Metabolism of p-Chlorophenyl N-Methylcarbamate in the Rat and Goat

Gaylord D. Paulson,* Mary V. Zehr, Margo M. Dockter, and Richard G. Zaylskie[1]

Rats and milking goats were given a single dose of either p-chlorophenyl-^{14}C(U) N-methylcarbamate (ring-label) or p-chlorophenyl N-methylcarbamate-^{14}C (carbonyl-label). Expiratory gases contained carbon-14 after the carbonyl-labeled compound was given but not after the ring-labeled compound was given. Most of the carbon-14 administered as the ring-labeled compound was excreted in the urine, with only trace amounts in the feces. Milk from the goat, as well as tissues from the rat and goat, contained trace levels of carbon-14 48 hr after dosing with the ring-labeled compound. Goat urine contained p-chlorophenol (A), p-chlorophenyl glucuronide (B), p-chlorophenyl sulfate (C), 4-chlorocatechyl 1-sulfate (D), and three minor unidentified ^{14}C-labeled metabolites. Goat milk collected 6 hr after dosing contained C and D. Metabolites A, B, and C were present in rat urine.

It has been reported that p-chlorophenyl N-methylcarbamate is a weak acetylcholinesterase inhibitor (Kolbezen *et al.*, 1954) and has some herbicidal activity (Herrett and Berthold, 1965). More recent reports indicate that p-chlorophenyl N-methylcarbamate prolongs the herbicidal activity of chloropropham [isopropyl N-(3-chlorophenyl)-carbamate] for dodder control (Dawson, 1969) and is a competitive inhibitor of hydrolytic enzyme systems which degrade a variety of pesticidal compounds (Technical Service Bulletin 105-F-1, PPG Industries, 1970). Thus p-chlorophenyl N-methylcarbamate may have potential as a synergist in pesticide formulations. Therefore, knowledge of the metabolic fate of this compound in animals is essential. The metabolism of this compound in the chicken has been reported previously (Paulson and Zehr, 1971). This study was initiated to determine the metabolic fate of p-chlorophenyl N-methylcarbamate in the rat and goat.

EXPERIMENTAL

Chemicals and Supplies. The p-chlorophenyl N-methylcarbamate, p-chlorophenyl-^{14}C(U) N-methylcarbamate, and p-chlorophenyl N-methylcarbamate-^{14}C were supplied by PPG Industries, Inc. The radioactive chemicals were shown to be greater than 99% pure, as previously described (Paulson and Zehr, 1971). The sources of other chemicals and supplies were as follows: $H_2{}^{35}SO_4$, New England Nuclear Corp.; 4-chlorocatechol, Pfaltz and Bauer, Inc.; dicyclohexylcarbodiimide, Eastman Organic Chemicals; dimethylformamide (DMF), Fisher Scientific Co.; Bio-Gel P-2 (100–200 mesh) and Cellex-GE, Bio-Rad Laboratories; polyethylene glycol (average molecular weight, 380–420), Matheson Coleman and Bell. The sources of other chemicals and supplies were as previously described (Paulson and Zehr, 1971).

Synthesis and Characterization of Chemicals. Potassium p-chlorophenyl sulfate, p-chlorophenyl glucuronide, p-chlorophenol-^{14}C(U), and p-chlorophenyl-^{14}C(U)-2-cyclopentyl acetate were synthesized as previously described (Paulson and Zehr, 1971). The method of Sullivan *et al.* (1967) was used to synthesize p-chlorophenyl N-acetyl N-methylcarbamate an 4-chloro-1,2-diacetoxybenzene.

