# Metabolic Fate of *O*-Ethyl *O*-[4-(Methylthio)phenyl-$^{14}C$] *S*-Propyl Phosphorodithioate (BAY NTN 9306) in a Lactating Cow

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A lactating Jersey cow was treated orally with *O*-ethyl *O*-[4-(methylthio)phenyl-$^{14}C$] *S*-propyl phosphorodithioate (BAY NTN 9306) at 0.12 mg/kg as a single oral dose. The administered radiocarbon was essentially quantitatively excreted during a 6-day posttreatment period; about 90% of the dose appeared in urine, about 0.1% in milk, and the rest in feces. Ten days after the first treatment, the animal was treated again with the radiochemical at 0.62 mg/kg. Twelve hours later, when radiocarbon residues in the blood were maximal, the cow was sacrificed, and tissue and digestive tract content samples were analyzed for metabolites. TLC studies indicated that metabolism of this insecticide by the cow involved oxidation of the methylthio sulfur to sulfoxide and sulfone derivatives and hydrolysis of the phosphorus *O*-phenyl ester to give phenolic metabolites that were excreted primarily in the urine in conjugated form. The low residues secreted into milk were also primarily conjugated phenols.

The organic phosphate insecticide *O*-ethyl *O*-[4-(methylthio)phenyl] *S*-propyl phosphorodithioate (BAY NTN 9306) is currently under development for use in controlling lepidopteran insects infesting cotton and other crops. The compound is effective against *Heliothis* spp., insects that have developed high levels of resistance to many other insecticides. Because the compound has potential for widespread use, it is important to determine its effects on and fate in both target and nontarget species. We have studied the metabolic behavior of BAY NTN 9306 in rats (Bull and Ivie, 1976), and the current studies were designed to evaluate the metabolic and residual behavior of this compound after exposure to a lactating ruminant.

## MATERIALS AND METHODS

**Chemicals.** BAY NTN 9306-$^{14}C$ (2.2 mCi/mmol, uniformly labeled in the phenyl ring) was supplied by the Chemagro Agricultural Division, Mobay Chemical Corp., Kansas City, Mo. Radiochemical purity of the compound as determined by thin-layer chromatography (TLC) was >99%. Unlabeled samples of BAY NTN 9306 (hereafter designated 9306) and certain analogs were also made available by Chemagro for use in TLC comparisons with radioactive metabolites. These compounds, and their trivial names used throughout this report, are as previously reported (Bull and Ivie, 1976).

**Thin-Layer Chromatography.** For TLC, precoated silica gel chromatoplates (Silplate-F22, 0.25 mm gel thickness, Brinkman Instruments, Westbury, N.Y.) were used. Metabolites were separated routinely by developing the plates two-dimensionally in solvent systems consisting of (A) 9:4:1 heptane–chloroform–methanol and (C) 2:2:1 hexane–ethyl acetate–methanol. Two additional systems, (B) 6:3:2 chloroform–hexane–glacial acetic acid and (D) 6:3:2 chloroform–hexane–acetone, were also used in cochromatography studies of radioactive metabolites and standards from synthesis. Radioactive regions on the developed plates were visualized by radioautography, and the unlabeled metabolite standards were detected by viewing the plates under short-wavelength ultraviolet light.

**Treatment and Sampling.** A 370 kg lactating Jersey cow producing about 10 kg of milk daily was obtained from a local dairy. The animal was held in a small pen for several days before treatment to allow adjustment to new surroundings and hand milking. A commercial dairy ration was fed at each milking (12-hr intervals), and water and alfalfa hay were available at all times. On the day before treatment, the cow was placed in a metabolism stall and catheterized, and an intravenous catheter was inserted into the juglar vein. For the treatment, the radiochemical was mixed with a small amount of crushed grain, and was administered orally with a balling gun. The first dose (0.3 mCi) was equivalent to 0.12 mg/kg of body weight. After treatment, blood samples were collected periodically, and total milk and urine were taken at 12-hr intervals, except that the samples were also collected 6 hr after dosing. Feces samples were collected at 24-hr intervals. Six days after the first treatment, sample collection was stopped, and the cow was transferred to a small holding pen. Four days later, the animal was stanchioned again and treated as before with 9306-$^{14}C$, except that the second dose (1.6 mCi) was equivalent to 0.62 mg/kg. After this treatment, blood samples were collected at hourly intervals, 1-ml portions were quickly dried (80–100°C), and the samples were analyzed for radiocarbon content by oxygen combustion and liquid scintillation counting (lsc). This procedure permitted quantitation of blood radiocarbon levels within 1 hr after sample collection. Analysis of blood collected from 8 to 11 hr after treatment indicated that radiocarbon residues in the blood had reached their maximum levels, and the cow was sacrificed 12 hr after treatment. Total urine and milk samples were collected at 3, 6, 9, and 12 hr after treatment. The animal was sacrificed at the time of highest radiocarbon residues in blood in order that maximum or near maximum tissue residues would be observed. Immediately after sacrifice, samples of major tissues were collected and frozen as soon as possible at −70°C. Samples of digestive tract contents were also collected and frozen for later analysis.

