates as herbicides (Casida et al., 1974, 1975a,b; Lay and Casida, 1976; Lay et al., 1975). Reaction of the thiocarbamate sulfoxides with GSH is catalyzed by GSH S-transferases of mouse liver and corn roots and leaves (Casida et al., 1974, 1975a,b; Lay and Casida, 1976; Lay et al., 1975). The S-(N,N-dialkyl-carbamoyl)glutathione derivative is detected in corn roots treated with a thiocarbamate sulfoxide (Lay and Casida, 1976) and is probably also formed in mammalian liver since the GSH level is transiently reduced in mice treated with thiocarbamates and thiocarbamate sulfoxides and the corresponding S-(N,N-dialkylcarbamoyl)-N-acetylcysteine derivatives (i.e., mercapturic acids) are rat urinary metabolites of thiocarbamates and their sulfoxides (Lay and Casida, 1976; Lay et al., 1975).

The studies indicated above suggest that carbamoylation reactions are important in the metabolism and possibly in the mode of action of thiocarbamate herbicides. To examine this hypothesis, the present investigation considers the metabolic fate of the N,N-dialkylcarbamoyl moiety of EPTC (S-ethyl N,N-dipropylthiocarbamate, I-Pr₂, Figure 1), butylate (S-ethyl N,N-diisobutylthio-carbamate, I-i-Bu₂), their sulfoxides (II-Pr₂ and II-i-Bu₂), and other thiocarbamates in rats and of EPTC and EPTC sulfoxide in corn seedlings.

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MATERIALS AND METHODS

Chromatography and Analysis. Unless otherwise stated, thin-layer chromatography (TLC) utilized pre-coated silica gel 60 F-254 20 × 20 cm chromatoplates with 0.25-mm layer thickness (EM Laboratories Inc., Elmsford, N.Y.) developed with the following solvent systems: BAW, 1-butanol–glacial acetic acid–water (6:1:1); EMPW, ethyl acetate–methanol–pyridine–water (13:3:3:1); EMW, ethyl acetate–methanol–water (13:3:1); HE, hexane–ethyl acetate (3:2); WBPA, water–1-butanol–pyridine–glacial acetic acid (70:65:65:3). In a few cases the WBPA solvent system was used with precoated MN 300F cellulose (normal) 20 × 20 cm chromatoplates with 0.25- or 0.50-mm layer thickness (Analtech, Inc., Newark, Del.). Methods for detection, quantitation, and TLC cochromatography of ^{14}C-labeled compounds and unlabeled standards were as previously reported (Casida et al., 1975b; Lay and Casida, 1976). The 5,5′-dithiobis(2-nitrobenzoic acid) (Nbs₂) reagent (Lay and Casida, 1976) was used as a spray to detect compounds with free thiol groups. Ninhydrin (1% w/v in ethanol) detected products with free amino groups as deep purple spots after heating the chromatoplates at 110 °C for 3 min.

Gas chromatography (GC) for analysis of mercapturic acids as their methyl esters employed glass columns (1.8 m × 2.0 mm i.d.) packed with 3.8% OV-101 on Chromosorb W (acid washed, DMCS treated, 60–80 mesh). Quantitative determinations utilized the Varian series 1400 gas chromatograph with a flame ionization detector (FID) under the following conditions: injector, 250 °C; detector, 310 °C; temperature programming from 100 to 290 °C at 4 °C/min; flow rates of 20, 20, and 250 ml/min for N₂, H₂, and air, respectively. The methyl esters of the mercapturic acids eluted at 280–290 °C. Comparable GC conditions were used for mass spectrometry (MS) with a Finnigan Model 9500 gas chromatograph coupled to a Finnigan Model 1015D mass spectrometer in combination with the System Industries Model 150 control system. Helium was used as the carrier gas at 20–25 ml/min and methane or isobutane as the reagent gas with a chemical ionization (CI) source pressure of 0.5–1.0 Torr.

The nuclear magnetic resonance (NMR) spectra were obtained with a Perkin-Elmer R12B 60 MHz or R32B 90 MHz spectrometer. Unless specified otherwise, samples were dissolved in DCCl₃ containing 1% tetramethylsilane
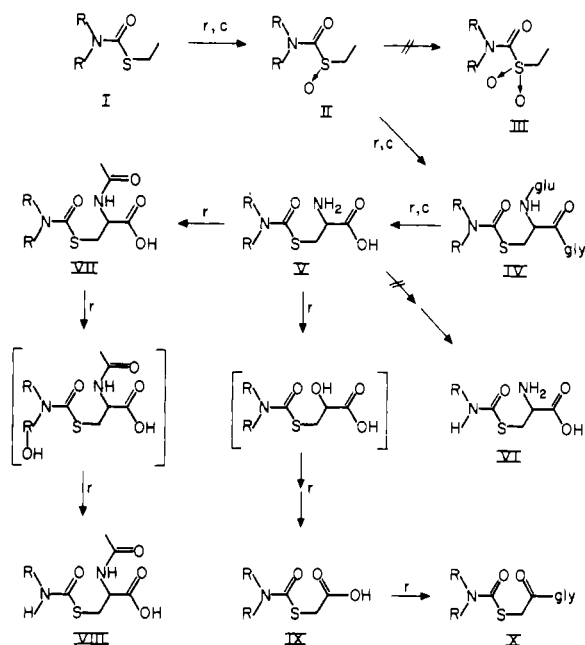
Figure 1. Partial metabolic pathways for EPTC (I, R = Pr) and butylate (I, R = i-Bu) in rats (r) and corn (c) showing compound designations (gly = glycine; glu = glutamic acid).

as the internal standard. Infrared (IR) spectra were recorded on a Perkin-Elmer 457 IR spectrophotometer using $HCCl_3$ solutions.

Synthesis of Unlabeled Compounds. The chemicals examined and their designations are shown in Figure 1. Methyl esters were prepared by treating the appropriate carboxylic acids in ether–methanol solution with diazomethane.

Thiocarbamates (I-Pr$_2$, I-i-Bu$_2$, and Others), Thiocarbamate Sulfoxides (II-Pr$_2$, II-i-Bu$_2$), and Thiocarbamate Sulfones (III-Pr$_2$, III-i-Bu$_2$). The thiocarbamate herbicides were supplied by Stauffer Chemical Co. (Richmond, Calif.) and their sulfoxide and sulfone derivatives were prepared by oxidation with m-chloroperoxybenzoic acid as previously reported (Casida et al., 1975a,b). A sample of S-ethyl N-propylthiocarbamate (N-depropyl-EPTC) was also provided by Stauffer.

Thiocarbamate sulfones (III-Pr$_2$, III-i-Bu$_2$) were used to prepare most of the thiocarbamate derivatives described below. These sulfones are more stable on storage and are much more reactive with thiols than the corresponding sulfoxides. Under comparable conditions, the N,N-dialkylcarbamoyl chlorides do not undergo any reaction.

S-(N,N-Dialkylcarbamoyl)glutathione (IV-Pr$_2$, IV-i-Bu$_2$). III-Pr$_2$ or III-i-Bu$_2$ (100 mg) was added to a solution of 100 mg of GSH in 15 ml of methanol and 5 ml of triethylamine. After stirring for 18 h at 25 °C and rotary evaporation to dryness, the residue was dissolved in 20 ml of water, which was then acidified to pH 2.0 with HCl and washed twice with ether, and the aqueous solution was lyophilized to dryness. Extraction of this residue with methanol gave IV-Pr$_2$ and IV-i-Bu$_2$ in ~50% yields.

S-(N,N-Dipropylcarbamoyl)glutathione (IV-Pr$_2$): NMR (90 MHz, D$_2$O, DSS internal standard) δ 3.34 [t, 4, J = 6 Hz, (CH$_3$CH$_2$CH$_2$)$_2$N–], 2.66 [m, 2, –CH$_2$CH$_2$CH(NH$_2$)–], 2.34 (t, 2, J = 6 Hz, –C(O)CH$_2$CH$_2$–], 1.53 [m, 4, (CH$_3$CH$_2$CH$_2$)$_2$N–], 0.88 [t, 6, J = 7 Hz, (CH$_3$CH$_2$CH$_2$)$_2$N–]. Dimethyl ester: NMR (60 MHz) δ 4.52 [m, 1, –NHCHC(O)NH–], 4.01 (d, 2, J = 7 Hz, –NHCH$_2$C(O)OCH$_3$], 3.72 [s, 6, –C(O)OCH$_3$], 3.13 [d, 2, J = 5 Hz, HCCH$_2$S–), 1.52 [m, 4, (CH$_3$CH$_2$CH$_2$)$_2$N–], 0.90 [t, 6, J = 7 Hz, (CH$_3$CH$_2$CH$_2$)$_2$N–].

S-(N,N-Diisobutylcarbamoyl)glutathione (IV-i-Bu$_2$) dimethyl ester: NMR (60 MHz) δ 6.22 [d, 1, J = 7 Hz, –C(O)NHCH], 4.53 [m, 1, –NHCHC(O)NH–], 4.04 [d, 2, J = 7 Hz, –NHCH$_2$C(O)OCH$_3$], 3.69 [s, 6, –C(O)OCH$_3$], 0.92 [d, 12, J = 7 Hz, (CH$_3$)$_2$CH–].