Dipotassium 4-chlorocatechyl disulfate was prepared by the method of Feigenbaum and Neuberg (1941). The method of Hoiberg and Mumma (1969) was modified slightly to prepare a mixture of dipotassium 4-chlorocatechyl disulfate, potassium 4-chlorocatechyl-1-sulfate, and potassium 4-chlorocatechyl-2-sulfate. Sixteen milliliters of DMF containing 9.6 g of dicyclohexylcarbodiimide was cooled on ice; then 1.4 g of 4-chlorocatechol in 12 ml of DMF was added. Two grams of $H_2{}^{35}SO_4$ in 12 ml of DMF was added and the solution stirred for 20 min. The precipitate which formed was removed by filtration, and the supernatant fluid was diluted to 100 ml with H_2O, neutralized with 1 *N* KOH, and then extracted with 150 ml of N-butyl alcohol. The alcohol phase was evaporated to dryness; the residue which remained was dissolved in a small amount of water and the ^{35}S-labeled products were purified by column chromatography, as outlined in Figure 1. Techniques for the preparation of chromatographic columns, elution of columns, monitoring of radioactivity, and collection of fractions are described in a later section dealing with purification of metabolites. The percentage of total radioactivity accounted for by fractions I, II, III, and IV was approximately 16, 62, 7, and 15%, respectively. Fractions I, II, III, and IV were all highly polar, contained ^{35}sulfur, and inspection of their infrared spectra indicated the presence of a sulfate ester (strong absorption bands at 1000 to 1650 cm^{-1} and 1200 to 1300 cm^{-1}). When acetylated (Paulson and Portnoy, 1970), all of the fractions were converted to 4-chloro-1,2-diacetoxybenzene; the diacetoxy derivative was identified by comparison of its infrared and mass spectra with those of an authentic sample. Fraction I was not characterized further; however, it seems likely that it was a pyrosulfate diester (Hoiberg and Mumma, 1969). Fraction II was identified as dipotassium 4-chlorocatechyl disulfate by comparison of its infrared spectrum with that of the compound synthesized by the method of Feigenbaum and Neuberg (1941). The aromatic region of the nmr spectra of fractions II, III, and IV and the parent catechol are shown in Figure 2. The proton assignments were made by examination of peak splitting. Since hydrogens 3 and 5 were meta to each other, their coupling was not as strong as the coupling between hydrogens 5 and 6. In this case, the meta coupling appeared partially as line broadening. Sulfate next to a hydrogen caused a strong downfield shift of the resonance (compare nmr of fraction II,

United States Department of Agriculture, Animal Science Research Division, ARS, Metabolism and Radiation Research Laboratory, Fargo, North Dakota 58102.

[1] Entomology Research Division, ARS, Metabolism and Radiation Research Laboratory, Fargo, North Dakota 58102.

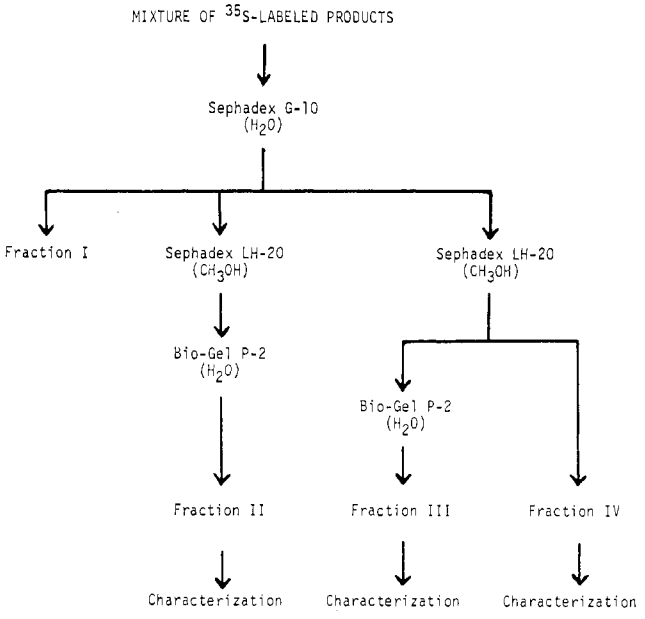

Figure 1. Separation and purification of ^{35}S-labeled 4-chlorocatechol derivatives