**Extraction and Analysis.** Aliquots (0.2 ml) of fresh milk and urine were subjected directly to lsc for radiocarbon quantitation. Feces and tissue samples were analyzed by air drying approximately 0.5-g portions and then combusting the samples under 1 atm of oxygen. The combustion gases were bubbled through a solution containing 1 part each of 2-aminoethanol and 2-methoxyethanol; then the trapping solution was subjected to lsc for radiocarbon quantitation.

Depending upon radiocarbon content, 10- to 25-ml

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volumes of urine were extracted four times with equal amounts of ethyl acetate; centrifugation was used to break emulsions where required. The combined organic extracts were dried over sodium sulfate, the solvent was removed under reduced pressure, and the samples were analyzed by TLC. Because of the very low radiocarbon levels observed in milk, only the 9- and 12-hr milk samples obtained after the second treatment were analyzed for metabolites. The entire samples, averaging about 1 l. each, were separated into cream and serum phases. The serum was extracted three times with equal volumes of ethyl acetate, and the organic extracts were combined, dried and concentrated, and then analyzed by TLC. The cream phase was extracted twice with hexane, and the combined hexane extracts were subsequently partitioned with acetonitrile. The acetonitrile fraction was then concentrated for TLC analysis.

Radiocarbon remaining in the aqueous phases of extracted urine samples was analyzed in the following manner. Approximately 5-ml samples of the extracted urine were lyophilized in 50-ml erlenmeyer flasks; then 5 ml of 0.1 *M* sodium acetate buffer (pH 4.5) was added to each flask. When required, the pH of the solutions was readjusted to 4.5 by the addition of dilute HCl. Subsequently, β-glucuronidase–aryl sulfatase (Calbiochem, Los Angeles, Calif.) was added to the flasks to make the total enzyme activity equivalent to 50000 Fishman units and 5000 Whitehead units/flask. The samples were then incubated for 24 hr at 37°C in a shaking water bath incubator. Samples were subsequently extracted with ethyl acetate as before, the two fractions were quantitated by lsc, and the organic fraction was analyzed by TLC.

Radiocarbon not converted to organic extractable compounds after enzyme treatment of the urine water-soluble samples was further analyzed as follows. The samples were adjusted to 1.0 *N* HCl and held for 30 min at 100°C in a boiling water bath, after which the samples were extracted with ethyl acetate and the radiocarbon in this phase was then analyzed by TLC. Radiocarbon residues remaining in the milk serum and cream phases after extraction were studied by subjecting the samples to acid hydrolysis followed by ethyl acetate extraction. Radiocarbon in the organic extracts was then analyzed by TLC. Samples of tissues, feces, and digestive tract contents were analyzed after homogenization and ethyl acetate extraction as described for tissue analysis of rats treated with 9306-$^{14}C$ (Bull and Ivie, 1975).

**Metabolite Characterization.** Radioactive metabolites were identified by comparing their TLC behavior with that of the authentic compounds from synthesis. The metabolites were isolated by scraping the appropriate gel regions from the developed TLC plates and eluting the $^{14}C$-labeled metabolites with acetone. The appropriate standard was added, and the mixture then spotted for TLC analysis. After development, the plates were examined for coincidence of standard and metabolite.

