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Metabolic Fate of *O*-[4-[(4-Chlorophenyl)thio]Phenyl] *O*-Ethyl *S*-Propyl Phosphorothioate in a Lactating Cow

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The organophosphorus compound O-[4[(4-chlorophenyl)thio]phenyl] O-ethyl S-propyl phosphorothioate (RH-0994 of the Rohm and Haas Co., Spring House, PA) has shown good insecticidal activity against phytophagous pests, particularly Heliothis on cotton. As part of our evaluation of the environmental behavior of this compound, we have previously reported on its fate in cotton (Bull and Ivie 1981), in soil (Bull and Ivie 1982), in boll weevil adults and tobacco budworm larvae (Bull et al. 1983), and in water in the dark (Ivie et al. 1981). We report here the results of studies aimed at defining the fate of [^{14}C] labeled RH-0994 after oral administration to a lactating cow.

MATERIALS AND METHODS

A radiocarbon-labeled preparation of RH-0994 (6.15 mCi/g) was provided by the Rohm and Haas Co. The [^{14}C] label was uniformly incorporated into the O-phenyl ring. Unlabeled samples of RH-0994 and some analogs considered as possible metabolites were also provided by Rohm and Haas. Prior to use, the radiochemical was purified on TLC (solvent system of benzene-ethanol, 99-1) to a radiochemical purity of >99%.

A lactating jersey cow (433 kg) from a local dairy was stanchioned in a metabolism stall and catheterized with a foley retention catheter. The animal was maintained on a twice daily milking schedule and was provided an appropriate dairy ration, with coastal bermuda grass hay and water available ad libitum. After allowing several days to adjust to its new surroundings, the animal was treated orally with a single dose of [^{14}C] RH-0994 at 10 mg RH-0994/kg of body weight (diluted with unlabeled RH-0994 to a specific activity of 420 dpm/µg. After treatment, total milk and urine samples were collected every 12 hours (after 2 days, urine samples were collected at 24-hour intervals), and fecal samples were collected at 24-hour intervals. Total radiocarbon in liquid samples was quantitated by liquid scintillation counting (LSC) of aliquots, that in feces by oxygen combustion and subsequent LSC analysis (Oehler and Ivie 1980). Seven days after treatment, the animal was euthanized and selected tissue samples were taken for quantitation of radiocarbon residues by oxygen combustion.

Samples of urine (10 ml) were acidified to pH ∿2.0 with HCl, then were extracted 5 times with equal volumes of ethyl acetate. Radiocarbon in the aqueous and combined organic fractions was quantitated, and the organic phase was dried over sodium sulfate and the radiocarbon-labeled components were resolved by TLC (vide infra).

Samples of feces (10 g) were extracted 3 times with 20 ml volumes of acetonitrile utilizing Polytron homogenization followed by centrifugation, and the liquid and solid phases were analyzed for radiocarbon content by LSC or oxygen combustion as appropriate. The feces extracts were then concentrated for TLC analysis.

Although only very small quantities of radiocarbon were present in any milk sample, studies were done on the milk collected 1/2 day after treatment in attempts to obtain some information on the nature of the radiocarbon present. Whole milk (125 ml) was acidified to pH <2.0 and then extracted 3 times with 150-ml volumes of ethyl acetate. The combined extracts were dried over sodium sulfate and concentrated to an oil which was partitioned between acetonitrile and hexane. The acetonitrile phase was concentrated by rotary evaporation for TLC analysis.

Samples of liver and kidney (5 g) were extracted 5 times with 15 ml volumes of acetone-acetonitrile (1-1) using Polytron homogenization. The extracts were concentrated, and then partitioned between acetonitrile and hexane--the acetonitrile fraction was subsequently analyzed by TLC. Samples of renal fat (5 g) were extracted 5 times with 15 ml volumes of hexane, cleaned up by partitioning between acetonitrile and hexane, and the acetonitrile phases analyzed by TLC.