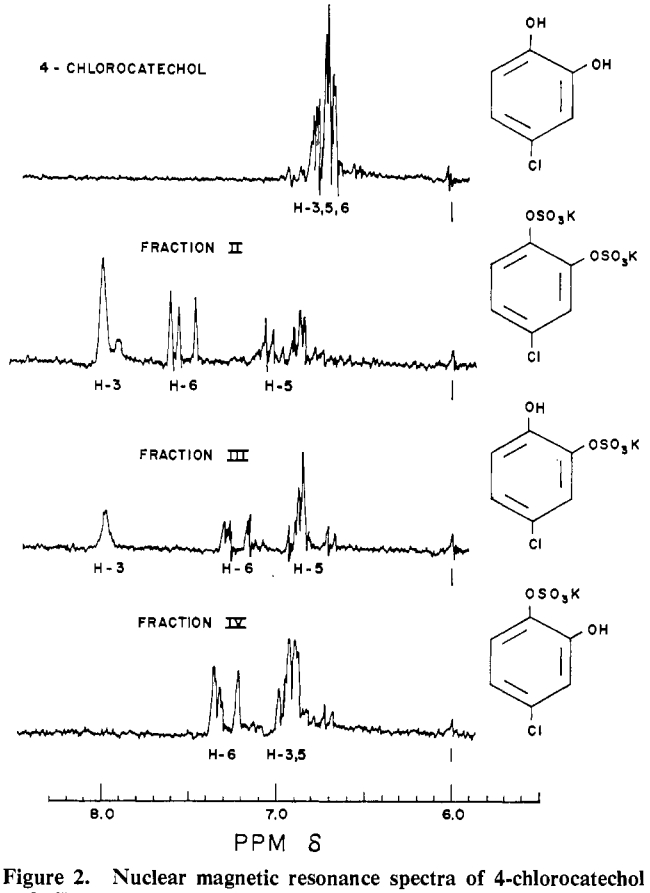

Figure 2. Nuclear magnetic resonance spectra of 4-chlorocatechol and ^{35}S-labeled 4-chlorocatechol derivatives. The spectra were taken in d_6-DMSO with D_2O added. The positions were determined as δ ppm, and were calibrated with a side band of TMS at δ 6.00

dipotassium 4-chlorocatechyl disulfate, with that of 4-chlorocatechol). The strong downfield shift shown by H-3 in the spectrum of fraction III indicated that the sulfate was on position 2. Hydrogen 6 in the spectrum of fraction IV showed the strongest downfield shift; hence, the structure potassium 4-chlorocatechyl-1-sulfate was assigned to that fraction.

Treatment of Animals. Three male and three female rats (average weight, 187 g) were given a single oral dose of *p*-chlorophenyl-^{14}C(U) *N*-methylcarbamate (ring-label) and three male and three female rats (average weight, 246 g) were given *p*-chlorophenyl *N*-methylcarbamate-^{14}C (carbonyl-label). The dose was dissolved in polyethylene glycol (average molecular weight, 380–420) and was administered by stomach tube. Smyth *et al.* (1955) reported that polyethylene glycols (PEG) may be considered inert when given orally to rats; Corbett *et al.* (1958) found no evidence for absorption of PEG from the alimentary tract of ruminants. The dose contained from 4 to 6 μCi of carbon-14 and was made to supply 10 mg of *p*-chlorophenyl *N*-methylcarbamate per kilogram of body weight by the addition of an appropriate amount of the unlabeled compound. Water and feed (Purina Laboratory Chow) were provided *ad libitum*. Feces, urine, and expiratory gases were collected (Bakke *et al.*, 1967) at 6, 24, and 48 hr after the dose was given; the rats were sacrificed 48 hr after dosing and tissues were removed.

A lactating goat (weight 40 kg) was given a single dose of *p*-chlorophenyl-^{14}C(U) *N*-methylcarbamate. The dose was dissolved in polyethylene glycol 400 and was given by rumen puncture. The dose contained 93.3 μCi of carbon-14 and was made to supply 10 mg of *p*-chlorophenyl *N*-methylcarbamate per kilogram of body weight by the addition of an appropriate amount of the unlabeled compound. Water, alfalfa hay, and oats were provided *ad libitum*. Feces, urine (*via* indwelling bladder catheter), milk, and expiratory gases were collected (Robbins and Bakke, 1967) at 6, 24, and 48 hr after the dose was given; the goat was sacrificed at 48 hr and tissues were removed.

