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.

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

Inder P. Kapoor* and Roger C. Blinn

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.

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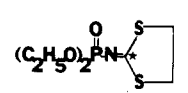
MATERIALS AND METHODS

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

silica gel plates and methylene chloride–ethyl acetate (1:1) as the solvent phase. The phosfolan was extracted with the developing solvent and its specific activity was found to be 48750 dpm/μg with a radiopurity of 99+% as determined by two-dimensional thin-layer chromatography.

Treatment of Animals. Twelve Royal Hart Wistar male rats each weighing 180 to 200 g were given oral dosages of 2 mg/kg of [^{14}C]phosfolan in corn oil. The animals were kept in metabolism cages and maintained by established procedures. The dosage level used in this study was equivalent to 20 ppm of the insecticide in the rats' daily food. Urine and feces were collected from the cages at 24, 48, 72, 96, and 192 h after treatment. At each of these time periods, two rats were sacrificed and blood, liver, kidney, muscle, fat, and gastrointestinal tract were collected. The samples from the two animals of each sacrifice period were pooled and stored in a freezer.

Because only 50.8% of the administered radioactivity was recovered in the excreta during 196 h, another experiment was designed to determine the presence of any volatile radioactivity in the animals' respired gases. Initially, the animals were dosed at the level of 2 mg/kg; however, because of the toxic symptoms displayed by these animals (LD_{50} = 6.7 mg/kg), others were dosed at 1 mg/kg and showed no toxic symptoms. This latter dosage rate was equivalent to 10 ppm of Cyolane in the feed. Immediately after dosing, each rat was placed in a Delmar-Roth metabolism cage (Perkin Elmer Corporation, Coleman Instruments Division, Maywood, Ill.) designed specifically for the purpose of collecting respiration gases and for separately collecting urine and feces. Urine and feces were collected separately for periods up to 192 h. The respiration gases were passed in series through two gas traps each containing 300 ml of ethanolamine. The ethanolamine was monitored periodically by radioassay.

Sample Processing. All tissues and feces samples were individually homogenized twice with 10 ml/g of methanol and once with 1% hydrochloric acid in methanol using a Virtis homogenizer. Fat samples were extracted with benzene and then with methanol. Solid residues (marcs) were air-dried before analysis. Aliquots of urine were diluted with 5 vol of methanol and centrifuged in order to remove inorganic salts and proteins.

Radioassay. Radioactivity recovered in methanol or benzene extracts was determined by mixing a small aliquot (0.5 to 1.0 ml) with Hydromix scintillation cocktail (Yorktown Research, New Hyde Park, N.Y.) and radioassaying with a Searle Analytic Mark III liquid scintillation counter. The counts were corrected for quenching by using the appropriate external standard pulse curve. Radioactivity trapped in the ethanolamine was determined by mixing 3-ml aliquots with 9 ml of methanol and 7 ml of the scintillator fluid (30 g of 2,5-diphenyloxazole and 2 g of *p*-bis(*o*-methylstyryl)benzene dissolved in 2 l. of toluene) used in the Packard Tricarb Model 305 sample oxidizer. This resultant mixture was equivalent to the scintillation solution from the oxidizer. Blood samples were spotted on filter paper and air-dried. These and 200 to 300 mg of solid residues (marcs) were burned in the oxidizer and the resulting trapped [^{14}C]carbon dioxide radioassayed. Scrapings from thin-layer plates were radioassayed directly in a gelled scintillation cocktail consisting of 4 ml of water and 11 ml of Aquasol (New England Nuclear, Boston, Mass.).

Chromatography and Analysis. Thin-layer chromatography was performed using 0.25-mm silica gel plates (E. Merck). Two different solvent systems were used. System I consisted of ethyl acetate–1-propanol–water–formic acid (30:50:15:5) vs. ethyl acetate–propanol–water–ammonium hydroxide (30:50:15:5), and system II consisted of benzene–methanol–formic acid (25:25:1) vs. ethyl alcohol. Either one- or two-dimensional thin-layer chromatography employing any of the above solvent systems was used for purification, isolation, and identification by cochromatography. Methanol extracts of tissues were chromatographed after concentration using a rotary evaporator. Urine samples were either chromatographed directly or partitioned by means of Amberlite XAD-2 resin (Applied Science Laboratories, State College, Pa.) using 4 g of resin per 25 ml of urine, into water-soluble and methanol-extractable fractions.