TLC studies utilized Brinkmann Silplate F-22, 20 x 20 cm, 0.25 mm gel thickness, with fluorescent indicator. Plates were developed either in 1 or 2 dimensions in 1 or more of the following solvent systems: A) benzene-ethanol-acetic acid, 93-7-1; B) hexane-ethyl acetate-methanol-acetic acid, 20-20-10-1; C) dichloromethane-methanol, 20-1; D) benzene-ether, 5-1; E) hexane-acetone-acetic acid, 50-50-1; F) chloroform-ethanol, 10-1; G) ether-hexane, 3-1; H) benzene-ethyl acetate-acetic acid, 90-9-1; I) benzene-ethanol, 20-1; J) ether-ethyl acetate-hexane, 3-1-1; and K) benzene-ethanol, 99-1. Initital resolutions with all samples were accomplished by 2-dimensional TLC (A x B). Radiolabeled components were visualized by autoradiography (Kodak No-Screen), and unlabeled RH-0994 and its analogs were visualized under short-wave ultraviolet light. For quantitative studies, appropriate gel areas were scraped for direct LSC measurements.

RESULTS AND DISCUSSION

Of the total radiocarbon given the cow, ∿90% was eliminated during the 7-day period after treatment (Figure 1). Most of this (∿67% of dose) was excreted in the urine, whereas ∿22% of the dose was eliminated in the feces. Trace levels of radiocarbon were detected in all milk samples collected, but total secretion of the

administered radiocarbon into the milk amounted to only 0.3% of the dose during the 7 day post-treatment period (Figure 1). Radiocarbon residues in tissues collected 7 days after treatment were highest in liver (2.2 ppm RH-0994 equivalent), kidney (1.8 ppm) and fat (1.4 ppm). With the exception of lung (0.5 ppm) and blood (0.3 ppm), none of the other tissues analyzed contained >0.2 ppm RH-0994 equivalent. These tissues included brain (<0.05), heart (0.1), muscle (<0.05), ovary (0.2), spleen (0.1), tongue (0.2), and udder (0.2 ppm).