A second lactating goat (weight 45 kg) was treated with a single dose of *p*-chlorophenyl-^{14}C(U) *N*-methylcarbamate (100 mg per kg of body weight, 143 μCi of carbon-14). Milk was collected 6 hr after dosing.

Previously described techniques were used to measure the carbon-14 in the expiratory gases, urine, feces, and tissues of the rat (Paulson and Feil, 1969) and goat (Robbins and Bakke, 1967).

Purification of Metabolites. The urine, collected from 0 to 6 hr after dosing, was extracted three times with an equal volume of diethyl ether. The ether-soluble metabolite was derivatized with 2-cyclopentylacetyl chloride, purified, and characterized as previously described (Paulson and Zehr, 1971). After ether extraction, the radio-labeled metabolites in the aqueous phase were separated and purified by a series of column chromatographic procedures outlined in Figure 3.

The milk collected 6 hr after dosing the goat (100 mg per kg of body weight) was freeze-dried; the milk solids were then extracted three times with methanol (total volume 4000 ml). The methanol extracts were combined and concentrated and then the radio-labeled metabolites were isolated by column chromatography as shown in Figure 4.

The preparation and use of Sephadex G-10 and Sephadex LH-20 columns, as well as the radioactivity monitoring and fraction collection systems, were as previously described (Paulson and Zehr, 1971). The 1- × 60-cm Cellex GE column was poured in water and the radio-labeled metabolites were eluted with a KBr gradient (100 ml of H_2O in chamber 1 and 100 ml of 0.1 *M* KBr in chambers 2 and 3; flow rate approximately 0.2 ml per minute). The 1- × 60-cm Bio-Gel P-2 column was poured in water, and the radio-labeled metabolites were eluted with water (flow rate approximately 0.2 ml per min). The column effluent containing the purified metabolites was concentrated to a small volume on a flash evaporator for characterization studies.

Table I. Cumulative Elimination of Carbon-14 in the Feces, Urine, and Expiratory Gases from the Rat After a Single Oral Dose of Radio-Labeled p-Chlorophenyl N-Methylcarbamate[a]

Time, hr	p-Chlorophenyl-^{14}C(U) N-methylcarbamate			p-Chlorophenyl N-methylcarbamate-^{14}C		
	Feces, % dose	Urine, % dose	Expiratory gases, % dose	Feces, % dose	Urine, % dose	Expiratory gases, % dose
6	1.05 ± 0.49[b]	70.71 ± 3.90	ND[c]	0.06 ± 0.05	6.14 ± 0.41	64.42 ± 2.57
24	1.77 ± 0.53	88.12 ± 5.06	ND	1.79 ± 0.52	11.23 ± 0.77	77.40 ± 2.07
48	2.39 ± 0.69	93.30 ± 1.42	ND	2.47 ± 0.56	12.46 ± 0.86	78.29 ± 2.04

[a] Dose, 10 mg per kg of body weight. [b] Mean ± standard error. [c] None detected.

