Identification of Thiocyanate as the Principal Metabolite of Oxamyl in Lactating Goat

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The metabolic fate of [1-^{14}C]oxamyl in a lactating goat was investigated. The test animal was administered five consecutive daily doses orally at 31 ppm oxamyl dietary burden. Most of the radioactivity was eliminated via urine (45.3%) and feces (7.2%). [^{14}C]Oxamyl equivalents in edible tissues (liver, kidney, muscle, and fat) and in milk accounted for 6.7 and 10.2% of the dose, respectively. A small percentage (1.9%) of the dose was exhaled as volatile metabolites (primarily $^{14}CO_2$). No oxamyl nor any closely related metabolites were detected in tissues, milk, or urine. Extensive degradation/metabolism of [1-^{14}C]oxamyl was observed. Radioactive thiocyanate was the major metabolite identified in the milk as well as in the methanol/water extracts for all tissue samples. Oxamyl-derived residues in the urine have been identified as thiocyanate, *N*-methyloxamic acid, oxamide, and *N*-methyloxamide.

Keywords: *Oxamyl; metabolism; goat; thiocyanate*

INTRODUCTION

Oxamyl [**1**, methyl 2-(dimethylamino)-*N*-[[(methylamino)carbonyl]oxy]-2-oxoethanimidothioate] is the active ingredient in DuPont's VYDATE insecticide/nematicide.

The metabolic fate of oxamyl in a variety of crop plants, as well as in the rat has been reported by Harvey et al. (1978) and by Harvey and Han (1978a). The degradation of oxamyl in water and its decomposition and movement in soil under laboratory and field conditions have also been reported (Harvey and Han, 1978b). Oxamyl was also metabolized rapidly by rumen microorganisms in vitro (Belasco and Harvey, 1980). Essentially all (99%) of the oxamyl had been metabolized within 6 h. The major metabolites after 24 h of incubation were oxime (**2**) and DMCF (**3**). In turn, DMCF was metabolized to *N*,*N*-dimethyloxamide (**4**), *N*,*N*-dimethyloxamic acid (**5**), and *N*-methyloxamic acid (**6**).

The findings reported in this paper describe the recent study of the metabolism of [1-^{14}C]oxamyl in lactating goat which further identified and characterized several metabolites to support the continued registration of oxamyl.

EXPERIMENTAL SECTION

Materials and Method. The test substance [1-^{14}C]oxamyl with radiochemical purity of 97.3% was synthesized by DuPont New England Nuclear Products (Boston, MA). It was adjusted to specific activity of 18.6 μCi/mg with unlabeled analytical grade oxamyl. [^{14}C]Potassium thiocyanate was purchased from Sigma Chemical Co. (St. Louis, MO). Non-radiolabeled authentic standards of oxamyl and potential metabolites (names and structures are listed in Figure 1) were synthesized at DuPont Agricultural Products (Wilmington, DE). All of the solvents used in this study were of HPLC grade or better.

Animal Handling and Dosing. A healthy, nonpregnant goat was dosed orally once per day for five consecutive days. Each gelatin capsule contained 59.3 mg (1103 μCi) of [1-^{14}C]-oxamyl. As such the treated animal was exposed to approximately 31 ppm of test material in the daily diet (average feed consumption 1.9 kg/day).