Radioautography was performed using Kodak SB-54 single-coated x-ray film as per the method of Blinn (1967).

Table I. Excretion of Radioactivity by Rats in Urine and Feces following a Single Oral Dose (2 mg/kg) of [^{14}C]Phosfolan

Sample	Time period, h	% of dose	Cumulative % of dose
Urine	0-24	20.1	20.1
	24-48	4.3	24.4
	48-72	3.2	27.6
	72-96	2.4	30.0
	96-192	2.2	32.2
Feces	0-24	4.6	4.6
	24-48	3.1	7.7
	48-72	2.1	9.8
	72-96	1.6	11.4
	96-192	4.1	15.5
Overall excretion:		Urine	32.2
		Feces	15.5
		Cage wash[a]	3.1
		Total	50.8

[a] Methanol wash of cage surfaces at end of study.

RESULTS AND DISCUSSION

Excretion of Radioactivity. The amount of radioactivity excreted by rats at various time intervals following a single oral dose of [^{14}C]phosfolan at 2 mg/kg, expressed as the percentage of dose, is given in Table I. A total of 50.8% of the administered radioactivity was excreted over a 192-h period; of this 32.2% was excreted in the urine, 15.5% in the feces, and the remaining 3.1% was recovered from cage washings. From these results and taking into consideration the position of the ^{14}C label, it was theorized that volatile radioactivity was being respired by the rat. Therefore, another experiment was undertaken. A rat was dosed with 2 mg/kg of [^{14}C]phosfolan and respired gases passed through ethanolamine and excreta collected for 192 h and a recovery of only 77.5% of the administered radioactivity was achieved. A second rat was similarly treated and its excretion pattern was also found to be similar, with 64.3% of the administered radioactivity excreted after 96 h. Both carcasses were analyzed for residual radioactivity by freezing, mincing in a Hobart food chopper, extracting with methanol, and combusting the air-dried residues (marcs). This resulted in an additional recovery of 13.0 and 23% for the 192- and 96-h studies. An average of 89% recovery of radioactivity was then achieved, as is shown in Table II.

A second study using the lower dose of 1 mg/kg was conducted because of the toxic effects noted at the higher dose. An average recovery of 98% was achieved with this study of 144 h, consisting of about 20% respired as gases trapped by ethanolamine, 48% in the excreta, 1% in the cage wash, and the residual 29% in the carcass. This is in good agreement with the earlier study where rats were dosed at the 2 mg/kg rate.

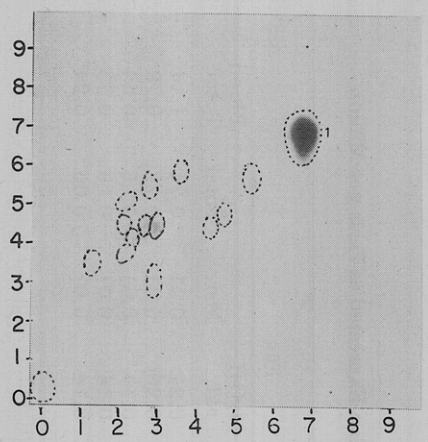


Figure 1. Thin-layer chromatography of radiometabolites in 0–24 h urine of rat following a single oral dose of [^{14}C]phosfolan. Solvent system of ethyl acetate–1-propanol–water–formic acid (30:50:15:5) vs. ethyl acetate–1-propanol–water–ammonium hydroxide (30:50:15:5). 1 indicates the thiocyanate ion.

Table II. Excretion of Radioactivity via Respiration Gases, Urine, and Feces by Rats Treated with a Single Oral Dose of [^{14}C]Phosfolan

	Radioact., % of dose			
	2 mg/kg		1 mg/kg	
	Trial I[a]	Trial II[b]	Trial I[c]	Trial II[c]
Respiration gases[d]	31.4	18.9	18.2	21.3
Urine	28.5	30.6	31.6	51.5[e]
Feces	15.2	13.7	13.3	
Cage wash	2.4	1.1	1.6	0.6
Carcass	13.0	23.4	33.1	24.2
Total	90.5	87.7	97.8	97.6
Average	89.1		97.7	

[a] Trial terminated after 192 h. [b] Trial terminated after 96 h. [c] Trial terminated after 144 h. [d] Trapped in ethanolamine. [e] Urine and feces became mixed and were determined together.