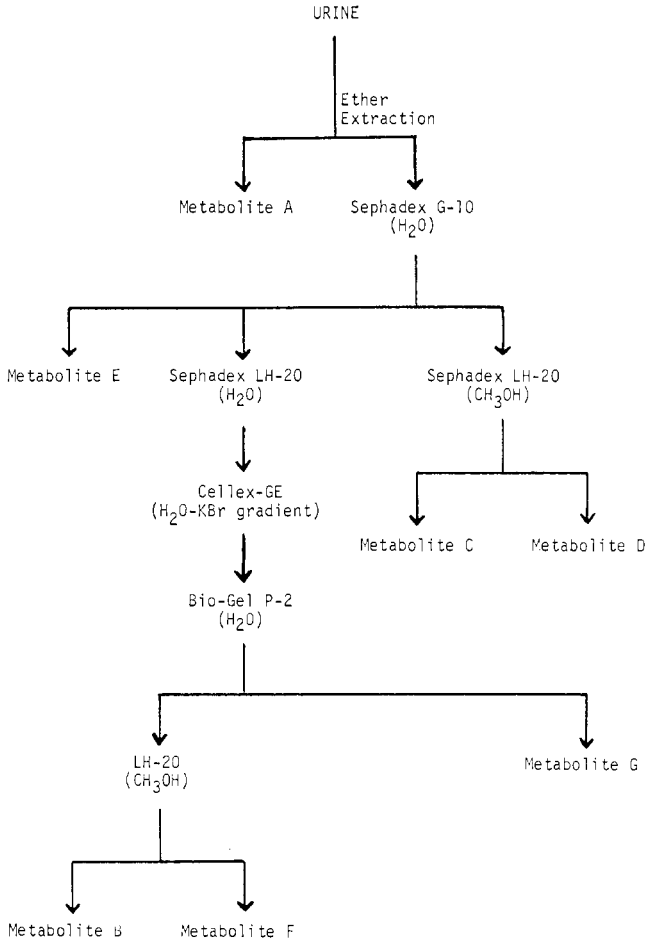

Figure 3. Separation and purification of metabolites of p-chlorophenyl N-methylcarbamate in urine

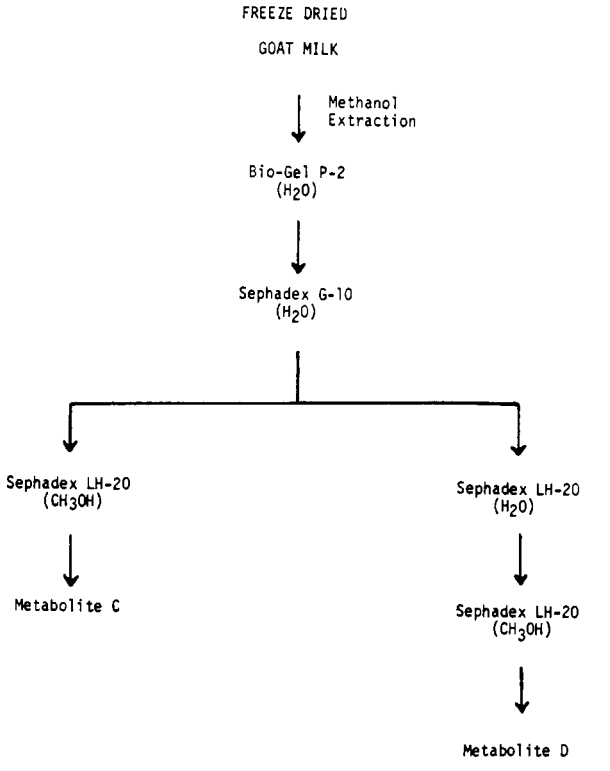

Figure 4. Separation and purification of metabolites of p-chlorophenyl N-methylcarbamate in goat milk

Gas–liquid chromatography (glc) of derivatized metabolites and synthetic products was performed with a Barber-Colman series 5000 gas chromatograph as previously described (Paulson and Zehr, 1971; Paulson *et al.*, 1970).

Hydrolysis of Metabolites. Enzymatic hydrolysis of metabolites was performed with a mixture of β-glucuronidase and aryl sulfatase (Paulson and Zehr, 1971). Direct acetylation of metabolites and synthetic products was carried out as previously described (Paulson and Portnoy, 1970).

Instrumental Analysis. Infrared spectra were taken with a model 337 Perkin-Elmer grating infrared spectrometer, using the micro KBr technique (1.5 mm disk with a 4× beam condenser). Mass spectra were taken with a Varian M-66 mass spectrometer equipped with a V5500 interface control console. Nuclear magnetic resonance spectra were taken with a Varian A-60A nmr spectrometer.