S-(N,N-Dialkylcarbamoyl)cysteine (V-Pr$_2$, V-i-Bu$_2$). To a stirred solution of 6 ml of methanol, 46 mg (0.46 mmol) of triethylamine, and 100 mg (0.82 mmol) of cysteine under a nitrogen atmosphere was added 100 mg (0.45 mmol) of III-Pr$_2$ or an equivalent amount of III-i-Bu$_2$. The reaction mixture was stirred for 24 h at 25 °C and then rotary evaporated to dryness. The solid from the III-Pr$_2$ reaction was washed with ether to remove most of the triethylamine and then extracted with 50 ml of chloroform to give V-Pr$_2$ in 50% yield. The product from the III-i-Bu$_2$ reaction was purified by ion-exchange chromatography on Bio-Rad AG 50W-X8 100–200 mesh hydrogen form (Bio-Rad Laboratories, Richmond, Calif.) with 0.4 M sodium formate (pH 4.5). V-i-Bu$_2$ was recovered in 35% yield from the appropriate fractions by lyophilization and extracting the residue with chloroform.

V-Pr$_2$ and V-i-Bu$_2$ gave positive ninhydrin tests for free amino groups and negative Nbs$_2$ tests for free thiol. The acetylated derivatives from reacting V-Pr$_2$ and V-i-Bu$_2$ with 1 molar equiv of acetic anhydride under basic conditions (2 molar equiv of NaOH in H$_2$O) were found to be identical with the S-(N,N-dialkylcarbamoyl)-N-acetylcysteine derivatives (VII-Pr$_2$, VII-i-Bu$_2$; described below) by two criteria: TLC cochromatography (BAW and EMPW); methylation and intercomparison of the methyl esters by GC–CI–MS.

S-(N-Propylcarbamoyl)cysteine (VI-Pr). Following slow addition of propyl isocyanate (13.5 g, 0.16 mol) to a stirred solution of 15.8 g (0.10 mol) of cysteine·HCl in 150 ml of dimethylformamide (DMF) at 0 °C, a drying tube was attached to the flask and the stirring continued for 70 h at 25 °C. The DMF was removed by distillation under vacuum and the residue dissolved in 200 ml of H$_2$O. After this solution was washed twice with ether and the pH adjusted to 6.5 with 1 N NaOH, the precipitate of VI-Pr was collected and dried (60% yield). As with V-Pr$_2$, the monopropyl derivative gave a positive ninhydrin test and a negative Nbs$_2$ test. The methyl ester of VI-Pr gave an appropriate NMR spectrum (90 MHz): δ 3.72 (s, 3), 1.60 (m, 2), 0.92 (t, 3, J = 7 Hz).

S-(N,N-Dialkylcarbamoyl)-N-acetylcysteine (VII-Pr$_2$, VII-i-Bu$_2$). III-Pr$_2$ (100 mg, 0.45 mmol) or an equivalent amount of III-i-Bu$_2$ was added to a stirred solution of 10 ml of methanol, 3 ml of triethylamine, and 100 mg (0.61 mmol) of N-acetylcysteine under a nitrogen atmosphere. After stirring the reaction mixture for 14 h at 25 °C, rotary evaporation to dryness, and extracting the solid with ether, the ether extract was washed twice with each of 1 N HCl and H$_2$O and rotary evaporated to dryness to give VII-Pr$_2$ and VIII-i-Bu$_2$ in 55–65% yields.

S-(N,N-Dipropylcarbamoyl)-N-acetylcysteine (VII-Pr$_2$): IR 2961, 2931, 2875, 1740, 1678, 1628, 1411, 1264, 1129, 911 cm^{-1}; NMR (60 MHz) δ 7.48 (d, 1, J = 7 Hz, –NH), 4.72 (complex multiplet, 1, –NCH–), 3.41 (d, 2, J = 6 Hz, –SCH$_2$–), 3.32 [t, 4, J = 7 Hz, (CH$_3$CH$_2$CH$_2$)$_2$N–], 2.08 [s, 3, –C(O)CH$_3$], 1.62 [m, 4, (CH$_3$CH$_2$CH$_2$)$_2$N–], 0.90 [t, 6, J = 7 Hz, (CH$_3$CH$_2$CH$_2$)$_2$N–]. Methyl ester: mass spectrum (CI–methane, relative intensity) 305 (1.9) [M + 1]$^+$, 172 (2.5), 162 (1.4), 144 (5.2), 129 (7.9), 128 (100), 126 (1.9), 112 (3.8), 102 (7.9), 100 (1.9); (high resolution EI) parent peak at mass 304.1453 (calcd for C$_{13}$H$_{24}$O$_4$SN$_2$, mass 304.1457).

S-(N,N-Diisobutylcarbamoyl)-N-acetylcysteine (VII-i-Bu₂): IR 2960, 2929, 2872, 1730, 1670, 1625, 1410, 1132, 911 cm⁻¹; NMR (60 MHz) δ 7.46 (d, 1, J = 7 Hz), 4.62 (m, 1), 3.40 (d, 2, J = 6 Hz), 3.21 (d, 4, J = 7 Hz), 2.06 (m, 2), 2.05 (s, 3), 0.94 (d, 12, J = 7 Hz). Methyl ester: mass spectrum (CI–methane, relative intensity) 333 (18.4) [M + 1]⁺, 172 (8.0), 158 (3.4), 157 (10.3), 156 (100), 144 (11.5), 130 (3.4), 128 (3.4), 102 (14.9), 100 (3.4).

S-(N-Alkylcarbamoyl)-N-acetylcysteine (VIII-Pr, VIII-i-Bu). To a stirred solution of 1.63 g (10 mmol) of N-acetylcysteine in 25 ml of DMF at 0 °C was slowly added 1.1 g (13 mmol) of propyl isocyanate or an equivalent amount of isobutyl isocyanate. A drying tube was attached and the solution stirred at 25 °C for 70 h. The residue following removal of the DMF by distillation under reduced pressure was dissolved in 0.5 N HCl and the product extracted with methylene chloride. The extract was dried with anhydrous MgSO₄ and rotary evaporated to give VIII-Pr and VIII-i-Bu in 80–90% yields. To help establish the structure of these compounds, the corresponding methyl esters were analyzed directly or after derivatization by GC–CI–MS with isobutane. Compounds VIII-Pr and VIII-i-Bu when examined directly as their methyl esters undergo thermal decomposition to the methyl ester of N-acetylcysteine so they were derivatized by reacting with trifluoroacetic anhydride in ethyl acetate for 1 h at 60 °C. GC–CI–MS analysis showed a small amount of the desired trifluoroacetate derivatives ([M + 1]⁺ of 359 and 373 for the derivatives from VIII-Pr and VIII-i-Bu, respectively) along with several decomposition products.

S-(N-Propylcarbamoyl)-N-acetylcysteine (VIII-Pr): IR 2998, 2960, 2928, 2876, 1497, 1384, 1192, 910 cm⁻¹; NMR (90 MHz) δ 6.88 (m, 1), 5.85 (m, 1), 4.80 (complex multiplet, 1), 3.38 (d, 2, J = 6 Hz), 3.32 (m, 2), 2.05 (s, 3), 1.60 (multiplet approximating a quartet, 2), 0.94 (t, 3, J = 7 Hz).

S-(N-Isobutylcarbamoyl)-N-acetylcysteine (VIII-i-Bu): NMR (90 MHz) δ 7.41 (m, 1), 6.54 (t, 1, J = 7 Hz), 4.71 (m, 1), 3.39 (d, 2, J = 6 Hz), 3.12 (d of d, 2, J₁ = 7 Hz, J₂ = 7 Hz), 2.06 (s, 3), 1.82 (m, 1), 0.91 (d, 6, J = 7 Hz).

S-(N,N-Dialkylcarbamoyl)mercaptoacetic acid (IX-Pr₂, IX-i-Bu₂). Into a flask equipped with a magnetic stirrer and a drying tube was placed 50 ml of methanol, 20 ml of triethylamine, 3.0 g (33 mmol) of mercaptoacetic acid, and 1.5 g (6.8 mmol) of III-Pr₂ or an equivalent amount of III-i-Bu₂. The reaction mixture was stirred for 12 h at 25 °C and rotary evaporated to remove the methanol and triethylamine. The product derived from the III-Pr₂ reaction was distilled under reduced pressure to remove the excess mercaptoacetic acid and then the residue was dissolved in hexane and cooled to –10 °C for 12 h and the hexane-soluble material rotary evaporated to give V-Pr₂ in 50% yield. In the reaction with III-i-Bu₂, the product was dissolved in chloroform and washed with 1 N HCl, rotary evaporated, distilled under vacuum to remove excess mercaptoacetic acid, and then dissolved in hexane for treatment as above to give a 40% yield.