## RESULTS

Analysis of whole blood samples collected after treatment of the cow with 9306-$^{14}C$ indicated that the radioactive compound was not rapidly absorbed by the animal. Treatment at 0.12 mg/kg resulted in maximum residues in venous blood 12–24 hr after treatment, but total $^{14}C$-labeled residues in all samples analyzed were very low, never exceeding 0.005 ppm (Table I). The second, higher level treatment with 9306-$^{14}C$ resulted in proportionately higher blood radiocarbon residues, and the data indicated that maximal blood radiocarbon levels were reached within 9 hr after treatment.

Table I. Radiocarbon Residues in Whole Blood of a Lactating Cow after Oral Treatment with 9306-$^{14}C$ at 0.12 and 0.62 mg/kg[a]

| hr after treatment | ppm of 9306 equiv at indicated treatment level[b] | |
|---|---|---|
| | 0.12 mg/kg | 0.62 mg/kg |
| 1 | <0.002 | 0.002 |
| 2 | 0.002 | 0.005 |
| 4 | 0.002 | 0.020 |
| 6 | 0.002 | 0.028 |
| 9 | 0.003 | 0.036 |
| 12 | 0.005 | 0.030[c] |
| 24 | 0.004 | |
| 36 | 0.002 | |
| 48 | <0.002 | |

[a] Treatment at 0.12 mg/kg followed 10 days later by treatment at 0.62 mg/kg. [b] Lower limit of sensitivity 0.002 ppm. [c] Animal sacrificed 12 hr after treatment.

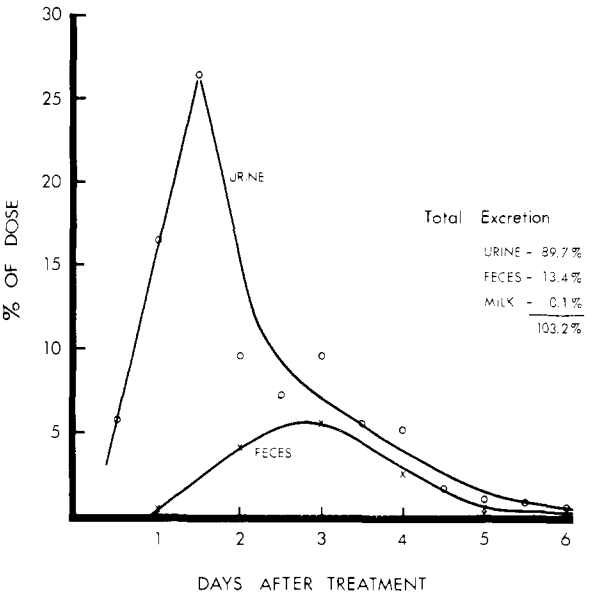


Figure 1. Radiocarbon excretion after oral treatment of a lactating cow with 9306-$^{14}C$ at 0.12 mg/kg.

**Radiocarbon Excretion and Tissue Residues.** After treatment of the cow at 0.12 mg/kg, most of the radiocarbon was excreted through the urine (Figure 1). Almost 90% of the dose was eliminated in the urine during a 6-day posttreatment period, about 13% was voided through the feces, and only about 0.1% was secreted into milk. Total accountability of administered radioactivity was about 103%. At the time of sacrifice 12 hr after the second treatment at 0.62 mg/kg, 17% of the administered dose had been eliminated in the urine.

After each of the 9306-$^{14}C$ treatments, radiocarbon secretion into the milk was very low. Maximum milk residues after the 0.12 mg/kg dose were 0.004 ppm of 9306 equivalents, detected in samples collected after 24 and 36 hr (Table II). Milk residues after the 0.62 mg/kg dose reached levels as high as 0.029 ppm, detected in the sample collected just before sacrifice.

Although the cow was sacrificed at the time in which tissue residues were predicted to be at or near their highest levels, combustion analysis of major tissues revealed that residues were low in all tissues (Table III). Kidney and liver contained the highest amounts of radiocarbon (0.21 and 0.11 ppm equiv, respectively), but all of the remaining samples contained much lower residues.