Chromatographic Analysis and Chemical Nature of Excreted Radioactivity. Thin-layer chromatographic analyses of the initial 24-h urine samples were performed using both one- and two-dimensional chromatography. Urine samples were fortified with 250 mg of nonradiolabeled potassium thiocyanate before thin-layer chromatography to avoid loss of radioactive thiocyanate ion by oxidation on the silica gel plate. The chromatographic pattern of metabolites in urine is presented in Figure 1. There are 15 or more metabolites visually present. The percentages of the major metabolites are presented in Table III. Thiocyanate ion constituted 76% of the urinary radioactivity. Two metabolites, which comprised 4 and 3% of the radioactivity, were not identified due to their absence in tissues and to their low levels in urine. The remaining metabolites contributed around 1% or less to the total radioactivity.

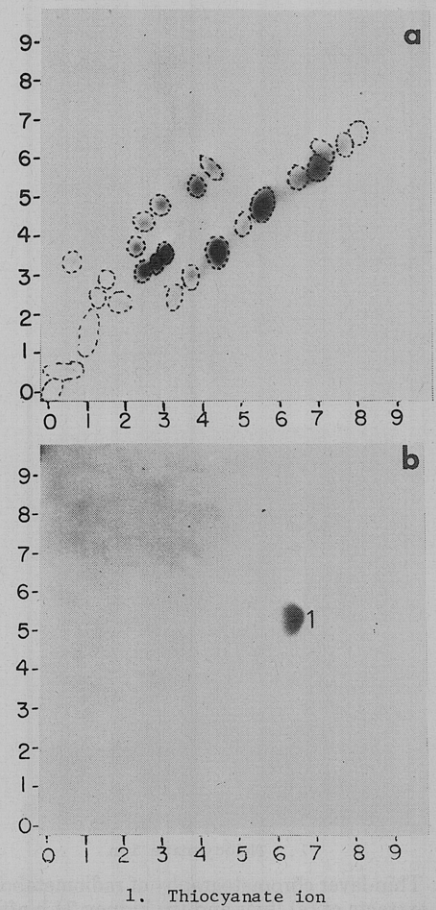
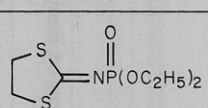


Figure 2. Thin-layer chromatography of radiometabolites in 0–24 h urine following partitioning with Amberlite XAD-2 resin: (a) methanol extract–TLC solvent system of ethyl acetate–1-propanol–water–ammonium hydroxide (30:50:15:5) vs. ethyl acetate–1-propanol–water–formic acid (30:50:15:5). (b) Water phase–TLC solvent system of ethanol vs. benzene–methanol–formic acid (25:25:1).

Upon partitioning of urine with Amberlite XAD-2 resin between methanol- and water-soluble radioactivity, 16% of the radioactivity stayed on the column and was extracted by methanol. Thin-layer chromatography of this methanol extract and of the water-soluble radioactivity revealed the patterns presented in Figure 2. The radioactivity in the methanol extract consisted of 26 or more metabolic products but none contributing more than 3%

Table III. Relative Amounts[a] of Phosfolan Metabolites in Rat Urine and Tissue Extracts

Compound			% in methanol extract			
Name	Structure	% in urine[b]	Liver	Kidney	Muscle	Fat
Phosfolan						
Thiocyanate	SCN^-	76.1	99 (97.9)[c]	99 (98.4)[c]	99 (97.4)[c]	99 (96.3)[c]
Unidentified migrating[d]		21.1[e]				
Unidentified nonmigrating[d]		2.8				

[a] Determined by scraping TLC plates and liquid-scintillation counting. [b] Urine chromatographed directly. [c] Percentages determined by precipitation as AgSCN. [d] Solvent system of ethyl acetate–1-propanol–water–formic acid (30:50:15:5) vs. ethyl acetate–1-propanol–water–ammonium hydroxide (30:50:15:5). [e] Consisted of 13 spots with the major one consisting of 3.8% of the urinary radioactivity.