Table II. Cumulative Elimination of Carbon-14 in the Feces, Urine, and Milk from the Goat after a Single Intraruminal Dose[a] of p-Chlorophenyl-^{14}C(U) N-Methylcarbamate

Time, hr	Urine, % dose	Feces, % dose	Milk, % dose
6	56.25	0.002	0.026
24	97.82	0.022	0.038
48	98.00	0.107	0.047

[a] Ten milligrams per kilogram of body weight.

RESULTS AND DISCUSSION

The single oral dose of p-chlorophenyl N-methylcarbamate did not appear to be toxic, as evaluated by outward appearance and gross postmortem examination.

Approximately 78% of the carbon-14 given to the rat as the carbonyl-labeled compound appeared in the expiratory gases of the rat during the 48-hr collection period, whereas no radioactivity from the ring-labeled compound was detected in the expiratory gases of the rat (Table I) or goat. Radioactivity from the ring-labeled compound was rapidly excreted in the urine by both the rat and the goat (Table II) during the first

Table III. Carbon-14 in Tissues from the Rat 48 Hr After a Single Oral Dose of Radio-Labeled *p*-Chlorophenyl *N*-Methylcarbamate[a]

Fraction	*p*-Chlorophenyl-^{14}C(U) *N*-methylcarbamate		*p*-Chlorophenyl *N*-methylcarbamate-^{14}C	
	% Dose/g, dry matter	% Dose in total fraction	% Dose/g, dry matter	% Dose in total fraction
GI contents	0.056 ± 0.010[b]	0.090 ± 0.029	0.172 ± 0.054	0.153 ± 0.045
Heart	0.012 ± 0.004	0.002 ± 0.001	0.268 ± 0.043	0.027 ± 0.004
Liver	0.023 ± 0.001	0.043 ± 0.005	0.206 ± 0.016	0.460 ± 0.033
Kidney	0.016 ± 0.002	0.006 ± 0.001	0.209 ± 0.034	0.081 ± 0.006
GI tract	0.014 ± 0.002	0.029 ± 0.003	0.095 ± 0.008	0.222 ± 0.012
Remainder of carcass	0.017 ± 0.003	0.691 ± 0.085	0.071 ± 0.008	4.030 ± 0.292
Total		0.861		4.973

[a] Dose, 10 mg per kg of body weight. [b] Mean ± standard error.

Table IV. Carbon-14 in the Tissues of the Goat 48 Hr After a Single Intraruminal Dose of *p*-Chlorophenyl-^{14}C(U) *N*-Methylcarbamate

Fraction	% Dose/g dry matter × 10^{-5}	% Dose in total fraction
Liver	14.0	0.029
GI contents	9.8	0.042
Lungs	7.1	0.006
Blood	5.1	0.011
Carcass	4.9	0.580
Spleen	4.6	0.003
Rumen	4.3	0.012
Kidney	4.1	0.001
Intestine	3.5	0.006
Abomasum	3.5	0.002
Heart	2.0	0.001
Total		0.693

6 hr after the dose was given; from 6 to 48 hr after dosing, progressively smaller amounts appeared in the urine. The cumulative 48 hr urinary excretion of carbon-14 from the ring-labeled compound was 93.3% for the rat and 98% for the goat; in contrast, the rat excreted only 12.5% of the carbon-14 given as the carbonyl-labeled compound in the urine. The feces were a minor route of excretion for both the rat and goat. The percentage of the radioactivity in the feces was similar when rats were dosed with the ring-labeled and the carbonyl-labeled compound.

Approximately 0.05% of carbon-14 (dose, 10 mg per kg of body weight) given to the goat as the ring-labeled compound was in the milk produced in the 48-hr collection period (Table II). When a second goat was given 100 mg per kg of body weight of the ring-labeled material, 0.11% of the carbon-14 was in the milk collected 6 hr after dosing; the second goat produced approximately 2.3 times as much milk as the first goat prior to and throughout the experimental period.