**Nature of Metabolites.** *Urine.* TLC analysis indicated that at least 18 metabolites were excreted into the urine after the 9306-$^{14}C$ treatments (Table IV). However, in

Table II. Radiocarbon Secretion into Milk of a Lactating Cow after Oral Treatment with 9306-$^{14}C$ at 0.12 and 0.62 mg/kg[a]

| hr after treatment | ppm of 9306 equiv[b] 0.12 mg/kg | ppm of 9306 equiv[b] 0.62 mg/kg | Cumulative % of dose 0.12 mg/kg | Cumulative % of dose 0.62 mg/kg |
|---|---|---|---|---|
| 3 | | <0.003 | | |
| 6 | <0.003 | 0.012 | | 0.01 |
| 9 | | 0.027 | | 0.02 |
| 12 | 0.003 | 0.029[c] | 0.01 | 0.03[c] |
| 24 | 0.004 | | 0.05 | |
| 36 | 0.004 | | 0.09 | |
| 48 | 0.003 | | 0.12 | |
| 60 | <0.003 | | 0.12 | |

[a] Treatment at 0.12 mg/kg followed 10 days later by treatment at 0.62 mg/kg. [b] Lower limit of sensitivity 0.003 ppm. [c] Animal sacrificed 12 hr after treatment.

Table III. Radiocarbon Residues in Tissues of a Lactating Cow 12 hr after Oral Treatment with 9306-$^{14}C$ at 0.62 mg/kg[a]

| Tissue | ppm of 9306 equiv[b] | Tissue | ppm of 9306 equiv[b] |
|---|---|---|---|
| Heart | 0.005 | Ovary | 0.012 |
| Kidney | 0.212 | Skin | 0.010 |
| Liver | 0.109 | Thyroid | 0.006 |
| Lung | 0.028 | Tongue | 0.010 |
| Mammary | 0.013 | | |

[a] Animal treated 10 days earlier with 9306-$^{14}C$ at 0.12 mg/kg. [b] The following tissues contained <0.005 ppm of 9306 equiv; the sensitivity of the combustion method used: brain, fat (omental), fat (subcutaneous), muscle (gracilis), muscle (longissimus dorsi), muscle (triceps), spleen.

all samples analyzed, most of the total radiocarbon was excreted as either free or conjugated phenol sulfide, phenol sulfoxide, or phenol sulfone. Traces of the parent compound were detected in most of the urine samples, but no other metabolites cochromatographed with any of the available standards containing the intact phosphorus–*O*-phenyl bond. In each of the urine samples, products remaining in the aqueous phase after exhaustive ethyl acetate extraction comprised about 90% of the total radiocarbon present. Because these water-soluble products were suspected to be mainly conjugated phenols, this phase was treated with β-glucuronidase–aryl sulfatase as described above. However, the extent of radiocarbon conversion to organic extractable products varied considerably among samples and, in general, the conversion efficiencies were only fair. For these reasons, an experiment was undertaken in an attempt to more effectively hydrolyze the urine water-soluble metabolites.

Results from this study, which involved multiple enzyme hydrolyses and subsequent acid treatment, are summarized in Table V. As the data show, the initial enzyme treatment of water solubles in the sample studied resulted in about 66% hydrolysis to organic extractable products, which were almost exclusively the three phenols. However, subsequent enzyme treatments (in which added enzymes were used) liberated additional radiocarbon, although in considerably reduced quantity. A final acid hydrolysis resulted in almost quantitative conversion of radioactivity into organic-extractable products, again primarily the three phenols. The acid treatment at 100°C did not result in any detectable radiocarbon loss. Results from this study indicated that enzyme hydrolysis alone was not sufficient to allow adequate characterization of water-soluble residues; thus, acid hydrolysis, either alone or after enzyme treatment, was used to study water-soluble metabolites in most of the samples analyzed during the study.

*Feces.* TLC analysis of feces extracts revealed only three products, unmetabolized 9306, 9306 sulfoxide, and phenol sulfoxide (Table VI). Greater than half of the total radiocarbon in feces samples was not extractable from the feces residue or water phase, but the total quantities of radiocarbon in these fractions were quite low, and the chemical nature of these products was not further investigated.