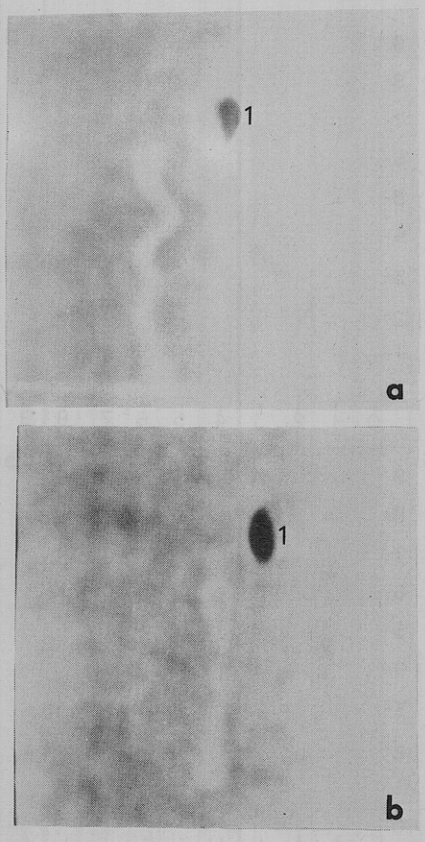


Figure 3. Thin-layer chromatography of radiometabolites in the methanol extracts of (a) liver and (b) kidney 24 h after a single oral dose of [^{14}C]phosfolan. Solvent system of ethanol vs. benzene–methanol–formic acid (25:25:1).

to the total urinary radioactivity. Thin-layer chromatography of the water phase showed only one major spot which cochromatographed with the thiocyanate ion. Confirmation of this finding was provided by precipitation of thiocyanate ion as silver thiocyanate, described by the following equation:

$$\overset{*}{SCN}^- + KSCN + AgNO_3 \dashrightarrow Ag\overset{*}{SCN} + KNO_3$$

where the asterisk denotes ^{14}C.

Thus, the percentage of thiocyanate ion found in the urine by precipitation as silver thiocyanate from the water phase from the XAD-2 resin was found to be 75%, which compares well with the value of 76% obtained when raw urine was thin-layer chromatographed directly and percentages determined by scraping the radiolabeled areas of silica gel for counting.

Residual Radioactivity in Tissues. The residual radioactivity levels in liver, kidney, muscle, fat, and blood, calculated as parts per million of phosfolan, at various time intervals following a single oral dose of 2 mg/kg are given in Table IV. The radioactivity is distributed throughout the body but is lower in concentration in muscle and fat. There is a steady decline in the levels of residual radioactivity throughout the 192 h of the study.

Chromatographic Analysis and Chemical Nature of Tissue Residual Radioactivity. Thin-layer chromatographic analyses of the methanol extracts of liver, kidney, muscle, and fat are presented in Table III. The thin-layer chromatograms of the radiometabolites of liver and kidney extracts are given in Figure 3. Radioautographs of muscle and fat extracts were similar to the other tissues and, therefore, have not been included. There is only one discernible metabolite in all tissues and it was

Table IV. Distribution of Residual Radioactivity in Rat Tissues following Administration of a Single Oral Dose of 2 mg/kg of [^{14}C]Phosfolan Expressed as Parts per Million Equivalent Phosfolan[a]

Time period, h	Liver				Kidney				Muscle			Fat			Blood Total[c]
	MeOH ext.	MeOH/HCl[b] ext.	Marc	Total	MeOH ext.	MeOH/HCl[b] ext.	Marc	Total	MeOH ext.	MeOH/HCl[b] ext.	Marc	Total	Benzene ext.	MeOH ext.	Marc	Total	
0–24	0.97	0.03	0.14	1.14	1.20	0.01	0.15	1.36	0.24	0.01	0.02	0.26	0.02	0.21	0.01	0.24	1.14
24–48	0.57	0.02	0.12	0.71	0.73	0.01	0.14	0.88	0.16		0.02	0.18	0.01	0.20	0.01	0.22	0.79
48–72	0.35	0.01	0.10	0.46	0.40	0.01	0.10	0.51	0.12	0.01	0.03	0.15	0.01	0.14	0.01	0.16	0.55
72–96	0.32	0.01	0.09	0.42	0.43	0.01	0.09	0.53	0.12	*d*	0.03	0.15	0.01	0.14	0.01	0.16	0.53
96–192	0.10	0.01	0.05	0.15	0.18	0.03	0.04	0.25	0.04	*d*	0.02	0.06	0.01	0.14	0.01	0.06	0.24

[a] Calculated on a fresh-weight basis. [b] 1% hydrochloric acid in methanol. [c] Determined directly. [d] Not determined.