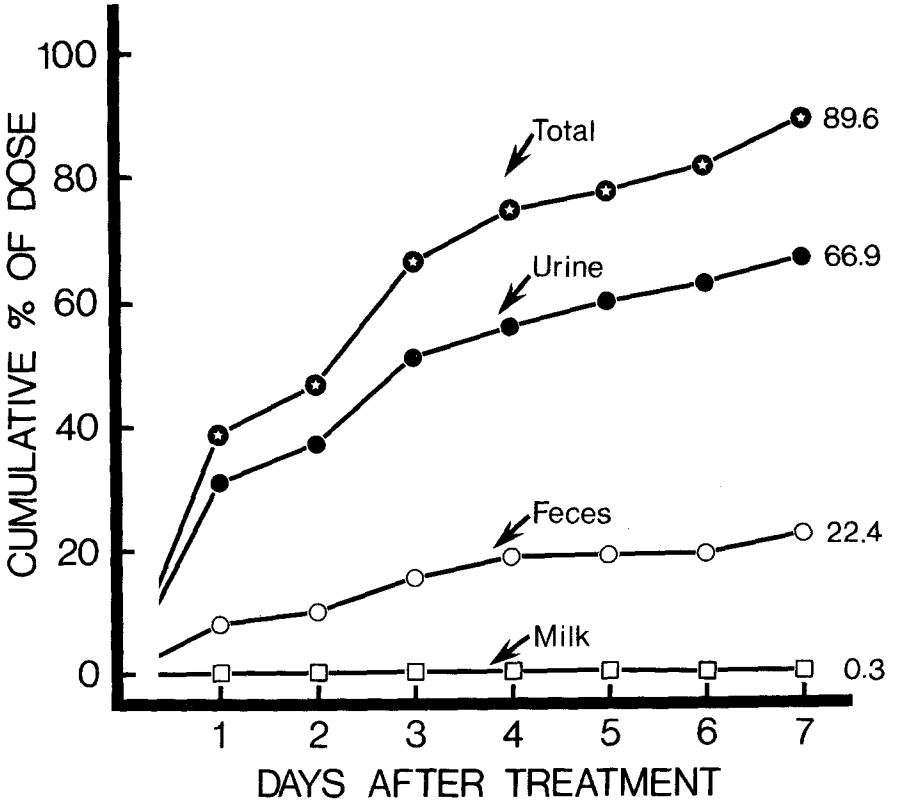

Figure 1. Elimination of radiocarbon after oral treatment of a lactating cow with [^{14}C] RH-0994 at a dosage equivalent to 10 mg/kg body weight.

Two-dimensional TLC analysis (A x B) of the urine extracts resolved the ethyl acetate extractable urinary radiocarbon into several components (Table 1). Three of these were identified by TLC co-chromatography studies in systems A,B, and G-J as RH-0994 phenol, its sulfoxide and sulfone analogs. These 3 phenols collectively comprised from <10% to >50% of the organic extractable radiocarbon in the urine, depending upon the sample (Table 1). In all urine samples, the organic extractable radiocarbon consisted in large part of quite polar compounds that remained at or near the origin on TLC (A,B). On the basis of chromatographic behavior,

Table 1. Metabolites in Urine from a Cow Treated With [^{14}C] RH-0994 as a Single Oral Dose Equivalent to 10.0 mg/kg Body Weight

Sample (Days After Dosing)	Metabolite or Fraction (% of Sample [^{14}C])[a]						
	Phenol[b]	Phenol Sulfoxide[b]	Phenol Sulfone[b]	Unk 1[c]	Unk 2[d]	Unk 3[e]	Water Soluble[f]
1[g]	20.7	19.1	12.1	1.9	1.7	37.8	6.7
2[g]	9.1	3.0	1.1	2.0	0.1	77.8	6.9
3	6.2	2.4	1.5	1.5	0.6	81.8	6.0
4	12.6	3.5	2.6	3.1	1.1	69.5	7.6
5	10.9	3.9	1.5	2.6	0.6	74.3	6.2
6	8.8	2.9	1.7	2.5	0	77.8	6.3
7	5.8	1.9	0.8	1.8	0	85.6	4.1

[a]As resolved by 2-dimensional TLC. [b]Identified on the basis of TLC co-chromatography with standards of known structure (see text). [c]Migrates slightly above the phenol on TLC in solvent system A and about the same as the phenol in solvent system B. [d]Migrates above the origin on TLC (A,B) but below any of the available standards of known structure. [e]Radiocarbon remaining at or streaking from the origin on TLC. [f]Radiocarbon remaining in the aqueous phase after ethyl acetate extraction of whole, acidified urine. [g]Composite whole-day samples obtained by combining appropriate volumes of half-day samples.

metabolite unk 3, the major constituent of all urine samples, appeared to be a rather complex mixture of products rather than a single component. Studies in which whole urine was acidified to 1N HCl, then heated for 30 minutes at 100°C, resulted in little differences in subsequent ethyl acetate partitioning characteristics from unheated, pH 2 urine. Further, TLC analysis of the extracts from acidified, heated urine, gave metabolite distributions very similar to those shown in Table 1. These observations suggest that the more polar metabolites in urine are largely not in the form of acid labile conjugates such as glucuronides or sulfates. The chemical nature of these polar urine metabolites is not known.

Extraction of selected fecal samples with acetonitrile resulted in every case in >80% partitioning of radiocarbon into the organic phase (Table 2). Two-dimensional TLC (A x B) of the fecal extracts resulted in the resolution of several radioactive products, with the major component being identified (A,B) as unmetabolized RH-0994 (Table 2). Small quantities of RH-0994 sulfoxide were also identified (A,B) in fecal extracts, as were appreciable quantities of the phenol and much lesser amounts of the phenol sulfoxide and phenol sulfone (Table 2).