*Milk.* Analysis of milk samples collected 9 and 12 hr after the second 9306-$^{14}C$ treatment indicated that 90–95% of the total milk radiocarbon consisted of the three

Table IV. Radiocarbon Residues in Urine of a Lactating Cow after Oral Treatment with 9306-$^{14}C$ at 0.12 and 0.62 mg/kg[a]

| Product | % of administered dose as indicated metabolite or fraction in each sample at hr[b] 24 | 48 | 72 | 96 | 120 | 144 | Total | 12[c] |
|---|---|---|---|---|---|---|---|---|
| 9306 | <0.1 | <0.1 | <0.1 | <0.1 | 0 | 0 | 0.1 | 0.1 |
| Phenol sulfide, free | 0.6 | 0.9 | 0.2 | 0.1 | <0.1 | <0.1 | 1.8 | 0.9 |
| Conjugate[d] | 3.3 | 5.4 | 2.5 | 1.7 | 0.8 | 0.6 | 14.3 | 1.4 |
| Phenol sulfoxide, free | 0.2 | 0.2 | 0.1 | <0.1 | <0.1 | <0.1 | 0.5 | 0.3 |
| Conjugate[d] | 12.2 | 19.9 | 9.8 | 5.6 | 1.2 | 0.8 | 49.5 | 8.2 |
| Phenol sulfone, free | 0.1 | 0.3 | <0.1 | <0.1 | <0.1 | <0.1 | 0.5 | 0.2 |
| Conjugate[d] | 3.4 | 4.2 | 2.0 | 1.7 | 0.2 | 0.1 | 11.6 | 2.7 |
| Unknown 1[e,f] | <0.1 | <0.1 | <0.1 | <0.1 | 0 | 0 | <0.1 | 0.1 |
| Unknown 2 | <0.1 | <0.1 | <0.1 | <0.1 | 0 | 0 | <0.1 | <0.1 |
| Unknown 3 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 |
| Unknown 4 | 0.5 | 1.2 | 0.2 | 0.2 | 0.1 | <0.1 | 2.2 | 0.4 |
| Unknown 5 | 0.2 | 0.3 | 0.1 | 0.1 | <0.1 | <0.1 | 0.7 | 0.1 |
| Unknown 6 | 0.1 | 0.1 | <0.1 | <0.1 | <0.1 | <0.1 | 0.2 | 0.1 |
| Unknown 7 | <0.1 | 0 | 0 | 0 | <0.1 | 0 | <0.1 | <0.1 |
| Unknown 8 | 0.3 | 0.4 | 0.2 | 0.2 | <0.1 | <0.1 | 1.1 | 0.1 |
| Unknown 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.1 |
| Unknown 10 | 1.1 | 2.1 | 1.0 | 0.6 | 0.1 | 0.1 | 5.0 | 1.3 |
| Water solubles[g] | 0.3 | 1.1 | 0.5 | 0.2 | <0.1 | 0 | 2.1 | 0.5 |

[a] Treatment at 0.12 mg/kg followed 10 days later by treatment at 0.62 mg/kg. [b] For metabolite analysis, aliquots of appropriate half-day samples were combined in proper proportion and analyzed as composite whole-day samples. [c] Composite 0- to 12-hr sample after second treatment. [d] Metabolites converted to organic extractable products during incubation of water-soluble radiocarbon with β-glucuronidase-aryl sulfatase, followed by acid hydrolysis (1.0 *N* HCl, 100°C, 30 min). [e] $R_f$ values of unidentified metabolites in TLC solvent systems A and C, respectively, are as follows: 1, 0.44 and 0.69; 2, 0.06 and 0.59; 3, 0.22 and 0.25; 4, 0.0 and 0.28; 5, 0.0 and 0.16; 6, 0.0 and 0.09; 7, 0.0 and 0.0; 8, 0.38 (system B); 9, 0.59 (system B); 10, 0.0 (system B). [f] Unknowns 1–7 are metabolites extracted directly from urine with ethyl acetate. Unknowns 8–10 are metabolites converted to organic extractable products during enzyme or acid treatment of the urine water solubles. [g] Radiocarbon remaining in the water phase after solvent extraction of the acid-treated samples.