S-(N,N-Dipropylcarbamoyl)mercaptoacetic acid (IX-Pr₂): NMR (90 MHz) δ 3.76 (s, 2), 3.27 (m, 4), 1.56 (m, 4), 0.93 (t, 7, J = 7 Hz). Methyl ester: IR 2968, 2936, 2875, 1745, 1660, 1293, 1121 cm⁻¹; NMR (90 MHz) δ 3.76 (s, 5), 3.27 (m, 2), 1.56 (m, 2), 0.93 (t, 3, J = 7 Hz); mass spectrum (CI–methane, relative intensity) 235 (6), 234 (40) [M + 1]⁺, 160 (1), 129 (8), 128 (100).

S-(N,N-Diisobutylcarbamoyl)mercaptoacetic acid (IX-i-Bu₂): IR 2960, 2930, 2878, 1729, 1660, 910 cm⁻¹; NMR (90 MHz) δ 3.76 (s, 2), 3.23 (d, 4, J = 7 Hz), 2.08 (complex multiplet, 2), 0.92 (d, 12, J = 7 Hz). Methyl ester: mass spectrum (CI–isobutane, relative intensity) 263 (40),

262 (100) [M + 1]⁺, 156 (80), 83 (40), 81 (20).

N-[S-(N,N-Dialkylcarbamoyl)mercaptoacetyl]glycine (X-Pr₂, X-i-Bu₂). To a stirred solution of 10 ml of methylene chloride, 600 mg (2.7 mmol) of IX-Pr₂ or an equivalent amount of IX-i-Bu₂ and 273 mg (2.7 mmol) of triethylamine at –20 °C was slowly added 308 mg (2.6 mmol) of thionyl chloride. After attaching a drying tube, the reaction mixture was stirred for 2 h at –20 °C. Then an excess of ethylglycine in methylene chloride was added dropwise and the stirring continued for 10 min. The reaction mixture was washed with 0.5 N HCl, dried with anhydrous MgSO₄, and rotary evaporated. The residue was distilled at 0.25 mm to give the ethyl esters of X-Pr₂ and X-i-Bu₂ in 80% yield. For hydrolysis, 235 mg (0.77 mmol) of the ethyl ester of X-Pr₂ or an equivalent amount of the ethyl ester of X-i-Bu₂ was added to a solution containing 0.85 mmol of NaOH in 6 ml of methanol and 4 ml of H₂O. After stirring for 30 min at 25 °C, the solution was poured into 50 ml of 1 N HCl and the product extracted with methylene chloride. The methylene chloride extract was dried with anhydrous MgSO₄ and rotary evaporated to give X-Pr₂ and X-i-Bu₂ in 95% yield.

Ethyl-N-[S-(N,N-dipropylcarbamoyl)mercaptoacetyl]glycine: IR 2960, 2930, 2873, 1740, 1672, 1634, 1410, 1375, 1195, 1127, 911 cm⁻¹; NMR (90 MHz) δ 7.30 (m, 1), 4.23 (q, 2, J = 7 Hz), 4.03 (d, 2, J = 6 Hz), 3.60 (s, 2), 3.33 (t, 4, J = 7 Hz), 1.60 (m, 4), 1.29 (t, 3, J = 7 Hz), 0.92 (t, 6, J = 7 Hz); mass spectrum (CI–isobutane, relative intensity) 306 (18), 305 (100) [M + 1]⁺, 202 (3), 145 (2), 128 (8), 100 (2); mass spectrum (high resolution EI) parent peak at mass 304.1456 (calcd for C₁₃H₂₄O₄SN₂, mass 304.1457). N-[S-(N,N-Dipropylcarbamoyl)mercaptoacetyl]glycine (X-Pr₂): IR 2960, 2930, 2875, 1725, 1670, 1630, 1410, 1128 cm⁻¹; NMR (90 MHz) δ 7.42 (m, 1), 4.08 (d, 2, J = 6 Hz), 3.62 (s, 2), 3.32 (t, 4, J = 7 Hz), 1.60 (m, 4), 0.91 (t, 6, J = 7 Hz).

Ethyl-N-[S-(N,N-diisobutylcarbamoyl)mercaptoacetyl]glycine: IR 2980, 2930, 2870, 1740, 1672, 1620, 1419, 1410, 1230, 1200, 1131, 910 cm⁻¹; NMR (90 MHz) δ 7.35 (t, 1, J = 6.5 Hz), 4.21 (q, 2, J = 7 Hz), 3.99 (d, 2, J = 6.5 Hz), 3.64 (s, 2), 3.24 (d, 4, J = 7 Hz), 2.08 (m, 2), 1.27 (t, 3, J = 7 Hz), 0.90 (d, 12, J = 7 Hz); mass spectrum (CI–isobutane, relative intensity) 335 (11), 334 (60), 333 (100) [M + 1]⁺, 332 (31), 230 (1), 156 (5), 91 (1). N-[S-(N,N-Diisobutylcarbamoyl)mercaptoacetyl]glycine (X-i-Bu₂): NMR (90 MHz) δ 7.45 (t, 1, J = 6.5 Hz), 4.06 (d, 2, J = 6.5 Hz), 3.64 (s, 3), 3.21 (d, 4, J = 7 Hz), 2.08 (m, 2), 0.90 (d, 12, J = 7 Hz).

S-(N,N-Dipropylcarbamoyl)-CoA (XI-Pr₂). CoASH (18 mg) was dissolved in D₂O in an NMR tube and then 18 mg of II-Pr₂ was added. After 5 h at 37 °C and extraction with ether, the NMR spectrum showed the presence of XI-Pr₂, i.e. the –S(O)CH₂CH₃ group was absent but the methyl group of the dipropylamine moiety was present. Tests with the Nbs₂ reagent indicated that an almost quantitative yield was obtained since no free thiol group remained and that on treatment with aqueous NaOH XI-Pr₂ hydrolyzed very slowly relative to acetyl-CoA.

Synthesis of ¹⁴CO-Labeled Compounds. The radiosynthesis procedures are based on the reactions described above for preparing the unlabeled standards. In each case the ¹⁴CO-labeled compound cochromatographed with the corresponding unlabeled standard in appropriate TLC solvent systems.

[¹⁴CO]EPTC and [¹⁴CO]Butylate ([¹⁴CO]I-Pr₂, [¹⁴CO]I-i-Bu₂). To an ampule containing 9.9 mg (0.1 mmol, 1 mCi) of [¹⁴C]phosgene in 2.5 ml of benzene was added a magnetic stirring bar, 20 mg of powdered activated

charcoal (PCB carbon), and 25 μl of ethanethiol. After stirring for 18 h at 25 °C, 50 μl of dipropylamine or 75 μl of diisobutylamine was added and the reaction mixture was stirred for an additional 30 min and then chromatographed on a Florisil column (Casida et al., 1975b) to give [^{14}C-O]I-Pr$_2$ (43% yield) or [^{14}CO]I-i-Bu$_2$ (21% yield). The radiochemical purity of each product was >99% based on TLC cochromatography (HE; R_f 0.71 for [^{14}CO]I-Pr$_2$ and 0.77 for [^{14}CO]I-i-Bu$_2$). The products were stable on storage as hexane solutions under nitrogen at -10 °C.

[^{14}CO]EPTC Sulfoxide ([^{14}CO]II-Pr$_2$) and Sulfone ([^{14}CO]III-Pr$_2$) and [^{14}CO]Butylate Sulfoxide ([^{14}CO]II-i-Bu$_2$) and Sulfone ([^{14}CO]III-i-Bu$_2$). These compounds were prepared by oxidation of [^{14}CO]I-Pr$_2$ and -i-Bu$_2$ in chloroform with 1 equivalent or 5 molar equivalent quantities of m-chloroperoxybenzoic acid according to Casida et al. (1975b). TLC purification (HE; R_f 0.14, 0.59, 0.26, and 0.64 for the designated chemicals, respectively) immediately before the compounds were used and extraction of the gel with methanol gave radiochemical purities of >99%.

S-(N-N-Dialkyl[^{14}CO]carbamoyl)glutathione and -cysteine ([^{14}CO]IV-Pr$_2$, [^{14}CO]IV-i-Bu$_2$, [^{14}CO]V-Pr$_2$, [^{14}CO]V-i-Bu$_2$). The carbamoylated GSH and cysteine derivatives were obtained in 60–70% yields by adding 50–500 μg of [^{14}CO]III-Pr$_2$ or [^{14}CO]III-i-Bu$_2$ to 50–75 mg of GSH or cysteine in 8 ml of methanol and 2 ml of triethylamine. The reaction mixtures were stirred for 18 h at 25 °C and the desired products isolated by TLC. The GSH derivatives were chromatographed on 0.50-mm cellulose MN chromatoplates with the WBPA system (R_f 0.63 for [^{14}CO]IV-Pr$_2$ and 0.69 for [^{14}CO]IV-i-Bu$_2$) and H$_2$O extraction for product recovery while the cysteine derivatives were chromatographed on silica gel chromatoplates with the BAW system and methanol extraction for product recovery. Radiochemical purities (>99%) and identities were confirmed by TLC on silica gel (BAW and EMW) immediately before use.

