plant spray. For our toxicity studies, the slide-dip was the method of choice, although we recognize that one of the techniques utilizing a natural substrate might give a better indication of control potential of the compounds under field conditions. Our major aim was to elucidate the intrinsic toxicity of each formamidine to the adult twospotted spider mite. Thus it was important to minimize those variables which could interfere with this measurement. With the slide-dip method the dosage is known more accurately than with a plant spray. Further, formamidines can undergo base–salt conversions on leaf surfaces, and there is some evidence that this can influence toxicity. Finally, with the mites adfixed to the glass slide, the end point can be determined more precisely than with other methods.

Metabolism studies of chlordimeform-^{14}C in twospotted spider mites indicated a rapid uptake of the compound accompanied by high internal levels of organosoluble radioactive material. Chlordimeform metabolites identified by cochromatography included demethylchlordimeform, didemethylchlordimeform, 4′-chloro-*o*-formotoluidide, and 4-chloro-*o*-toluidine. The unknown at R_f 0.09 may be 3-(4-chloro-*o*-tolyl)urea. Benezet and Knowles (1976b) have recently elucidated a urea pathway for chlordimeform metabolism in rats and mice.

Uptake of demethylchlordimeform-^{14}C by twospotted spider mites also was rapid, and levels of internal organosoluble radioactive materials were quite high. Demethylchlordimeform metabolites identified included didemethylchlordimeform, 4′-chloro-*o*-formotoluidide, and 4-chloro-*o*-toluidine.

Chlordimeform and demethylchlordimeform were not rapidly metabolized by twospotted spider mites. This slow metabolism of chlordimeform also has been observed in other chlordimeform-susceptible insects and acarines, such as the cabbage looper, *Trichoplusia ni* (Hübner) (Crecelius and Knowles, 1976), the cattle tick *Boophilus microplus* (Canestrini) (Knowles and Schuntner, 1974), and the rice steam borer, *Chilo suppressalis* Walker (Morikawa et al., 1975) and is in contrast to the rapid metabolism observed with the chlordimeform-tolerant house fly, *Musca domestica* L. (Knowles and Shrivastava, 1973). It appears that differential metabolism plays a role in the selective toxicity of chlordimeform to insects and acarines.

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Metabolism of *O*-Ethyl *S*-4-Chlorophenyl Ethanephosphonodithioate (N-2596) in the Rat

John B. Miaullis,* Larry J. Hoffman, Jack R. DeBaun, and Julius J. Menn

The experimental insecticide, *O*-ethyl *S*-4-chloro[U-^{14}C]phenyl ethanephosphonodithioate ([^{14}C]N-2596), is rapidly metabolized to detoxification products in rats receiving a single oral dose of [^{14}C]N-2596. Within 4 days of administration, the radiocarbon had been excreted mostly in the urine as conjugates of 4-chloro-3-hydroxyphenyl methyl sulfone. The primary metabolic biotransformations arise from scission of either N-2596 or its oxygen analogue to yield 4-chlorothiophenol which is then S-methylated, sulfoxidized, and ring hydroxylated. Neither N-2596 nor its oxygen analogue were detected in the urine of treated rats. Tissue residues based on total ^{14}C analysis decreased rapidly to negligible levels during an 8-day holding period.

Several phosphonate esters (organophosphorus esters with a P–C bond) have gained acceptance in recent years as highly effective pesticides. The metabolism of these compounds was recently reviewed by Menn and McBain (1974).

Included in this group of compounds is the experimental soil insecticide, N-2596 (*O*-ethyl *S*-4-chlorophenyl ethanephosphonodithioate). It has shown particular efficacy

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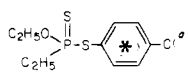
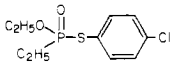
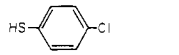
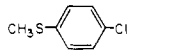
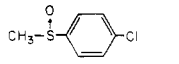
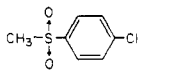
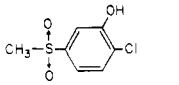
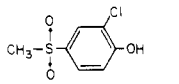
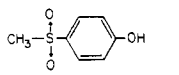
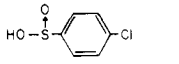
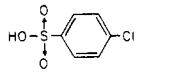
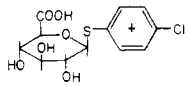
Table I. Standard and Reference Compounds and Their Chromatographic Characteristics

Compd and Code	Structure	Chromatographic solvent systems and R_f values							
		HC	BE	CEA	CAM	EAFA	EAMW	BEW	BAW
O-Ethyl *S*-4-chloro[*U*-^{14}C]-phenyl/ethanephosphono-dithioate ([^{14}C]N-2596)		0.7	0.9	0.9	0.9	1.0	0.9	1.0	1.0
O-Ethyl *S*-4-chlorophenyl ethanephosphonothioate (N-2596-oxon)		0.1	0.3	0.6	0.9	0.9	0.9	1.0	0.9
4-Chlorothiophenol (4-ClPhSH)		1.0	1.0	0.9	1.0	1.0	1.0	1.0	1.0
4-Chlorophenyl methyl sulfide (4-ClPhSMe)		1.0	1.0	1.0					
4-Chlorophenyl methyl sulfoxide (4-ClPhSOMe)		0.0	0.1	0.3	0.8	0.6	0.8	0.8	0.8
4-Chlorophenyl methyl sulfone (4-ClPhSO$_2$Me)		0.1	0.6	0.7	0.9	0.9	0.9	1.0	0.9
4-Chloro-3-hydroxyphenyl methyl sulfone 4-Cl-3-OHPhSO$_2$Me		0.0	0.3	0.6	0.9	0.9	0.9	1.0	0.9
3-Chloro-4-hydroxyphenyl methyl sulfone 3-Cl-4-OHPhSO$_2$Me			0.2	0.5	0.9				
4-Hydroxyphenyl methyl sulfone (4-OHPhSO$_2$Me)			0.2	0.6	0.9				
4-Chlorobenzene sulfinic acid (4-ClPhSO$_2$H		0.0	0.0	0.0	0.1	0.5	0.4	0.7	0.7
4-Chlorobenzene sulfonic acid (4-ClPhSO$_3$H)			0.0	0.0	0.2	0.1	0.6	0.8	0.7
4-Chlorothio[*U*-^{14}C]phenyl *S*-glucosiduronic acid (4-ClPhS-gluc)						0.7	0.4	0.6	0.7