Table V. Hydrolysis of Water-Soluble Radiocarbon in Cow Urine by Sequential Enzyme and Acid Treatments[a]

| Treatment | Water-soluble radiocarbon as indicated metabolite or fraction (cumulative %) | | | | |
|---|---|---|---|---|---|
| | Phenol sulfide | Phenol sulfoxide | Phenol sulfone | Other[b] | Water soluble[c] |
| First enzyme treatment | 10.2 | 32.8 | 22.0 | 1.2 | 33.8[d] |
| Second enzyme treatment | 14.3 | 40.3 | 23.4 | 2.2 | 19.8[e] |
| Third enzyme treatment | 14.9 | 42.3 | 23.5 | 2.5 | 16.8[f] |
| Acid hydrolysis | 17.1 | 50.3 | 25.1 | 5.7 | 1.8 |

[a] 12-hr urine water solubles from cow treated with 9306-$^{14}C$ at 0.62 mg/kg. [b] Unidentified products extracted from water phase. [c] Radiocarbon remaining in water phase after indicated treatment. [d] Radioactive residues in the extracted aqueous phase after the first enzyme treatment were subjected to a second enzyme treatment. [e] Radioactive residues in the extracted aqueous phase after the second enzyme treatment were subjected to a third enzyme treatment. [f] Radioactive residues in the extracted water phase after the third enzyme treatment were subjected to acid hydrolysis (see text for enzyme and acid hydrolysis procedures).

Table VI. Radiocarbon Residues in Feces of a Lactating Cow after Oral Treatment with 9306-$^{14}C$ at 0.12 mg/kg

| Product | % of administered dose as indicated metabolite or fraction in each sample at hr[a] | | | |
|---|---|---|---|---|
| | 48 | 72 | 96 | Total |
| 9306 | 0.5 | 0.7 | 0.4 | 1.6 |
| 9306 sulfoxide | 0.6 | 0.6 | 0.4 | 1.6 |
| Phenol sulfoxide | 0.4 | 0.3 | 0.1 | 0.8 |
| Water soluble | 0.4 | 0.7 | 0.2 | 1.3 |
| Unextractable | 2.2 | 3.2 | 1.2 | 6.6 |

[a] Samples collected 24 and 120–144 hr after treatment collectively contained 1.5% of the administered radiocarbon, but at concentrations insufficient for analysis.

Table VII. Nature of Radiocarbon Residues in Milk of a Lactating Cow after Oral Treatment with 9306-$^{14}C$ at 0.62 mg/kg[a]

| Product | % of radiocarbon present in milk at indicated times, hr | |
|---|---|---|
| | 9 | 12 |
| 9306 | 2.6 | 1.7 |
| 9306 sulfoxide | 0.9 | 0.3 |
| 9306 sulfone | 0.8 | 0.2 |
| Phenol sulfide, free | 0 | 0.4 |
| Conjugate[b] | 7.6 | 11.2 |
| Phenol sulfoxide, free | 1.2 | 1.8 |
| Conjugate[b] | 35.2 | 32.7 |
| Phenol sulfone, free | 0.8 | 1.6 |
| Conjugate[b] | 45.2 | 49.7 |
| Unknown 5[c] | 0.7 | 0.4 |
| Unknown[d] | 0.6 | 0 |
| Unknown[e] | 4.4 | 0 |

[a] Animal treated 10 days earlier with 9306-$^{14}C$ at 0.12 mg/kg. [b] Liberated by acid hydrolysis of the extracted milk serum and extracted cream phases. [c] Unidentified metabolite extractable from milk serum, possibly identical with unknown 5 in urine based on similar chromatographic behavior. [d] Radiocarbon not extractable from the cream phase after acid hydrolysis. [e] Radiocarbon not extractable from the milk serum phase after acid hydrolysis.

phenolic metabolites and that these were primarily in the form of conjugates (Table VII). The parent compound and its sulfoxide and sulfone metabolites were also present in milk but collectively comprised less than 5% of the total milk residues.

*Tissues.* Only kidney and liver samples contained sufficient radiocarbon for metabolite analysis. Most of the $^{14}C$-labeled residues in each of these organs were conjugates of the three phenols, but in liver samples, the parent compound and its sulfone metabolite were also present in low amounts (Table VIII). Traces of the parent compound were also detected in kidney samples.