S-(N,N-Dipropyl[^{14}CO]carbamoyl)-CoA ([^{14}CO]XI-Pr$_2$). [^{14}CO]III-Pr$_2$ (20 μg) was added to 5 mg of CoASH in 2 ml of methanol and 2 ml of H$_2$O. After stirring for 24 h at 25 °C the desired product was obtained in 50% yield by TLC (0.25 mm MN cellulose, WBPA, R_f 0.4–0.5), extraction of the cellulose with H$_2$O, and lyophilization to dryness. The radiochemical purity of [^{14}CO]XI-Pr$_2$ was 80%, the 20% impurity moving free from the origin of a silica gel chromatoplate on development with the BAW solvent system.

Metabolism in Rats. Male, albino Sprague-Dawley rats (190–210 g, Horton Laboratories Inc., Oakland, Calif.) were individually treated orally by stomach tube with ^{14}CO-labeled preparations (6–12 μCi) of I-Pr$_2$, I-i-Bu$_2$, II-Pr$_2$, and II-i-Bu$_2$ in methoxytriglycol (50–60 μl) or intraperitoneally (ip) with ^{14}CO-labeled preparations (0.3–2.3 μCi) of IV-Pr$_2$, IV-i-Bu$_2$, V-Pr$_2$, V-i-Bu$_2$, and XI-Pr$_2$ in H$_2$O (120–240 μl). A methoxytriglycol rinse (50 μl) of the stomach tube provided more quantitative introduction of the desired dose. The rats were held in all-glass metabolism cages (rat metabolism unit Model MC 3000-1, Stanford Glassblowing Labs, Inc., Palo Alto, Calif.) for 48 h prior to sacrifice with analyses of ^{14}CO$_2$, urinary and fecal radiocarbon, and tissue residues as previously described (Gaughan et al., 1977). The urinary metabolites were analyzed by directly spotting 50 μl of urine on each silica gel chromatoplate and two-dimensional TLC (BAW × EMW and EMPW × BAW). The possible presence of glucuronide and sulfate conjugates was examined by incubating urine samples with β-glucuronidase and aryl sulfatase to determine any

changes in chromatographic patterns (TLC) attributable to conjugate cleavage (method based on Gaughan et al., 1977).

In another study, rats were administered unlabeled I-Pr$_2$, I-i-Bu$_2$, II-Pr$_2$, II-i-Bu$_2$, benthiocarb, cycloate, molinate, and pebulate at 1.0 mmol/kg using the ip route and methoxytriglycol as the carrier vehicle. The 0–24-h urine (after centrifugation to remove particulate material) was lyophilized to dryness, the residue extracted with methanol, and the products therein methylated with diazomethane. Analysis by GC–CI–MS identified the mercapturic acid (VII-Pr$_2$, VII-i-Bu$_2$) and mercaptoacetic acid (IX-Pr$_2$) conjugates as their methyl esters. Mercapturic acid quantitation by GC–FID utilized appropriate internal standards introduced prior to the lyophilization step.

In order to examine metabolites in the liver, rats treated ip with III-Pr$_2$ (1000 μmol/kg) and II-i-Bu$_2$ (1000 μmol/kg) in methoxytriglycol (75 μl) or with methoxytriglycol alone were sacrificed 30–135 min after treatment. The liver was homogenized in H$_2$O and the homogenate was centrifuged at 15 000g to obtain the supernatant fraction. For determination of IV-Pr$_2$, the supernatant was lyophilized, the residue extracted with methanol, and the methanol extract analyzed by TLC with ninhydrin for product detection [R_f 0.62 in WBPA and 0.56 in H$_2$O–1-butanol–pyridine (1:1:1)]. To analyze for VII-i-Bu$_2$, the supernatant was adjusted to pH 1 with HCl and extracted with chloroform, and this extract after treatment with diazomethane was subjected to GC-CI-MS.

Metabolism in Corn. Corn seedlings (DeKalb XL-66-H variety, 3.5-day following germination) that had been exposed for 24 h to N,N-diallyl-2,2-dichloroacetamide (R-25788) solutions (0 or 30 ppm) were rinsed and transferred for an additional 24 h into 1.85 μM solutions of [^{14}CO]II-Pr$_2$. The roots were then cut off, rinsed, and homogenized in distilled H$_2$O and the homogenate was extracted with chloroform (Lay and Casida, 1976). The aqueous phase was adjusted to pH 2 with HCl and lyophilized to dryness and the methanol-soluble portion was analyzed by two-dimensional TLC (BAW × EMW and EMPW × BAW).

Leaves and stems (25–35 cm length) from older corn seedlings were also examined, comparing untreated plants with those exposed for 4 days to R-25788 added to the soil at a rate equivalent to 1 kg/ha. The plants were removed from the soil, the roots and stems immersed in Hoagland's solution (Hoagland and Arnon, 1938), and the roots cut off. While keeping the cut end immersed, the stem was placed into a 5.5-ml vial from which solution was removed until 1.0 ml remained. After bubbling oxygen through the solutions for 2 min, [^{14}CO]I-Pr$_2$ (10 μg) or [^{14}CO]II-Pr$_2$ (7 μg) in 25 μl of ethanol was introduced into the Hoagland's solution and the upper end of the vial was sealed around the stem with parafilm. The plants were held for 48 h under greenhouse conditions adding Hoagland's solution as necessary to keep the cut end of the stem immersed. The plants were then removed and the 1-cm end directly exposed to the ^{14}CO-labeled compounds was cut off, rinsed with methanol, and analyzed for total radiocarbon by combustion. The remainder of the stem and leaves was homogenized in H$_2$O and the homogenate filtered. After extracting the filtrate with ether, the aqueous phase was adjusted to pH 2 with HCl and lyophilized and the residue extracted with methanol–water (4:1) to obtain an extract for analysis by one-dimensional (EMW) and two-dimensional TLC (EMPW × BAW and BAW × WPBA). Residual ^{14}C-labeled compounds in the vial and in the methanol rinse of the stem segment were analyzed for total

radiocarbon and for individual components by TLC (HE).

Toxicity to Mice. Male albino mice (18–20 g, Horton Laboratories Inc.) were treated ip with the test compounds using 50 μl of methoxytriglycol as the carrier unless specified otherwise. LD_{50} values are based on determinations with 12 mice per dose and a 1.5-fold magnitude of dose differential.

RESULTS

Radiocarbon Distribution 48 h following Administration to Rats of ^{14}CO-Labeled Preparations of EPTC, Butylate, Their Sulfoxides, and the Corresponding GSH and Cysteine Conjugates. There is a large difference between the thiocarbamates and the other compounds in the proportion of radiocarbon expired as $^{14}CO_2$ relative to that excreted in the urine (Table I). The thiocarbamates yield large amounts of $^{14}CO_2$ (46–52% with I-Pr$_2$ and 61–64% with I-i-Bu$_2$) relative to urinary radiocarbon (34–45% with I-Pr$_2$ and 27–32% with I-i-Bu$_2$). In contrast, the thiocarbamate sulfoxides (II-Pr$_2$, II-i-Bu$_2$) and the GSH and cysteine conjugates (IV-Pr$_2$, V-Pr$_2$, IV-i-Bu$_2$, V-i-Bu$_2$) yield relatively little $^{14}CO_2$ (7–10% for the dipropylcarbamoyl derivatives and 22–28% for the diisobutylcarbamoyl derivatives) and large amounts of urinary radiocarbon (82–87% for the dipropylcarbamoyl derivatives and 60–65% for the diisobutylcarbamoyl derivatives) (Table I). These differences do not appear to be attributable to variations in dose (i.e., similar findings with two widely varying doses of EPTC and butylate) or route of administration (i.e., similar results with II administered orally and IV and V administered ip in both the EPTC and butylate series). Relatively little of the radiocarbon is excreted in the feces within 48 h. The radiocarbon is rapidly eliminated from the body such that of the total amount expired or excreted within 48 h, about half is lost by 6 h and 93–99% by 24 h.

Tissue analyses at 48 h (Table I) show a preferential retention of radiocarbon in the blood with EPTC sulfoxide and the high dose of EPTC. However, in the other cases (including butylate sulfoxide and the high dose of butylate) the liver and kidney generally retain the highest parts per billion equivalents of the administered compounds.

Rat Urinary Metabolites of ^{14}CO-Labeled Preparations of EPTC, Butylate, Their Sulfoxides, and the Corresponding GSH, Cysteine, and CoASH Conjugates. Almost identical TLC patterns and proportions of individual metabolites are obtained with the ^{14}CO-labeled preparations of I-Pr$_2$, II-Pr$_2$, IV-Pr$_2$, and V-Pr$_2$ (Figure 2, Table II) and with ^{14}CO-labeled I-i-Bu$_2$, II-i-Bu$_2$, IV-i-Bu$_2$, and V-i-Bu$_2$ (Figure 2, Table III). Several metabolites are tentatively identified by TLC in each of two different two-dimensional solvent systems as follows: in the EPTC (I-Pr$_2$) series—the cysteine conjugate (V-Pr$_2$) and the mercapturic acid (VII-Pr$_2$) and mercaptoacetic acid derivatives (IX-Pr$_2$); in the butylate (I-i-Bu$_2$) series—the mercapturic acid (VII-i-Bu$_2$), the N-deisobutylmercapturic acid (VIII-i-Bu), and the mercaptoacetic acid derivative (IX-i-Bu$_2$). The glycine conjugate (X-i-Bu$_2$) in the butylate series is also tentatively identified but only on the basis of one-dimensional TLC cochromatography (BAW) and an appropriate chromatographic position on two-dimensional TLC (BAW × EMW and EPMW × BAW). Two mercapturic acids (VII-Pr$_2$, VII-i-Bu$_2$) and one mercaptoacetic acid conjugate (IX-Pr$_2$) were further identified by GC–CI–MS using metabolites derivatized by methylation of the methanol extract of lyophilized urine.