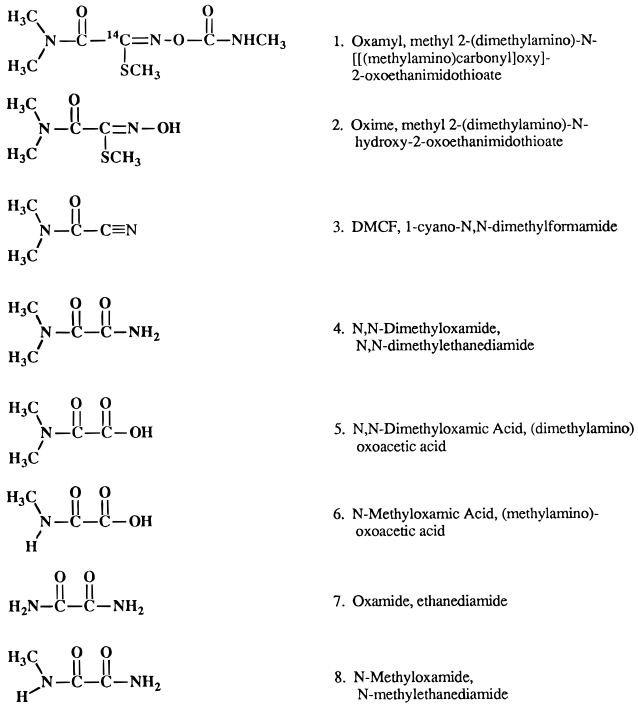

Figure 1. Names and structures of oxamyl and related compounds.

The treated animal was kept in a metabolism cage designed to monitor respired air. Acidic volatiles, such as CO_2, were trapped in a 2 N NaOH solution, and other potential organic volatiles were trapped in MeOH solutions on ice and dry ice. The trapping solutions were changed daily. Urine and feces were collected once daily and milk was collected twice daily. The animal was sacrificed 21 h after the last dosing. Tissue samples collected at necropsy were blood, liver, kidney, fat (omental and renal), muscle (longissimus dorsi, triceps, and gastrocnemius), gallbladder contents, stomach (rumen, omasum, and abomasum) and contents, and small and large intestines and contents.

Chromatography and Radioassay. Radioactivity contained in liquid samples and in sample extracts was quantified

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S0021-8561(96)00548-1 CCC: $14.00

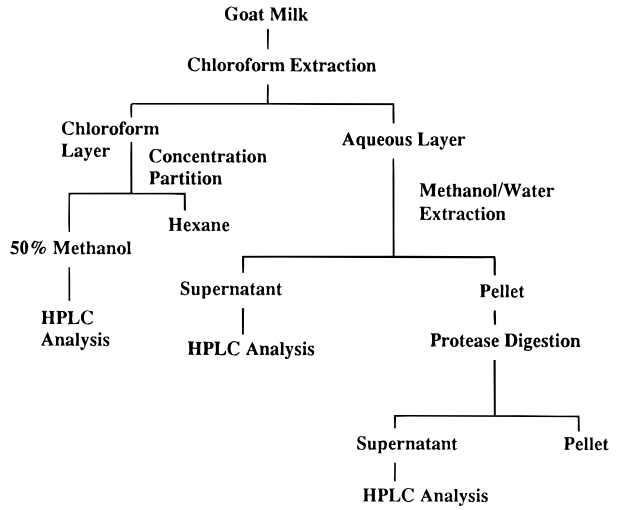

Figure 2. Flow sheet for the extraction and characterization of goat milk.

by direct liquid scintillation counting (LSC) on either a Packard TRI-CARB 2500 (Packard, Downers Grove, IL) or a Tracor MARK III (TM Analytical Inc., Elk Grove, IL) liquid scintillation counting system. Radioactivity contained in solid samples was quantified by oxidative combustion on a Packard Model 306 sample oxidizer (Packard, Downers Grove, IL) followed by LSC.

Extracts and aqueous samples were routinely analyzed by high-performance liquid chromatography (HPLC). HPLC analysis was performed on Waters 600E solvent delivery system with a Waters 996 photodiode array detector and a Raytest RAMONA 92 radiochemical detector. Three HPLC methods were employed. Method 1 used a Hamilton PRP-1 column (7 μm, 7 mm i.d. x 305 mm, Hamilton Co., Reno, NV) with H_2O and CH_3CN as the gradient system at a flow rate of 3.0 mL/min. Method 2 used a porous graphite carbon HYPERCARB column (10 μm, 10.0 mm i.d. x 250 mm, Keystone Scientific, Inc., Bellefonte, PA) with 0.1% trifluoroacetic acid in water and methanol as the gradient system at a flow rate of 3.0 mL/min. For both HPLC methods, a fraction collector was used to collect column effluent (1 min fraction) followed by LSC analysis. HPLC method 1 was used for detecting oxamyl and potential metabolites (see Figure 1). HPLC method 2 was employed for analyzing polar metabolites and fractions. The analysis of lactose was performed on ALLTECH carbohydrate column (10 μm, 4.1 mm i.d. x 300 mm, Alltech Associates, Inc., Deerfield, IL).