*Digestive Tract Contents and Bile.* Combustion analysis of the food matter in various regions of the alimentary canal revealed that a large part of the administered radiocarbon had not been absorbed 12 hr after 9306-$^{14}C$ treatment of the cow at 0.62 mg/kg. Radioactive residues, on a parts per million basis, were as follows: rumen (7.4), omasum (5.1), abomasum (7.9), small intestine (0.8), and large intestine (<0.1). Lsc analysis of bile samples collected upon sacrifice of the animal showed residues equivalent to 0.4 ppm of 9306 equivalents. Extraction and TLC analysis of these samples indicated that the parent compound was present throughout the digestive tract, but considerable quantities of other products were observed as well (Table IX). In the rumen, omasum, and abomasum, 9306 and its sulfoxide and sulfone derivatives were the only products identified, but about half of the radioactivity in these samples was not extractable from the particulate matter. Radiocarbon present in the small intestine contents consisted partly of intact 9306 and its sulfoxide metabolite but was primarily conjugated phenols. In the bile, no metabolites were observed which contained the intact phosphorus–*O*-phenyl ester, and the identified products were exclusively phenols in both the free and conjugated states.

Table VIII. Nature of Radiocarbon Residues in Kidney and Liver of a Lactating Cow 12 hr after Oral Treatment with 9306-$^{14}C$ at 0.62 mg/kg[a]

| Product | % of sample radiocarbon as indicated metabolite or fraction | |
|---|---|---|
| | Kidney | Liver |
| 9306 | 0.1 | 6.5 |
| 9306 sulfone | 0 | 1.5 |
| Phenol sulfide, free | 17.6 | 9.8 |
| Conjugate[b] | 31.9 | 14.9 |
| Phenol sulfoxide, free | 1.9 | 6.2 |
| Conjugate[b] | 8.2 | 29.7 |
| Phenol sulfone, free | 8.5 | 7.8 |
| Conjugate[b] | 13.6 | 17.4 |
| Unknown 4[c] | 1.5 | 0 |
| Unknown 7 | 6.1 | 3.1 |
| Unknown 11 | 1.1 | 1.5 |
| Unknown 12 | 0 | 1.6 |
| Unknown 13 | 9.5 | 0 |
| Water residue[d] | 0 | 0 |

[a] Animal treated 10 days earlier with 9306-$^{14}C$ at 0.12 mg/kg. [b] Liberated by acid hydrolysis of the extracted water-residue slurry. [c] Unknowns 4 and 7 are possibly identical with the same numbered unknowns in urine (Table IV). $R_f$ values of other unidentified metabolites in TLC solvent systems A and C, respectively, are as follows: unknown 11, 0.23 and 0.45; unknown 12, 0.26 and 0.50; unknown 13, 0.90 (system B). Because unknown 13 was not consistently observed in acid hydrolysates of kidney samples, it may be an artifact generated by acid degradation of the identified phenols. [d] Radiocarbon not extractable from the water-residue slurry after acid hydrolysis.

During the metabolite isolation and cochromatography studies with products in milk, urine, feces, and tissues, we

Table IX. Nature of Radiocarbon Residues in Digestive Tract Contents and Bile of a Lactating Cow 12 hr after Oral Treatment with 9306-$^{14}C$ at 0.62 mg/kg[a]

| Product | % of sample radiocarbon as indicated metabolite or fraction[b] | | | | |
|---|---|---|---|---|---|
| | Rumen | Omasum | Abomasum | Small intestine | Bile |
| 9306 | 17.9 | 13.5 | 25.1 | 17.6 | 0 |
| 9306 sulfoxide | 36.0 | 36.4 | 13.4 | 8.7 | 0 |
| 9306 sulfone | 0.3 | 0.1 | 0.1 | 0 | 0 |
| Phenol sulfide, free | 0 | 0 | 0 | 0 | 0.2 |
| Conjugate[c] | | | | 1.0 | 0.5 |
| Phenol sulfoxide, free | 0 | 0 | 0 | 8.3 | 4.8 |
| Conjugate[c] | | | | 44.5 | 44.6 |
| Phenol sulfone, free | 0 | 0 | 0 | 1.5 | 2.6 |
| Conjugate[c] | | | | 3.1 | 13.4 |
| Unknown[d] | 0 | 0 | 0.1 | 0.7 | 0 |
| Water soluble[e] | 0.6 | 0.1 | 0.1 | 12.1 | 33.9 |
| Unextractable | 45.2 | 49.9 | 61.2 | 2.5 | 0 |

[a] Animal treated 10 days earlier with 9306-$^{14}C$ at 0.12 mg/kg. [b] Samples of large intestine contents contained insufficient radioactivity for analysis. [c] Liberated during incubation of water-soluble radiocarbon with β-glucuronidase-aryl sulfatase. [d] Radiocarbon remaining at the origin following chromatoplate development. [e] Radiocarbon remaining in the water phase following solvent extraction of the enzyme-treated samples. Water solubles from rumen, omasum, and abomasum samples were not subjected to enzyme treatment due to very low radiocarbon levels.

observed in several instances that certain $^{14}C$-labeled metabolites degraded to one or more additional products. The degradations were, in most cases, to known compounds of which standards were available; thus, the observed decomposition patterns strengthened the identification of the original metabolites.