Compounds that do not cochromatograph with the urinary metabolites are also shown by chromatographic position in Figure 2. The urine does not appear to contain

Table I. Radiocarbon Distribution 48 h following Administration to Rats of [^{14}CO]Thiocarbamates, [^{14}CO]Thiocarbamate Sulfoxides, and the Corresponding S-(N,N-Dialkyl[^{14}CO]carbamoyl)glutathione and -cysteine Derivatives

Compound	Dose (mg/kg)	Dose (μmol/kg)	Route	Radiocarbon recovery, % of admin. dose — CO_2	Urine	Feces	Body	Total	Tissue radiocarbon, ppb equiv of admin. compd — Blood	Kidney	Liver	Others[a]
S-[*N,N*-(Dipropylcarbamoyl)] Derivatives												
EPTC (I-Pr$_2$)	13.5[b]	71	Oral	46.0	44.7	0.7	3.3	94.7	1 340	1000	2980	200–960
EPTC (I-Pr$_2$)	132.5	701	Oral	52.0	33.9	1.0	1.4	88.3	14 740	5500	7810	890–4870
EPTC sulfoxide (II-Pr$_2$)	13.0	63	Oral	9.2	81.6	0.6	2.7	94.1	2 820	1140	1730	109–1340
GSH conjugate (IV-Pr$_2$)	0.49	1.13	ip	7.1	86.8	0.9	0.7	95.5	4	11	7	1–9
Cysteine conj. (V-Pr$_2$)	0.13	0.52	ip	10.0	86.9	1.7	0.9	99.5	2	3	3	0.3–4
S-[*N,N*-(Diisobutylcarbamoyl)] Derivatives												
Butylate (I-i-Bu$_2$)	12.3[b]	57	Oral	60.9	31.5	3.3	2.4	98.1	276	524	710	182–545
Butylate (I-i-Bu$_2$)	156.0[b]	719	Oral	64.0	27.3	4.7	2.2	98.2	2 076	5320	7720	1720–5560
Butylate sulfoxide (II-i-Bu$_2$)	6.15	26	Oral	24.2	63.0	2.7	3.3	93.2	172	141	436	30–243
GSH conjugate (IV-i-Bu$_2$)	0.08	0.17	ip	21.6	65.3	3.8	1.2	91.9	1	2	1	0.3–1
Cysteine conjugate (V-i-Bu$_2$)	0.13	0.47	ip	27.9	60.1	2.3	2.7	93.0	1	3	3	1–3

[a] Range for brain, fat, heart, lung, muscle, spleen, and testes. [b] Data are average for two rats. In all other cases a single rat was used.

Table II. Urinary Metabolites 48 h following Administration to Rats of [14CO]EPTC, [14CO]EPTC Sulfoxide, and the Corresponding S-(N,N-Dipropyl[14CO]carbamoyl)glutathione and -cysteine Derivatives

	Urinary radiocarbon, %a			
TLC system and metabolite	EPTC (I-Pr$_2$)	EPTC sulfoxide (II-Pr$_2$)	GSH conjugate (IV-Pr$_2$)	Cysteine conjugate (V-Pr$_2$)
Identified Metabolites				
BAW × EMW				
V-Pr$_2$	18.6[b]	18.0[b]	23.6[b]	26.6[b]
VII-Pr$_2$	38.7	60.0	66.3	58.5
IX-Pr$_2$	5.9	8.7	0.8	1.8
EMPW × BAW				
V-Pr$_2$	15.2[c]	13.4[c]	17.6[c]	25.8[c]
VII-Pr$_2$	50.7	59.7	62.3	37.1
IX-Pr$_2$	3.4	8.9	1.1	3.5
Unidentified Metabolites				
BAW × EMW				
1	1.8	0.2	1.4	2.0
2	3.3	0.2	0.4	0.0
3	1.7	2.3	1.0	1.7
4	1.0	0.5	0.4	0.9
5	3.9	0.5	0.7	1.8
6	9.6	1.4	2.0	3.5
Others	15.5	8.2	3.4	3.2
EMPW × BAW				
1	0.0	1.1	0.0	0.1
2	0.1	1.0	0.0	0.1
3	1.3	0.1	1.5	3.1
4	2.0	0.1	0.0	0.0
5	2.1	2.6	0.9	2.8
6	0.9	0.8	1.4	2.9
7	3.3	1.5	3.4	5.9
8	0.0	0.6	1.5	2.9
9	8.8	0.6	1.2	2.0
10	2.6	1.0	2.4	3.5
Others	9.6	8.6	6.7	10.3

a See Table I for treatment conditions and Figure 2 for separation of metabolites in indicated TLC systems. EPTC data are average from two dosage levels since similar results were obtained. b Includes at least two unidentified metabolites. c Includes at least one unidentified metabolite.

Table III. Urinary Metabolites 48 h following Administration to Rats of [14CO]Butylate, [14CO]Butylate Sulfoxide, and the Corresponding S-(N,N-Diisobutyl[14CO]carbamoyl)glutathione and -cysteine Derivatives

	Urinary radiocarbon, %a			
TLC system and metabolite	Butylate (I-i-Bu$_2$)	Butylate sulfoxide (II-i-Bu$_2$)	GSH conjugate (IV-i-Bu$_2$)	Cys conjugate (V-i-Bu$_2$)
Identified Metabolites				
BAW × EMW				
VII-i-Bu$_2$	3.1	6.9	7.7	9.8
VIII-i-Bu$_2$	24.7[b]	28.7[b]	39.1[b]	38.7[b]
IX-i-Bu$_2$	0.4	2.4	6.0	6.6
X-i-Bu$_2$	6.2	3.1	1.8	0.7
EMPW × BAW				
VII-i-Bu$_2$	4.3	7.5	7.4	10.2
VIII-i-Bu	17.1[b]	23.6[b]	30.1[b]	36.2[b]
IX-i-Bu$_2$	0.8	3.2	7.1	6.7
X-i-Bu$_2$	11.7	4.4	1.9	0.8
Unidentified Metabolites				
BAW × EMW				
1	0.9	1.9	0.3	0.0
2	0.5	3.1	0.0	0.6
3	2.5	6.3	6.1	7.0
4	1.8	2.6	3.5	3.4
5	0.0	9.9	11.6	6.4
6	0.5	0.0	3.4	2.1
7	1.2	1.4	0.6	0.1
8	3.7	1.1	0.0	0.0
9	19.3[b]	4.5[b]	4.0[b]	2.6[b]
Others	35.2	28.1	15.9	22.0
EMPW × BAW				
1	1.6	9.5	9.3	10.9
2	1.2	0.0	0.0	0.0
3	0.0	7.8	11.9	7.7
4	2.5	3.1 }	} 4.9	{ 2.8
5	1.7	1.5 }		{ 1.0
6	0.3	1.2	0.8	0.6
7	0.4	1.5	1.2	1.6
8	4.1	0.0	0.0	0.0
9	2.0	4.5	1.6	3.9
10	0.8	2.2	0.0	0.2
11	17.6	3.8	4.5	3.6
12	1.2	0.8	2.4	4.0
13	14.5 }	9.8 }	2.3	1.9
14	8.8 }			
Others	9.4	15.6	14.6	7.9

a See Table I for treatment conditions and Figure 2 for separation of metabolites in indicated TLC systems. Butylate data are average from two dosage levels since similar results were obtained. b Includes at least one unidentified metabolite.

glucuronide and sulfate conjugates derived from [14CO]-EPTC or -butylate since the TLC patterns (HE, EMW, and BAW) and proportions of the metabolites remain unchanged on incubating the urine with β-glucuronidase and aryl sulfatase. Additional compounds not present in the urine (TLC R_f values higher than those of any of the metabolites) are EPTC and butylate, their sulfoxides and sulfones, and N-depropyl-EPTC. In contrast, Ong and Fang (1970) report the presence of EPTC in urine under comparable treatment conditions.