Gas chromatographic/mass spectrometric (GC/MS) analyzes of sample extracts and of the corresponding authentic standards after derivatization were accomplished using a Finnigan MAT 4500 quadruple mass spectrometer interfaced with a Finnigan Model 9610 gas chromatograph. Chromatographic separation was achieved using a DB-5 (J & W Scientific) (phenylmethyl)silicone, bonded phase fused-silica capillary column (30 m $\times$ 0.25 mm i.d.) with helium gas. Gas chromatographic separation involved a splitless injection, with an injector temperature of 225 °C and a column oven temperature program of 70 °C for 2 min, then 70−260 °C at 15 °C/min and at 260 °C until acquisition completed. The mass spectrometer was run in EI mode or CI/CH_4 mode using the scan mode of m/z 46−650.

Analysis of Milk. The total radioactive residue in milk was determined by LSC analysis of milk aliquots. Milk (24, 72, and 120 h) samples were extracted and analyzed as illustrated in Figure 2. The unextractable residue was subjected to PRONASE E (type XXV, Sigma, St. Louis, Mo.) digestion in 0.01 N sodium phosphate buffer (pH 7.5) at 37 °C for 20−24 h. HPLC analysis was carried out for the extracts and the released radioactivity using methods 1 and 2. Lactose was analyzed by ALLTECH carbohydrate column.

The identification of thiocyanate was accomplished by GC/MS after derivatization with pentafluorobenzyl bromide as

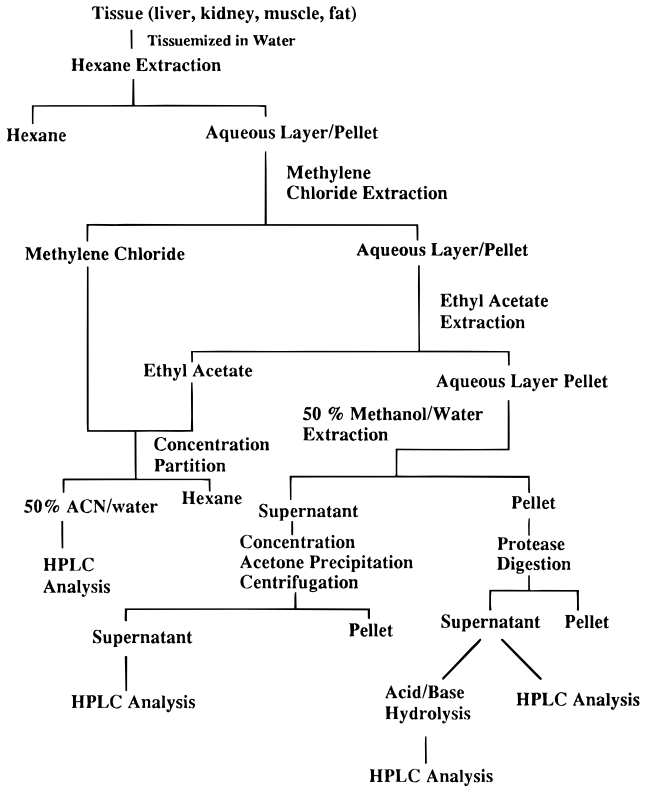

Figure 3. Flow sheet for extraction and characterization of tissue samples.

follows. Milk (120 h methanol/water fraction) was adjusted to pH 8.0 with diluted NaOH solution, and an equal volume of chloroform was added. After the addition of pentafluorobenzyl bromide and 1% tetrabutylammonium bromide, the mixture was stirred vigorously at room temperature for 3 h. The organic layer was separated, and the aqueous layer was extracted with chloroform three times. The organic solvent was evaporated, and the residue was analyzed by GC/MS.