## DISCUSSION

The metabolic fate of 9306 in the cow was quite similar to that observed in rats (Bull and Ivie, 1975) in that most of the administered radiocarbon was excreted in the urine as conjugated phenolic metabolites. In rats, however, oxidative desulfuration pathways were apparent which were not observed in the cow. Thus, the O-analog of 9306 and the O-analog sulfoxide and sulfone derivatives were seen as intermediate metabolites in the liver of rats treated with high doses of 9306-$^{14}C$ but were not observed in any tissues of the cow. This may have been due to the lower dosage given the cow, but at any rate, these products probably were generated as short-lived intermediates in the metabolism of 9306 by the cow but were not detected because of the extremely low tissue residues observed. Although the major metabolic pathways of 9306 were the same in rats and the cow, metabolism by the cow resulted in both a greater number of uncharacterized metabolites and their occurrence in greater quantity. However, of the total radiocarbon given the cow, more than 80% was recovered in the excreta as identified products. Most of the unidentified metabolites were seen in very minor quantity, and no single uncharacterized metabolite comprised more than 5% of the total dose. Urinary excretion of metabolites in nonconjugated form was considerably greater in the cow (5–10% of the dose) than in rats (only traces).

Absorption from the gastrointestinal tract is apparently the rate-limiting step in the metabolism and excretion of 9306 by ruminants, and radiocarbon absorption was much slower than that observed in rats (Bull and Ivie, 1975). The relatively slow absorption of radiocarbon from the digestive tract of the cow and the fact that residues in blood and tissues were very low indicated that, once absorbed, the compound was rapidly metabolized and excreted.

Analysis of digestive tract contents indicated that 9306-$^{14}C$ had degraded appreciably before absorption into the body. Apparently, less than 25% of the radiocarbon within the digestive tract at the time of sacrifice was in the form of unmetabolized 9306 (Table IX). And at that time, probably 75% or more of the total dose was unabsorbed because cumulative urine excretion amounted to only 17% of dose, and residues in blood and tissues were extremely low. In some cases, 9306 was oxidized to the sulfoxide derivative in generally low amounts during extraction and TLC analysis. Thus, the levels of 9306 and its sulfoxide reported in digestive tract contents (Table IX) and also in milk (Table VII) and in feces (Table VI) may not reflect the true relative concentrations of these two products.

Most of the radiocarbon in the feces after oral treatment of the cow with 9306 was not extractable from the residue-water phase and was not characterized. However, because conjugated phenolic metabolites were excreted through the bile, part of the unidentified feces radiocarbon probably consisted of phenol conjugates. In milk, less than 5% of the total radioactivity present was not characterized. Most of this material was as radiocarbon remaining in the aqueous phase of the 9-hr sample after acid hydrolysis and subsequent solvent extraction (Table VII). This material may well have been residual phenol conjugates not cleaved by the acid treatment because acid hydrolysis of the 12-hr milk sample resulted in quantitative conversion of the radiocarbon into organic extractable products, almost totally phenols (Table VII).

Results from the studies reported here show that 9306 is readily susceptible to metabolic degradation by ruminants and that the major metabolites are likely to be of considerably reduced toxicological significance. Further, 9306 and its metabolites show little tendency toward secretion into milk or toward retention and accumulation within the body.

## ACKNOWLEDGMENT

For invaluable technical assistance during this study, we thank Judy Pennington and Michael Imhoff of the Veterinary Toxicology and Entomology Research Laboratory. The cooperation of D. R. Flint and T. B. Waggoner, Chemagro Agricultural Division, Mobay Chemical Corp., Kansas City, Mo., is gratefully acknowledged.

## LITERATURE CITED

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