The urine from rats treated with [14CO]EPTC or its derivatives contain at least ten other labeled metabolites present in >1% amount relative to the urinary radiocarbon with one or more of the administered compounds (Figure 2, Table II). Most if not all of these metabolites are formed from each of I-Pr$_2$, II-Pr$_2$, IV-Pr$_2$, and V-Pr$_2$. Several additional metabolites are detected when a lower proportion of the administered dose is set as the discriminating level. The position of the cysteine conjugate (V-Pr$_2$) is clearly defined by cochromatography but the quantitative values include at least one additional metabolite that is not adequately separated by TLC. It is notable that the N-depropylmercapturic acid (VIII-Pr) and the glycine conjugate of the mercaptoacetic acid (X-Pr$_2$) are not detected as metabolites in the EPTC series.

The butylate series gives a more complex metabolite pattern with at least 14 products present in >1% amount relative to the urinary radiocarbon in addition to the identified compounds referred to above (Figure 2, Table III). One of these metabolites (designated as 2 in the EMPW × BAW solvent system; detected at <1% with BAW × EMW) arises only from butylate and therefore is formed without the sulfoxide as an intermediate. The N-deisobutylmercapturic acid (VIII-i-Bu), although clearly cochromatographing with the authentic standard, is not adequately resolved on TLC from at least one other metabolite for quantitation; however, the spot intensities on the radioautogram indicate that about half of the tabulated amount is actually VIII-i-Bu. No evidence was obtained for excretion of the cysteine conjugate (V-i-Bu$_2$).

On ip administration of S-(N,N-dipropyl[14CO]carbamoyl)-CoA ([14CO]XI-Pr$_2$) at 0.17 μmol/kg, about half of the radiocarbon appears in the urine within 24 h. Nine

Table IV. Urinary S-(N,N-Dialkylcarbamoyl)mercapturic Acids Extracted within 24 h following Intraperitoneal Administration of Thiocarbamate Herbicides or Their Sulfoxides to Rats at 1.0 mmol/kg with Amounts and Chemical Ionization Mass Spectra Determined on the Methyl Esters

			Mercapturic acid, methyl ester		
			CI-MS (methane), m/e (rel. intensity)		
Compd administered		Amount in urine, %[b]	$[M + 1]^+$	$[R_1R_2NC(O)]^+$	Fragments at 172 and 144, resp.
Name	Structure[a]				
Compounds with n-Alkyl Substituents on the Nitrogen					
Benthiocarb	$Et_2NC(O)SCH_2PhCl-4$	35	277 (4.3)	100 (100)	(2.4) (5.3)
EPTC (I-Pr$_2$)	$Pr_2NC(O)SEt$	18[c] }	305 (1.9)	128 (100)	(2.5) (5.2)
EPTC sulfoxide (II-Pr$_2$)	$Pr_2NC(O)S(O)Et$	36 }			
Pebulate	$Bu(Et)NC(O)SPr$	16	305 (16.1)	128 (100)	(4.9) (6.3)
Compounds with Branched-Alkyl or Cyclic Substituents on the Nitrogen					
Butylate (I-i-Bu$_2$)	(i-Bu)$_2$NC(O)SEt	4–6[c] }	333 (18.4)	156 (100)	(8.0) (11.5)
Butylate sulfoxide (II-i-Bu$_2$)	(i-Bu)$_2$NC(O)S(O)Et	6 }			
Cycloate	Cy(Et)NC(O)SEt	12 ·	331 (20.0)	154 (100)	(8.0) (8.0)
Molinate	$(CH_2)_6NC(O)SEt$	2	303 (14.3)	126 (100)	(<10) (14.3)

[a] Substituent designations: Et = ethyl; Pr = propyl; Bu = butyl; i-Bu = isobutyl; Cy = cyclohexyl; Ph = phenyl. [b] Quantitation by GC-FID using VII-Pr$_2$ methyl ester as the internal standard for butylate, butylate sulfoxide, and cycloate and VII-i-Bu$_2$ methyl ester as the internal standard for the other compounds. The results are given as the molar equivalent amounts relative to the administered dose. [c] Comparable values for orally administered compounds are 24% for EPTC and 4-6% for butylate.
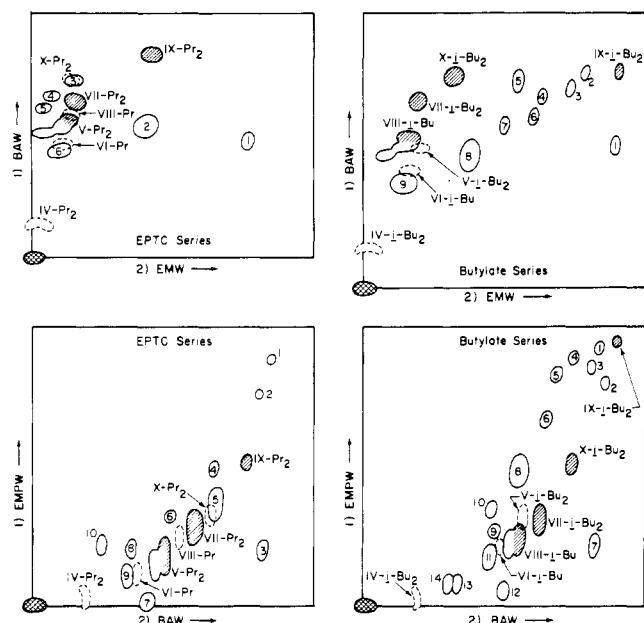

Figure 2. TLC chromatographic patterns for rat urinary ^{14}C-labeled metabolites of [^{14}CO]thiocarbamates, [^{14}CO]thiocarbamate sulfoxides, and the corresponding S-(N,N-dialkyl[^{14}CO]carbamoyl)glutathione and -cysteine derivatives. For structures of compounds designated by Roman numerals see Figure 1. Dotted circles are positions of standards not detected as metabolites. Circles with diagonal lines indicate identified metabolites. Open circles with Arabic numbers are unidentified metabolites. Circle with cross-hatch is origin. For quantitative data see Tables II and III.

metabolite regions are detected by TLC (BAW, R_f from 0.45 to 0.90, each >1% of the urinary radiocarbon), no one of which cochromatographs with any metabolite of [^{14}CO]EPTC.

The feces from both dose levels of EPTC and butylate contain a small proportion (<1% of the total fecal radiocarbon) of unmetabolized compound based on TLC (HE) of volatile or hexane-extractable materials.

Mercapturic Acid Conjugates in the Urine of Thiocarbamate- and Thiocarbamate Sulfoxide Treated Rats. From 2 to 36% (GC-FID) of the ad-

ministered thiocarbamate and thiocarbamate sulfoxide is excreted in the 0–24-h urine as the corresponding mercapturic acid (GC–CI–MS on methylated extracts) (Table IV). The amount is greater (16–36%) for compounds with n-alkyl substituents (Et, Pr, Bu) on the nitrogen than for those with branched-alkyl (i-Bu, Cy) or cyclic substituents [(CH$_2$)$_6$N] on the nitrogen (2–12%). The yields of mercapturic acids from the orally administered unlabeled compounds determined by the GC-FID method (24% for I-Pr$_2$, 4–6% for I-i-Bu$_2$; Table IV) fall in the same range as those for the orally administered ^{14}CO-labeled compounds determined by TLC (18% for I-Pr$_2$, 1.1% for I-i-Bu$_2$; Tables I–III).

GSH and Mercapturic Acid Conjugates in the Liver of Treated Rats. The liver of rats receiving EPTC sulfone (III-Pr$_2$) contains detectable (TLC) S-(N,N-dipropyl-carbamoyl)glutathione (IV-Pr$_2$) at 30 and 135 min after treatment, with lesser amounts appearing in the 135-min sample. Following butylate sulfoxide (II-i-Bu$_2$) administration, S-(N,N-diisobutylcarbamoyl)mercapturic acid (VII-i-Bu$_2$) is identified in the liver (GC–CI–MS on methylated extracts) at 30, 60, and 120 min after administration, with the highest level at 60 min.

Corn Metabolites of [^{14}CO]EPTC and [^{14}CO]EPTC Sulfoxide. ^{14}C-Labeled metabolites of [^{14}CO]EPTC sulfoxide in the methanol-soluble fraction from the roots of seedlings pretreated with 0 and 30 ppm of R-25788 constitute 9 and 48%, respectively, of the root radiocarbon content (Lay and Casida, 1976). Two of the products in each case cochromatograph with the GSH (IV-Pr$_2$) and cysteine (V-Pr$_2$) conjugates and the additional 13–15 products, although not identified, appear to be the same compounds but in slightly varying proportions in the control and antidote-treated plants (Figure 3, Table V). By considering the amount of methanol-soluble radiocarbon the antidote-treated plants contain two and five times more GSH and cysteine conjugates, respectively, than the control plants. It, therefore, appears that the antidote increases the extent of GSH conjugation of EPTC sulfoxide in corn roots.