Extraction of acidified whole 1/2 day milk with ethyl acetate and subsequent cleanup of the extract by partitioning between acetonitrile and hexane resulted in 14% as hexane soluble, 77% as acetonitrile soluble, and 9% of the sample radiocarbon not extracted from the aqueous phase. TLC (A x B) resolution and co-chromatography studies with the acetonitrile fraction and long-term exposure (7 months) to X-ray film showed that the major component (81%) in the milk extract was unmetabolized RH-0994. In addition, RH-0994 sulfoxide, sulfone, and the phenol, phenol sulfoxide, and phenol sulfone were also present in small amounts (none exceeding 5% of the radiocarbon in the extract), as confirmed by TLC co-chromatography (A,B) with appropriate standards. Three unidentified metabolites were resolved from the milk extracts which collectively comprised about 8% of the total radiocarbon present.

Extraction of liver and kidney with acetone-acetonitrile (1-1) recovered about 70% of the [^{14}C] in liver and about 74% of that in the kidney. Subsequent partitioning of these samples (concentrated to oily residues) between acetonitrile and hexane resulted in essentially all of the radiocarbon residing in the acetonitrile phase. TLC resolution and co-chromatography (A-F) studies showed that liver extracts contain the phenol sulfoxide (19%), phenol sulfone (34%), and unidentified polar component(s) (47%). Kidney extracts contain the phenol (31%), phenol sulfoxide (5%), phenol sulfone (9%), and unidentified polar product(s) (55%). Extraction of fat samples with hexane recovered >85% of the radiocarbon present and more than 90% of this partitioned into acetonitrile during the cleanup procedure. TLC studies revealed the presence of only 2 [^{14}C] components in the extracts of fat, which were identified on the basis of TLC co-chromatography studies (A-F) as intact RH-0994 (85%) and RH-0994 sulfoxide (15%).

Table 2. Metabolites in Feces of a Lactating Cow Treated With [^{14}C] RH-0994 as a Single Oral Dose Equivalent to 10.0 mg/kg Body Weight

Sample (Days After Dosing)	Metabolite or Fraction (% of Radiocarbon in Sample)[a]						
	RH-0994[b]	RH-0994 Sulfoxide[b]	Phenol[b]	Phenol Sulfoxide[b]	Phenol Sulfone[b]	Unknown[c]	Residue[d]
1	41.8	2.5	31.9	1.9	2.8	7.6	11.5
2	51.4	0.8	32.0	1.6	3.1	8.7	2.4
3	65.6	0.4	23.5	0.6	2.8	3.3	3.8
4	63.9	0.4	15.4	0.2	2.6	1.0	16.5
7	63.9	0.5	24.0	0	0.4	0.9	10.3

[a]As resolved by 2-dimensional TLC. [b]Identified on the basis of TLC co-chromatography studies with authentic standards (see text). [c]Cumulative totals for 1-5 unidentified metabolites, depending upon the sample. [d]Radiocarbon not extracted from the feces residues.

Our studies indicate that RH-0994 is absorbed, metabolized, and excreted after oral administration to a lactating cow. Most of the dose is ultimately eliminated in the urine (Figure 1), indicating that absorption is quite extensive. Only a very small amount of the administered radiocarbon is secreted into the milk. Retention of residues by tissues, to the extent that it occurs, appears to be maximum in liver, kidney, and fat. Unmetabolized RH-0994 and its sulfoxide are the only detected [^{14}C] residues in fat, but in kidney and liver, no intact ester metabolites are detected and only RH-0994 hydrolysis products are identified. The trace levels of radiocarbon secreted into milk consist primarily of unmetabolized RH-0994, but metabolites arising through sulfur oxidation and ester hydrolysis are also secreted into milk.