The specific activity and percentage of the dose in various rat and goat tissues 48 hr after dosing are shown in Tables III and IV. The specific activity of liver tissue was highest; however, the remainder of the carcass, due to its much larger mass, contained most of the carbon-14 which remained in the animals. Less than 1% of the carbon-14 remained in the body of the rat and goat 48 hr after the ring-labeled compound was given. When the carbonyl-labeled compound was given to the rat, a larger portion (4.97% of total carbon-14) was retained in the body.

The total recovery of carbon-14 (ring-labeled) given to the goat was 98.8% of the dose; the recoveries of carbon-14 in rats from the ring-labeled and carbonyl-labeled compounds were 96.6 and 98.3%, respectively.

Ether extracted only one radio-labeled compound (metabolite A) from the urine of the rat and the goat. Metabolite A was identified as *p*-chlorophenol by preparing and characterizing its 2-cyclopentyl acetate derivative (Paulson and Zehr, 1971).

Metabolite B was identified as *p*-chlorophenyl glucuronide and metabolite C as *p*-chlorophenyl sulfate by comparing their infrared spectra with those of authentic samples synthesized as previously described (Paulson and Zehr, 1971).

Metabolite D was acetylated (Paulson and Portnoy, 1970) and the acetylation product purified by glc; the derivative was identified as 4-chloro-1,2-diacetoxybenzene by comparing its infrared and mass spectra with those of an authentic sample.

Table V. Metabolites of *p*-Chlorophenyl *N*-Methylcarbamate in Rat Urine,[a] Goat Urine,[a] and Goat Milk[b]

Metabolite	Structure	Rat		Goat	
		Female, % of ^{14}C in urine	Male, % of ^{14}C in urine	% of ^{14}C in urine	% of ^{14}C in milk[b]
A	*p*-Chlorophenol	2.5	4.2	0.2	ND
B	*p*-Chlorophenyl glucuronide	30.5	26.8	6.5	ND
C	*p*-Chlorophenyl sulfate	67.0	69.0	75.2	97.9
D	4-Chlorocatechyl-1-sulfate	ND[c]	ND	17.1	2.1
E	Unknown	ND	ND	0.1	ND
F	*p*-Chlorophenol ring structure Conjugate not determined	ND	ND	0.6	ND
G	Unknown	ND	ND	0.3	ND

[a] Urine was collected for 6 hr after dosing (10 mg per kg body weight) with *p*-chlorophenyl-^{14}C(U) *N*-methylcarbamate. [b] Dose, 100 mg/kg body weight; 81% of the carbon-14 in the milk was extracted by CH_3OH; values show per cent distribution of activity extracted by CH_3OH. [c] Not detected.

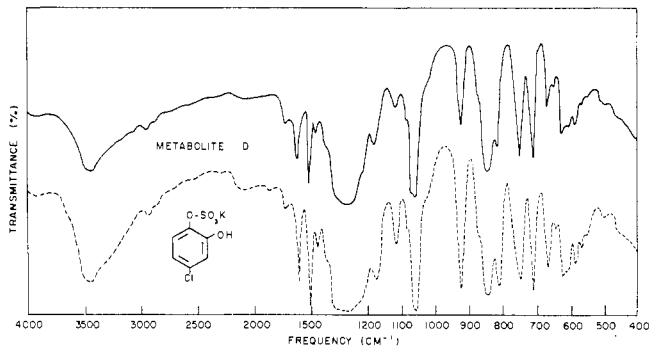

Figure 5. Infrared spectra of metabolite D and potassium 4-chlorocatechyl-1-sulfate

Inspection of the infrared spectrum of metabolite D indicated the presence of a sulfate group (strong absorption bands at 1000 to 1060 cm^{-1} and 1200 to 1300 cm^{-1}); hence, the three different sulfate derivatives of 4-chlorocatechol were prepared. Metabolite D was converted to its potassium salt form (Paulson *et al.*, 1970) and identified as potassium 4-chlorocatechyl-1-sulfate by comparison of its infrared spectrum with that of the known compound (Figure 5).