The uptake and metabolism of [^{14}CO]EPTC and -EPTC sulfoxide were also examined with the aerial portions of corn plants. After 48 h relatively little of the radiocarbon

Table V. ^{14}C-Labeled Metabolites of [^{14}CO]EPTC Sulfoxide in Roots of Corn Seedlings Pretreated with 0 or 30 ppm of R-25788

| Metabolite designation | Radiocarbon in methanol-soluble fraction, % at indicated ppm of R-25788 | | | |
| | BAW x EMW[a] | | EMPW x BAW[a] | |
	0	30	0	30
IV-Pr$_2$	16.2	6.3	17.3	6.7
V-Pr$_2$	9.5	8.6	6.9	6.4
1	10.7	10.9	9.9	10.9
2	9.8	26.0	11.1	27.3
3	1.8	1.2	2.0	1.5
4	4.8	4.9	5.3	5.5
5	8.5	2.9	8.3	2.9
6	7.6	7.9	7.9	7.4
7	1.7	0.8	2.0	0.8
8	4.9	2.2	3.7	3.7
9	4.6	6.3	2.9	3.2
10			2.2	2.0
11	3.2	4.1	3.1	2.1
12			3.5	2.3
13	1.7	2.1	1.4	1.2
a[b]	2.1	2.1		
b[b]	1.1	0.7		
Others	11.8	13.0	12.5	16.1

[a] See Figure 3 for separation of metabolites in indicated TLC systems. [b] Mixtures of two or more products that are resolved in the EMPW x BAW system but individually account for <1% of the radiocarbon in the methanol-soluble fraction.

remained in the vial, most of which was starting material with [^{14}CO]EPTC and unidentified compounds with [^{14}CO]EPTC sulfoxide (Table VI). Plants pretreated with R-25788 retain a much larger amount of the radiocarbon from [^{14}CO]EPTC in the exposed stem section than control plants, an antidote-related effect which is much less important with [^{14}CO]EPTC sulfoxide. The ether-soluble fraction of the translocated portion is mostly starting material from [^{14}CO]EPTC and an unidentified metabolite (unknown a) from [^{14}CO]EPTC sulfoxide. TLC analysis (HE) revealed no EPTC sulfone or N-depropyl-EPTC (R_f 0.56) in the EPTC-treated plants. Trace amounts of EPTC sulfoxide (II-Pr$_2$) are detected in plants treated with either [^{14}CO]EPTC or its sulfoxide. The fraction most
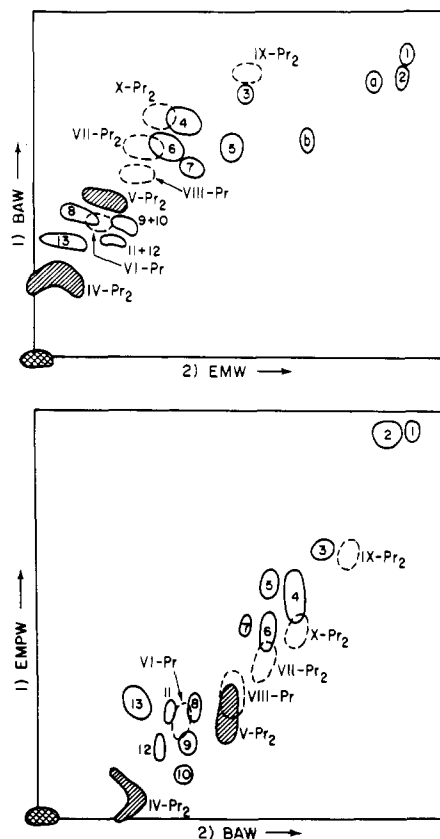

Figure 3. TLC chromatographic patterns for corn root ^{14}C-labeled metabolites of [^{14}CO]EPTC sulfoxide. For designations see Figure 1 and legend of Figure 2. For quantitative data and nature of a and b see Table V.

greatly affected by the antidote is the methanol-H$_2$O extract in plants treated with [^{14}CO]EPTC sulfoxide. Although this indicates increased conjugate formation, no further analyses are available on the components because of low radiocarbon levels.

The products in the methanol-soluble fraction from the aerial portion of EPTC-treated seedlings are shown in Figure 4. Only the GSH and cysteine conjugates are identified. Several thiocarbamate conjugates found as
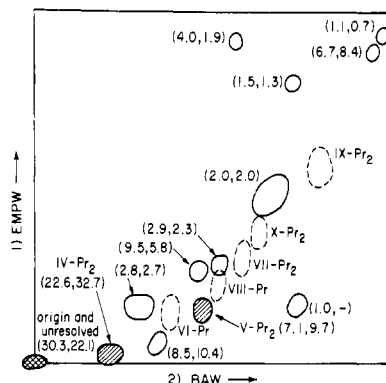
Table VI. Radiocarbon Distribution 48 h following Introduction of the Aerial Portion of Corn Seedlings Pretreated with 0 or 1 kg/ha R-25788 into Solutions of [^{14}CO]EPTC and [^{14}CO]EPTC Sulfoxide

| Sample or metabolite | TLC R_f, HE | Radiocarbon recovery, % of initial amount, at indicated kg/ha R-25788 | | | |
| | | EPTC | | EPTC sulfoxide | |
		0	1	0	1
Remaining in vial		7.0[a]	10.9[a]	15.6[b]	17.3[b]
Retained in exposed stem section		8.9	26.8	10.0	12.8
Translocated in plant					
Ether extract					
I-Pr$_2$	0.71	2.4	5.3	0.0	0.0
Unk. a	0.57	0.0	0.0	5.1	2.7
Unk. b	0.38	0.0	0.0	0.5	1.9
Unk. c	0.28	1.7	1.6	0.0	0.0
Unk. d	0.17	0.0	0.0	0.2	0.2
II-Pr$_2$	0.14	0.2	0.1	0.2	0.1
Origin and unresolved		0.8	0.8	1.7	1.1
Aqueous phase after lyophilization					
Methanol-H$_2$O sol.[c]		32.9	30.3	7.1	13.0
Methanol-H$_2$O insol.		5.8	3.2	1.2	1.4
Loss (not in plant or vial)		40.3	21.0	58.4	49.5

[a] TLC analysis (HE) detected I-Pr$_2$ (82%), II-Pr$_2$ (1.5%), and unknown c (4.6%) with the remaining ^{14}C-labeled compounds unresolved and no significant effect of R-25788. [b] TLC analysis (HE) detected II-Pr (<1%), unknown a (63%), unknown b (7%), and unknown d (5%) with the remaining ^{14}C-labeled compounds unresolved and no significant effect of R-25788. [c] Results of TLC analysis (EMPW x BAW) are shown in Figure 4.

Figure 4. TLC chromatographic pattern for corn leaf ^{14}C-labeled metabolites of [^{14}CO]EPTC. For designations see Figure 1 and legend of Figure 2. Numbers in parentheses are percentage amounts for individual metabolites from plants without and with R-25788 treatment, respectively.

mammalian metabolites are absent in corn. More GSH conjugate appears in the antidote-treated than in control plants (32.7 vs. 22.6%) but there is little if any effect of the antidote on the proportionate amount of most of the other metabolites (Figure 4). Other TLC analyses verified these findings: the BAW × WBPA system, which separates the GSH conjugate from an impurity in the same region, gives 25.3% GSH conjugate in antidote-treated and 18.3% in control plants; the EMW system on one-dimensional development provides six distinct metabolite bands, each a mixture of metabolites, with little variation between the control and antidote-treated plants. The overall effects of the antidote, although relatively small, appear to include increasing the reaction in the exposed stem section immediately on entering the plant, increasing the conjugate fraction in EPTC sulfoxide treated plants, and increasing the amount of GSH conjugate in EPTC-treated plants.

Toxicity to Mice of Thiocarbamates, Their Metabolites, and Derivatives. Compounds in the EPTC series (Pr$_2$) are more toxic to ip-treated mice than those in the butylate series (i-Bu$_2$) (Table VII). The sulfones (III) are the most toxic derivatives. EPTC has the largest dose differential in the 1- and 7-day LD$_{50}$ values. There is little if any change in the LD$_{50}$ values with any of the compounds on holding the treated mice longer than 7 days. When administered in corn oil, the 7-day LD$_{50}$ for EPTC is >500 mg/kg. Additional compounds assayed including some metabolites are generally less toxic than the thiocarbamates and their sulfoxides and sulfones.