The elimination curves in Figure 1 indicate that radiocarbon residues, at low levels, continued to be eliminated by the cow at the time it was sacrificed 7 days after treatment. Such an observation might well not reflect the normal disposition of RH-0994 at lower dosages. This is because the animal, although appearing normal for several days prior to treatment, may have been

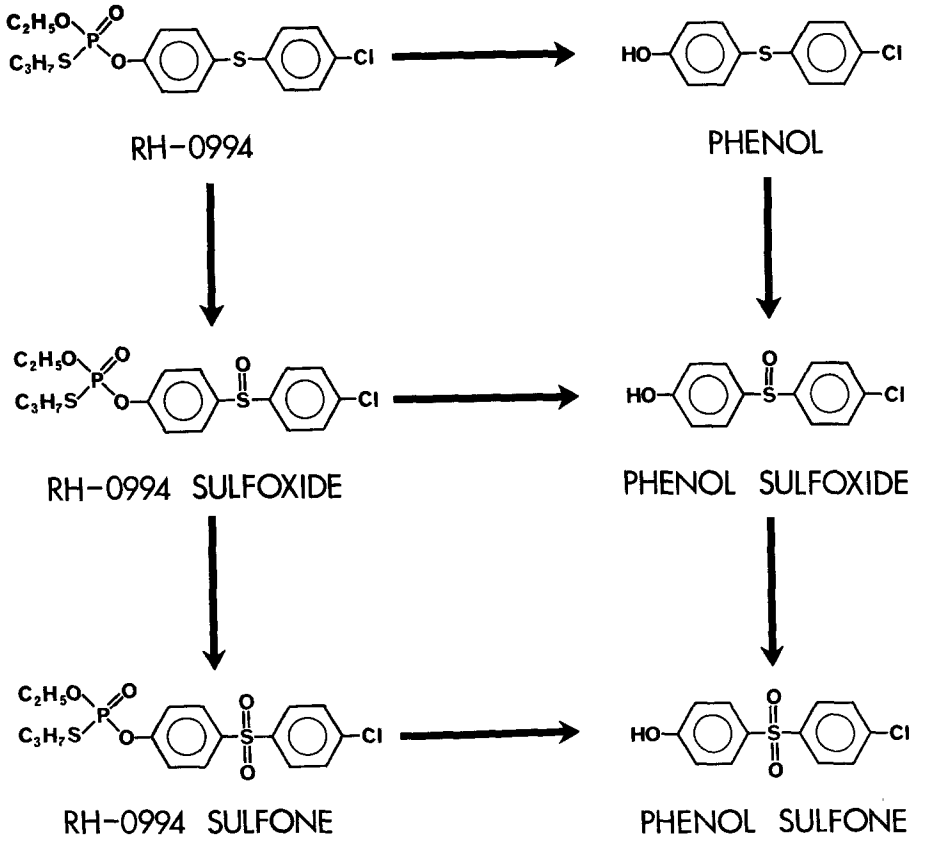

Figure 2. Identified metabolites of RH-0994 in a lactating cow.

affected adversely by the oral 10 mg/kg RH-0994 dose. No signs of organophosphorus poisoning were observed, but the animal experienced a considerable curtailment of feed consumption within 24 hours after dosing, and the cow had not returned to a normal feed consumption pattern by the end of the experiment. Generation of excreta, particularly feces, was considerably diminished, and thus the gastrointestinal tract was probably not "cleared" of the administered dose as rapidly as would have occurred under more normal circumstances. The fact that detectable levels of unmetabolized RH-0994 were seen in feces for as long as 7 days after treatment supports the view that persistence of residues in our study animal was quite possibly increased by rumen or other digestive tract dysfunction.

These studies establish that the biotransformation mechanisms for RH-0994 in the cow include hydrolysis of the phosphorus-O-phenyl ester linkage and oxidation of the sulfide sulfur to sulfoxide and sulfone analogs (Figure 2). The chemical identities of the more polar metabolites observed are not known. Data from tissue and milk analyses suggest that unmetabolized RH-0994 and/or its intact ester oxidation products will occur as appreciable residues only in lipid rich tissues.

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