Analysis of Tissues. Tissues (liver, kidney, muscle, and fat) were analyzed as shown in Figure 3. Unextractable residue was digested with PRONASE E as described for milk samples. The released radioactivity was further subjected to acid hydrolysis with 6 N HCl for 16 h. HPLC analysis of extracts and released radioactivity was performed using methods 1 and 2.

Analysis of Urine. Urine (72 h) was filtered and analyzed directly by HPLC methods 1 and 2. Preparative HPLC was carried out to isolate individual metabolites. These purified isolates were dissolved in acetonitrile and stirred with *N*-methyl-*N*-(*tert*-butyl-dimethylsilyl)trifluoroacetamide (TBDMSTFA) containing 1% *tert*-butyldimethylchlorosilane as the catalyst at 60 °C for 1 h. The solution was analyzed by GC/MS. Corresponding reference standards were derivatized using similar procedures and analyzed by GC/MS under the same conditions.

RESULTS

Distribution of [1-^{14}C]Oxamyl Residue. Total radioactive recovery was 80.2% with the majority found in urine (45.3%, urine and cage rinse). Fecal elimination accounted for 7.2% of the dose which was not further analyzed. A small percentage of the dose was exhaled primarily as $^{14}CO_2$ (1.9%). The total radioactivity found in milk accounted for 10.2% of the dose. Tissue (liver, kidney, muscle, and fat) accounted for 6.7% of the dose with the highest level in liver (8.39 μg/g; 1.9% of dose), followed by kidney (4.57 μg/g; 0.2%), muscle (1.29 μg/g; 3.4%), and fat, (0.67 μg/g; 1.2%).

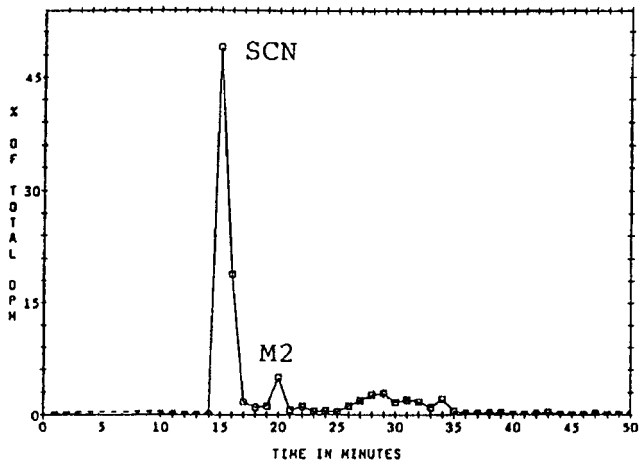

Figure 4. HPLC (HYPERCARB column, method 2) analysis of 120 h goat milk (methanol/water extract).

Table 1. Distribution (%) of ^{14}C-Residue in 24, 72, and 120 h Milk

	24 h	72 h	120 h
chloroform	2.17	1.98	2.13
methanol/water	67.1	67.6	72.5
unextractable	30.7	30.5	25.3

Nature of [1-^{14}C]Oxamyl Residue in Milk. Milk samples (24, 72, and 120 h) were extracted and analyzed as shown in Figure 2. The distribution of radioactivity in different fractions is shown in Table 1. Most of the extractable radioactivity was found in the methanol/water fraction. Oxamyl was not detected in any fractions. The major radioactivity was not retained by HPLC method 1 and could only be retained on a HYPERCARB column (method 2, Figure 4).

The major component in the methanol/water fraction had the same retention time as [^{14}C]potassium thiocyanate. This fraction was reacted with pentafluorobenzyl bromide, and the product was analyzed by GC/MS. The MS spectrum was identical with that of the standard pentafluorobenzyl thiocyanate (PBT). The similarities in retention time and characteristic ions between authentic PBT standard and the derivatized milk sample demonstrated the presence of thiocyanate as the major metabolite in the milk extract (Figure 5).

The unextractable residue of milk was digested with PRONASE E. Most of the radioactivity was released after digestion. HPLC analysis revealed that the major component was also thiocyanate. A small percentage was tentatively found to be lactose. Overall, the major metabolite in goat milk was found to be thiocyanate and the total amount varied from 32 to 49% of total radioactive residue in different day's milk (Table 3).