DISCUSSION

The present investigation and our earlier studies (see introductory statement) establish that an important pathway for metabolism of EPTC and butylate in rats involves mfo oxidation to the thiocarbamate sulfoxide (II), transfer of the carbamoyl group to GSH (IV) assisted by the GSH S-transferase system, and cleavage of the GSH conjugate to the cysteine derivative (V) which is then acetylated to the mercapturic acid (VII) or further degraded to the mercaptoacetic acid derivative (IX), a portion of which undergoes conjugation with glycine (X) (Figure 1). The N-dealkylmercapturic acid (VIII) may be formed from the mercapturic acid (VII) or less likely from an earlier intermediate. This overall pathway is established by identification of relevant metabolites as follows: II-Pr$_2$ and -i-Bu$_2$ from I-Pr$_2$ and -i-Bu$_2$ in mouse liver mfo systems; II-Pr$_2$ from I-Pr$_2$ in the liver of mice; VII-i-Bu$_2$ from II-i-Bu$_2$ in the liver of rats; V-, VII-, and IX-Pr$_2$ as

Table VII. Mouse Intraperitoneal LD$_{50}$ Values for EPTC, Butylate, and Their Metabolites or Derivatives

Compound[a]	LD$_{50}$, mg/kg, at indicated days after treatment	
	1	7
EPTC (I-Pr$_2$)	> 500	58
EPTC sulfoxide (II-Pr$_2$)	325	215
EPTC sulfone (III-Pr$_2$)	130	40
Butylate (I-i-Bu$_2$)	> 500	365
Butylate sulfoxide (II-i-Bu$_2$)	440	250
Butylate sulfone (III-i-Bu$_2$)	200	80

[a] The 1-day LD$_{50}$ values are > 500 mg/kg for the following additional compounds using the indicated carrier solvents: V-Pr$_2$, 150 μl of H$_2$O; V-i-Bu$_2$, 100 μl of methoxytriglycol; VI-Pr, 100 μl of Tween 80–H$_2$O (1:4); VII-Pr$_2$, VII-i-Bu$_2$, VIII-Pr, VIII-i-Bu, X-Pr$_2$, and X-i-Bu$_2$, each with 50 μl of methoxytriglycol. The 7-day LD$_{50}$ values fall in the range of 300–500 mg/kg except for V-Pr$_2$, VI-Pr, and VIII-Pr where they are >500 mg/kg.

rat urinary metabolites of I-, II-, IV-, and V-Pr$_2$; VII-, IX-, and X-i-Bu$_2$ and VIII-i-Bu as rat urinary metabolites of I-, II-, IV-, and V-i-Bu$_2$.

The thiocarbamates, their sulfoxides, and the corresponding GSH and cysteine conjugates give the same urinary metabolites, both identified and unidentified. This indicates that thiocarbamate metabolism via the sulfoxide and GSH conjugate accounts for 34–45% of the EPTC metabolites and 27–32% of the butylate metabolites. It further suggests that the unidentified urinary metabolites are probably mono- and dialkylcarbamoyl derivatives. An additional 7–10% of the [^{14}CO]EPTC dose and 22–28% of the [^{14}CO]butylate dose expired as ^{14}CO$_2$ may also go through the thiocarbamate sulfoxide → GSH conjugate pathway. Based on analogy with studies on metabolism of S-methyl-L-cysteine in rats (Barnsley, 1964; Sklan and Barnsley, 1968), some of the unidentified thiocarbamate metabolites in urine may be sulfoxide derivatives of the identified compounds and 2-hydroxy-3-(N,N-dialkylcarbamoylthio)propionic acids.

It is proposed that there is also a second major pathway for EPTC and butylate metabolism in rats which does not involve the sulfoxide intermediate. This pathway is invoked to account for the 40% greater amount of ^{14}CO$_2$ liberated from the thiocarbamates than from the sulfoxide derivatives and the corresponding GSH and cysteine conjugates. There is insufficient information at present to determine if this pathway involves carbon hydroxylation at a SCH$_2$ or NCH$_2$ position, further oxidation at the sulfur, or another mechanism.

There are several indications that the available metabolic pathways in rats differ for thiocarbamates with n-alkyl substituents on the nitrogen as opposed to those with branched-alkyl or cyclic substituents on the nitrogen. First, the yield of mercapturic acids is greater with the former than with the latter group of compounds. Second, a greater number of metabolites are formed from butylate than from EPTC. Third, the cysteine conjugate is detected from EPTC but not from butylate. Finally, the N-dealkylmercapturic acid is a major metabolite with butylate but is not detected with EPTC. These differences may result from the greater number and variety of sites for carbon hydroxylation in compounds with branched-alkyl or cyclic substituents on the nitrogen.

The metabolism of EPTC in corn also involves oxidation to the sulfoxide, conjugation with GSH, and cleavage to the cysteine conjugate. The relevant metabolites are identified in corn leaves following EPTC treatment and

in roots exposed to EPTC sulfoxide. Most of the cysteine conjugate in corn is not metabolized by the pathways utilized in rats since none of the other identified urinary metabolites are found in corn. Atrazine metabolism in corn or sorghum yields a GSH derivative and a cysteine conjugate, the latter compound undergoing a nonenzymatic rearrangement and then conjugation to a lanthionine derivative (Shimabukuro, 1975). The present studies do not evaluate the possibility that similar reactions with the thiocarbamates are a possible source of some of the unidentified metabolites.

The antidote R-25788 increases the formation of the GSH and cysteine conjugates in corn roots treated with EPTC sulfoxide and it may increase conjugate formation in EPTC-treated leaves. Identical metabolites are present with or without antidote treatment. R-25788 appears to have a greater effect on the metabolism in roots than in leaves.

Thiocarbamate sulfoxides are carbamoylating agents for thiol groups, including those of GSH and CoASH and potentially those of other tissue components including proteins. Carbamoylation of CoASH does not constitute a significant pathway in the metabolism of EPTC in rats. [^{14}CO]EPTC sulfoxide and a high dose of [^{14}CO]EPTC give a greater radiocarbon retention in the blood than in other tissues of rats, suggesting the possibility that EPTC sulfoxide carbamoylates a blood constituent.

These studies verify the rapid biodegradation of thiocarbamate herbicides and their sulfoxide metabolites. They also establish that the available metabolites with a carbamoyl moiety are generally less toxic to mice on an acute basis than the parent compounds. Rapid sulfoxidation and conjugation with GSH are significant detoxification pathways.

LITERATURE CITED

Ashton, F. M., Crafts, A. S., "Mode of Action of Herbicides", Wiley-Interscience, New York, N.Y., 1973, p 289.

Barnsley, E. A., Biochim. Biophys. Acta 90, 24 (1964).

Casida, J. E., Gray, R. A., Tilles, H., Science 184, 573 (1974).

Casida, J. E., Kimmel, E. C., Lay, M., Ohkawa, H., Rodebush, J. E., Gray, R. A., Tseng, C. K., Tilles, H., Environ. Qual. Safety, Suppl. Vol. 3, 675 (1975a).

Casida, J. E., Kimmel, E. C., Ohkawa, H., Ohkawa, R., Pestic. Biochem. Physiol. 5, 1 (1975b).

Fang, S. C., in "Herbicides. Chemistry, Degradation and Mode of Action", 2nd ed, Kearney, P. C., Kaufman, D. D., Ed., Marcel Dekker, New York, N.Y., 1975, p 323.

Gaughan, L. C., Unai, T., Casida, J. E., J. Agric. Food Chem., 25, 9 (1977).

Hoagland, D. R., Arnon, D. I., Calif. Agric. Exp. Stn. Circ., 347 (1938).

Ishikawa, K., Nakamura, Y., Kuwatsuka, S., J. Pestic. Sci. 1, 49 (1976), and references cited therein.

Ishikawa, K., Okuda, I., Kuwatsuka, S., Agric. Biol. Chem. 37, 165 (1973).

Lay, M.-M., Casida, J. E., Pestic. Biochem. Physiol. 6, 442 (1976).

Lay, M.-M., Hubbell, J. P., Casida, J. E., Science 189, 287 (1975).

Ong, V. Y., Fang, S. C., Toxicol. Appl. Pharmacol. 17, 418 (1970).

Shimabukuro, R. H., Environ. Qual. Safety 4, 140 (1975).

Sklan, N. M., Barnsley, E. A., Biochem. J. 107, 217 (1968).

Absorption, Excretion, and Metabolism of Cyolane (Phosfolan) Systemic Insecticide [(Diethoxyphosphinyl)dithioimidocarbonic Acid, Cyclic Ethylene Ester] in the Rat

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When rats were given a single oral dose of [^{14}C]phosfolan (Cyolane, a registered trademark of American Cyanamid Co.) they excreted approximately 50% of the administered radioactivity and respired about 20% as ^{14}CO$_2$ within 144 h. The radioactive residues in the various tissues were found to be distributed throughout the body with lower concentrations residing in fat and muscle. Chromatographic analysis of urinary and tissue radioactivity showed the presence of only one significant metabolite, which was identified as thiocyanate ion.

Cyolane systemic insecticide [phosfolan; (diethoxyphosphinyl)dithioimidocarbonic acid, cyclic ethylene ester] is useful for the control of several cotton, cabbage, and tobacco insects. It is effective against many species of Spodoptera, such as the Egyptian cotton leafworm, S. littoralis. It is effective against leafhoppers, aphids, thrips, mites, whiteflies, lygus bug, leaf miners, cutworms, flea beetles, and alfalfa weevil.

This investigation was initiated to study the excretion, tissue residue behavior, isolation, and identification of major metabolites in excreta and tissues of rats following

a single oral dose of [^{14}C]phosfolan. This information is needed in evaluating the significance of crop residues.

MATERIALS AND METHODS

Radiolabeled Phosfolan. [^{14}C]Phosfolan with the label in the imino carbon position was obtained from New England Nuclear, Boston, Mass. (The asterisk denotes ^{14}C.)
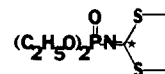
Two-dimensional thin-layer chromatography with chloroform–acetone (9:1) vs. methylene chloride–ethyl acetate (1:1) showed that the compound was 86.6% pure. It was purified by preparative chromatography using 2-mm

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