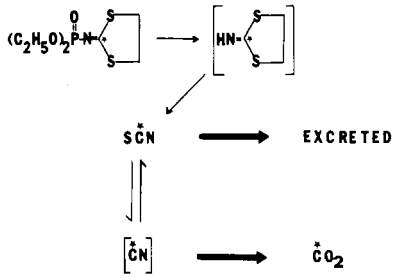


Figure 4. Pathways of metabolism of [^{14}C]phosfolan in the rat.

shown by cochromatography to be thiocyanate ion; it constituted greater than 99% of the extractable tissue radioactivity. Confirmation of this finding was provided by precipitation as silver thiocyanate which gave values of 98, 98, 97, and 96% for liver, kidney, muscle, and fat extracts, respectively.

Thin-layer chromatography of methanol extracts of rat carcass also showed only one metabolite which was identified as thiocyanate ion.

CONCLUSION

The excretory pattern and residue behavior of [^{14}C]-phosfolan administered orally to rats at 1 and 2 mg/kg have been studied. The compound is highly degradable and is excreted from the body as carbon dioxide in the respiration gases and as highly polar ionic metabolites in the urine. The only urinary and tissue metabolite that occurred in significant amounts has been identified as thiocyanate ion. Formation of 2-imino-1,3-dithiolanes as an intermediate step in the formation of thiocyanate has been postulated by Siegel and Rosenblatt (1958), although no salts were actually isolated or identified. Addor (1970) has reported isolation of 2-imino-1,3-dithietane hydrochloride from acid hydrolysis of the related imidocarbonic acid, (diethoxyphosphinyl)dithio-, cyclic methylene ester, which on titration with base yielded thiocyanate and thioformaldehyde polymer. A metabolic pathway of phosfolan in the rat is proposed in Figure 4. The metabolic fate of thiocyanate and cyanide has already been established (Williams, 1959).

ACKNOWLEDGMENT

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COMMUNICATIONS

Varietal Differences in Aroma Essence from Maize Kernels (*Zea mays* L.)

Aroma essences of reproducible composition were isolated from maize kernels (*Zea mays* L.) by steam distillation, extraction of the distillate, and slow evaporation of the extract. Gas chromatography revealed 91 components in the aroma essences. The quantitative composition of the essences differed significantly among five varieties of maize investigated.

The composition of the volatile aroma materials of some vegetable foodstuffs has been shown to be dependent upon plant variety or geographical location, or both, as reported for cranberries (Anjou and von Sydow, 1967), onions (Chua et al., 1968), cocoa beans (Bailey et al., 1962), tea (Bondarovich et al., 1967), coffee, bananas, oranges, tomatoes, potatoes, peppermint oils, spearmint oils, and peanuts (reviewed by Pattee and Singleton, 1972). More recently, cereal grains have been investigated, with identification of aroma compounds from corn (Goeckner, 1958), rice (Yasumatsu et al., 1966), wheat (McWilliams and Mackey, 1969), and triticale (Lorenz and Maga, 1972). Corn samples of "good" and "bad" (musty, sour, "insect") odor have been distinguished by gas chromatography (Dravnieks and Watson, 1973).

We have earlier reported varietal differences in the composition of headspace vapor from maize and wheat (Hougen et al., 1971). The present report similarly indicates that varietal differences can be observed in the composition of aroma essences prepared by steam distillation of maize. The work was part of a study on the quality of maize for use in the alcoholic fermentation industry. Steam distillation was used to simulate the industrial cooking process.

EXPERIMENTAL SECTION

Aroma essences were prepared from maize, essentially as described by Heinz et al. (1966), by steam distillation of ground kernels followed by extraction of the distillate with methylene chloride and slow evaporation of the solvent. About 15 μl of aroma essence was obtained from samples of 1250 g of maize. An internal standard (2,2,-4-trimethylpentane) was added to the essence preparations to ensure that subsequent chromatographic analyses would be independent of the volume of residual solvent in the essence and of the sample volume injected for analysis.

Samples were analyzed with a Varian gas chromatograph Series 1800, with a flame ionization detector and a stainless steel column (4.57 m × 1.8 mm i.d., packed with OV 225 on Chromosorb W AW DMCS, 80–100 mesh, 1:20 by weight). The column oven was held at 60 °C for 20 min, increased 2 °C/min for 10 min, held at 80 °C for 15 min,