Nature of [1-^{14}C]Oxamyl in Tissues. Tissues (liver, kidney, muscle, and fat) were extracted sequentially with a series of solvents as shown in Figure 3. The distribution of radioactivity was demonstrated in Table 2. The major extractable radioactivity was found in the methanol/water extract. Oxamyl was not found in any fractions. The chromatographic profiles for different tissues were similar, and the separation was only achieved on a HYPERCARB column (method 2). Thiocyanate was found in all the tissue extracts as one of the major metabolites. The concentration varied from 3 to 31% of total radioactive residues in respective tissues (Table 3).

The unextractable residues of tissues were subjected to PRONASE E digestion. Most of the radioactivity was

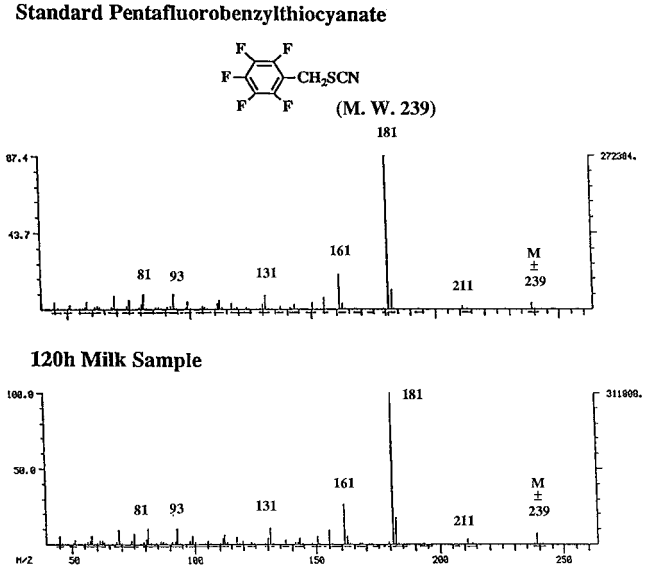

Figure 5. Mass spectra of standard pentafluorobenzyl thiocyanate and milk-derivatized metabolite (120 h milk, methanol/water extract).

Table 2. Distribution (%) of ^{14}C-Residue in Liver, Kidney, Muscle, and Fat

	liver	kidney	muscle	fat
hexane	0.02	0.04	0.08	0.20
methylene chloride	0.21	0.87	0.23	0.86
ethyl acetate	0.45	1.09	1.21	0.74
methanol/water	29.7	36.1	55.6	66.9
unextractable	69.6	61.9	42.9	31.3

Table 3. Percentage of Total Radioactive Residue Accounted for as Thiocyanate in Milk, Urine, and Tissues

sample	% TRR
24h milk	35.4
72 h milk	31.9
120 h milk	48.5
kidney	9.1
muscle	12.6
fat	31.0
liver	2.8
urine (72 h)	2.5

released, and oxamyl was not detected in the released radioactivity. Unlike the milk sample, thiocyanate was not detected in the released radioactivity. The released radioactivity was not retained by HPLC method 1, and further 6 N HCl acid hydrolysis revealed that the final products were different from those generated by oxamyl under the same condition. Further characterization was attempted, but definitive identification was not achieved.

Nature of [1-^{14}C]Oxamyl in Urine. A goat urine sample (72 h) was filtered and analyzed by HPLC directly. Oxamyl was not found in this sample. About 85% of the radioactivity was unretained in method 1. The polar fraction was further analyzed by HPLC method 2, and at least 11 fractions were detected. Five major fractions were further purified and identification was attempted. The other fractions were all below 3% of total radioactive residues in urine and were not further identified.