Metabolite F was hydrolyzed with a mixture of β-glucuronidase and aryl sulfatase, and the 2-cyclopentyl acetate derivative of the aromatic hydrolysis product was prepared; the derivative was purified by glc and identified as *p*-chlorophenyl-2-cyclopentyl acetate by comparative infrared spectrometry. The nature of the conjugating group in metabolite F was not established. Metabolites E and G, which occurred in only trace amounts in goat urine, were not identified.

The predominant metabolite in the urine of both the rat and goat was *p*-chlorophenyl sulfate (Table V). The rat produced sizable quantities of *p*-chlorophenyl glucuronide, whereas the glucuronide was a relatively minor metabolite in goat urine. The goat urine also contained 4-chlorocatechyl-1-sulfate and three minor unidentified metabolites which were not detected in rat urine. There was very little difference between the metabolite distribution in male and female rat urine.

Only two radio-labeled metabolites were detected in the goat milk (Figure 4). The major metabolite in the milk (Table V) was *p*-chlorophenyl sulfate and the minor metabolite was 4-chlorocatechyl-1-sulfate; both metabolites were identified by comparison of their infrared spectra with those of the known compounds.

These studies indicated that the metabolism of *p*-chlorophenyl *N*-methylcarbamate in the rat was similar to its metabolism in the chicken (Paulson and Zehr, 1971). The carbamate side chain was readily cleaved, and the ring moiety was quickly excreted in the urine as *p*-chlorophenol and its conjugation products, *p*-chlorophenyl glucuronide and *p*-chlorophenyl sulfate. Cleavage of the carbamate side chain and elimination of *p*-chlorophenol and its conjugation products was the predominant pathway in the goat also; however, metabolism in the goat also involved hydroxylation of the ring at the 2 position and excretion of the conjugate 4-chlorocatechyl-1-sulfate.

LITERATURE CITED

Bakke, J. E., Robbins, J. D., Feil, V. J., J. AGR. FOOD CHEM. **15,** 628 (1967).
Corbett, J. L., Greenhalgh, J. F. D., Gwynn, P. E., Walker, D., *Brit. J. Nutr.* **12,** 266 (1958).
Dawson, J. H., *Weed Sci.* **17,** 295 (1969).
Feigenbaum, J., Neuberg, C. A., *J. Amer. Chem. Soc.* **63,** 3529 (1941).
Herrett, R. A., Berthold, R. V., *Science* **149,** 191 (1965).
Kolbezen, M. J., Metcalf, R. L., Fukuto, T. R., J. AGR. FOOD CHEM. **2,** 864 (1954).
Hoiberg, C. P., Mumma, R. O., *J. Amer. Chem. Soc.* **91,** 4273 (1969).
Paulson, G. D., Feil, V. J., *Poultry Sci.* **48,** 1593 (1969).
Paulson, G. D., Portnoy, C. E., J. AGR. FOOD CHEM. **18,** 180 (1970).
Paulson, G. D., Zaylskie, R. G., Zehr, M. V., Portnoy, C. E., Feil, V. J., J. AGR. FOOD CHEM. **18,** 110 (1970).
Paulson, G. D., Zehr, M. V., J. AGR. FOOD CHEM. **19,** 471 (1971).
Robbins, J. D., Bakke, J. E., *J. Anim. Sci.* **26,** 424 (1967).
Symth, H. F., Carpenter, C. P., Weil, C. S., *J. Amer. Pharm. Ass.* **44,** 27 (1955).
Sullivan, L. J., Eldridge, T. M., Knaak, J. B., J. AGR. FOOD CHEM. **15,** 927 (1967).
Technical Service Bulletin 105-F-1, Biochemicals Industrial Chemical Division, PPG Industries, Inc., Pittsburgh, Pennsylvania, 1970.

Received for review June 3, 1971. Accepted August 9, 1971. Reference to a company or product name does not imply approval or recommendation of the U.S. Department of Agriculture to the exclusion of others that may be suitable.