Thiocyanate was also detected as one of the polar components (2.5%) of urine. Other urinary metabolites identified were oxamide (**7**, 10.2%), *N*-methyloxamic acid (**6**, 13.0%), and *N*-methyloxamide (**8**, 5.4%). The identification was achieved by GC/MS after derivatization of purified isolates with TBDMSTFA in the pres-

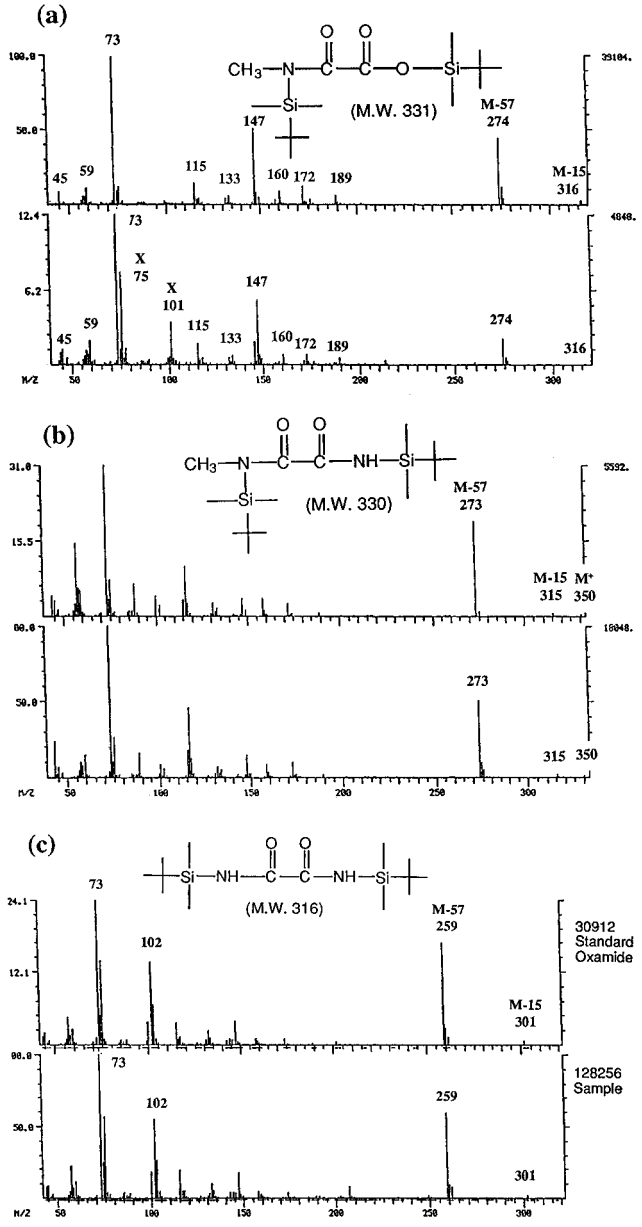

Figure 6. Mass spectra of TBDMS derivatives (a) *N*-methyloxamic acid standard (top) and urine metabolite (bottom), (b) *N*-methyloxamide standard (top) and urine metabolite (bottom), and (c) oxamide standard (top) and urine metabolite (bottom).

ence of trimethyldichlorosilane. The corresponding standards were derivatized in the same manner and showed the identical mass spectra (Figure 6). The other major fractions were not very stable, and attempts to identify these urine metabolites were unsuccessful.

DISCUSSION

The metabolism of oxamyl in the goat rapidly produces polar, readily eliminated molecules, such as carbon dioxide, thiocyanate, *N*-methyloxamic acid, *N*-methyloxamide, and oxamide. The proposed metabolic pathway of oxamyl in the lactating goat is presented in Figure 7. This pathway is consistent with those reported previously for in vitro rumen microorganisms (Belasco and Harvey, 1980), rat liver microsomes, and in vivo rat experiments (Harvey and Han, 1978a). Polar radioactive compounds, which could not be identified, were reported in all previously published oxamyl

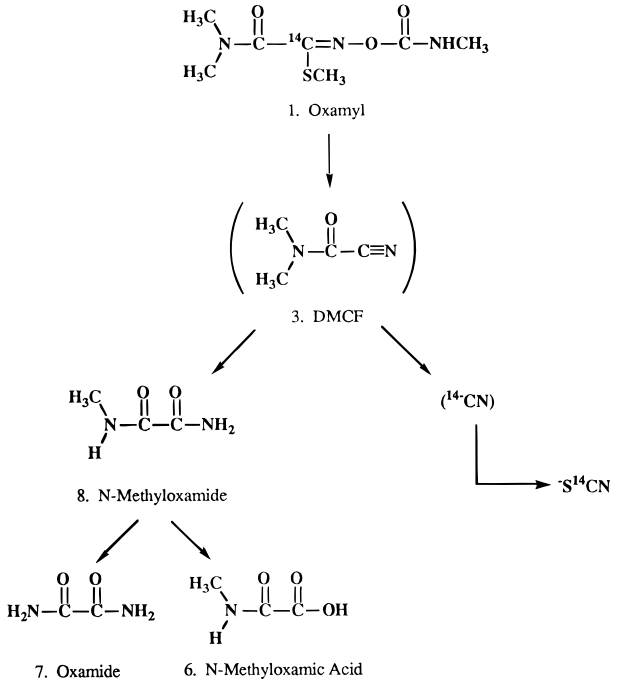

Figure 7. Proposed metabolic pathway in goat.

metabolism studies. An unpublished goat metabolism study confirmed that the major radioactive component in milk was polar and readily lost from acidic solutions, but the metabolite could not be identified. The successful identification of several new metabolites in our work was accomplished through the use of analytical techniques capable of isolating and identifying polar metabolites. The HYPERCARB column packed with graphitized carbon made possible the separation of these polar metabolites, including thiocyanate, which were not retained on typical reversed phase columns.

The generation of thiocyanate from cyanide through the reaction with thiosulfate catalyzed by the enzyme rhodanese has been well documented in the literature (Solomonson, 1981). Studies show that acetonitrile as well as many other alkyl or arylnitriles are metabolized quickly in the rat to give cyanide, which is excreted as thiocyanate (Silver et al., 1982). As shown in previous studies, DMCF is an intermediate in the metabolism of oxamyl, and we propose that DMCF is further degraded to give thiocyanate through enzymatic reactions which result in formation of thiocyanate from other nitriles.

Cyanide is a common metabolite which can be formed from a variety of precursors that are widely distributed in nature. Vegetables such as cassava, lima beans, sorghum, linseed, kernels of fruits, sweet potatoes, maize, millet, and bamboo shoots contain cyanogenic glycosides (Solomonson, 1981). The ability to detoxify cyanides through formation of thiocyanate has been observed in several mammalian species, and we expect that the metabolism of oxamyl in other animal species will result in thiocyanate as a principal metabolite. A recent unpublished poultry metabolism study (Scott, 1994) has shown that thiocyanate was the major metabolite in eggs and tissues.

The detoxification of oxamyl appears to be a facile process resulting in products that are readily eliminated. Toxic metabolites of oxamyl, such as radiolabeled cyanide, were not observed in this study. There were no apparent toxic effects on the treated goat as determined by clinical observations and by measure-

ment of body weight, food consumption, and milk production.

Previous goat metabolism studies demonstrated that some radioactivity was incorporated into natural products, such as lactose in milk and protein in milk and tissues. In this study, the finding of radiolabeled thiocyanate, CO_2 in expired air, and other small molecular weight compounds demonstrated the possibilities of incorporation of radioactive residues into natural products. The small amount of radioactivity found in lactose is consistent with previous findings and supports our contention that the radiolabeled carbon will be incorporated into natural products. Identification of a series of oxamyl degradation products in urine agrees well with the finding of radioactive thiocyanate and elucidates the oxamyl metabolic pathway in the goat. The lack of oxamyl detected in the milk and tissues and the multitude of metabolic products recovered in the urine support the conclusion that oxamyl residues do not accumulate and are extensively metabolized in food-producing animals.

ACKNOWLEDGMENT

We thank D. L. Ryan for many helpful discussions regarding the project and W. Zimmerman for synthesizing the standard references. The technical assistance of K. Watson and W. Stigler was greatly appreciated.

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Received for review July 22, 1996. Accepted December 3, 1996.[⊗]

JF960548I

[⊗] Abstract published in *Advance ACS Abstracts,* February 1, 1997.