[a] Asterisk denotes position of ^{14}C label. [b] TLC solvent abbreviations: HC, hexane–chloroform; BE, benzene–ether; CEA, chloroform–ethyl acetate (1:1); CAM, chloroform–acetone–methanol (5:4:1); EAFA, ethyl acetate–formic acid (30:1); EAMW, ethyl acetate–methanol–water (5:4:1); BEW, 1-butanol–ethanol–water (4:1:1); BAW, 1-butanol–acetic acid–water (4:1:1).

as a soil insecticide with moderate residual activity (Genung and Janes, 1970; Harris and Svec, 1973; Harris et al., 1974; and Libby et al., 1974). Gas–liquid chromatographic procedures for the determination of N-2596 and its oxygen analogue in crops, soils, milk, and animal tissues have been developed (Bussey et al., 1977).

Our laboratory has previously reported the metabolic fate of the phosphonate insecticide, Dyfonate, a member of this phosphonodithioate series having an unsubstituted phenyl ring (Hoffman et al., 1971; McBain et al., 1971). These studies established that the thioaryl phosphonates undergo metabolism via several pathways, including desulfuration to the oxygen analogue and cleavage to thiophenol, phosphonothioic acid, and phosphonic acid. The thiophenol released from Dyfonate was metabolized to several products via S-methylation, sulfoxidation, ring hydroxylation, and finally O-conjugation before excretion (McBain and Menn, 1969). This study describes the metabolic fate of phenyl-labeled N-2596 in rats receiving an oral dose of the radiolabeled insecticide.

MATERIALS AND METHODS

Reagents. Chemical names, structures, abbreviations, thin-layer chromatography (TLC) solvent systems, and TLC characteristics of reference compounds and possible metabolites are described in Table I. All the compounds were synthesized by Stauffer Chemical Company or purchased from commercial sources. TLC was run on 20 × 20 cm glass plates coated with 250 or 1000 μm layers of silica gel G or GF (Analtech, Newark, Del.). Unlabeled compounds were detected with I_2 vapor or by the quench of the fluorescent indicator in the GF TLC plates.

O-Ethyl *S*-4-chloro[U-^{14}C]phenyl ethanephosphonodithioate ([^{14}C]N-2596) was synthesized by J. Kalbfeld, Stauffer Chemical Company, Richmond, Calif. The specific activity was 5.0 mCi/mmol. The preparation was purified by preparative TLC on 1000 μm layer silica gel using the hexane–chloroform 3:2 (HC) and benzene–hexane 1:1 (BH) solvent systems. The radioactive areas were located on the developed plates by autoradiography using Royal X-Omat x-ray film (Kodak RP/R54). Radioactive areas were scraped free from the supporting glass plate and eluted from the silica gel with ether. The resulting [^{14}C]N-2596 was 98.5% radiochemically pure as determined in both solvent systems.

The standard 4-ClPhS-gluc was synthesized from [U-^{14}C]4-chlorothiophenol and uridine 5′-diphosphoglucuronic acid, ammonium salt (Sigma Chemical Co., St. Louis, Mo.) using rat liver microsomes according to the method of Mehendale and Dorough (1971). Preparative TLC with 1-butanol–ethanol–water 4:1:1 (BEW) was used to purify the radiolabeled compound.

A preconditioning solution of unlabeled N-2596 (94.5%) was prepared with 230 μg of the insecticide dissolved in 20 mL of corn oil. A dosing solution of [^{14}C]N-2596 was prepared in corn oil and the radiocarbon content of this solution was determined from weighed aliquots assayed by liquid scintillation counting (lsc). The dose given each animal was determined by weighing the syringe prior to and after dosing the animal and calculating the value from the known dpm/mg of solution.

Treatment of Rats. Eight female and eight male Simonsen albino rats (Gilroy, Calif.) were dosed by stomach intubation with 0.2 mL of the preconditioning solution daily for 4 days (11.5 μg kg^{-1} day^{-1}). Three days after the last preconditioning dose, the animals were all dosed with [^{14}C]N-2596 (average 3.3 μCi/animal; 1.2 mg/kg) dose. Treated animals were held for 4 days after the [^{14}C]N-2596 dosing and were housed in glass–stainless steel metabolism cages designed to collect urine, feces, and expired $^{14}CO_2$ (in 10% KOH) separately. Animals held for 1, 2, and 8 days were housed in plastic cages (Maryland Plastics, Federalsburg, Md.) which have no provision for collecting expired $^{14}CO_2$. Immediately after the collection of the last samples, the rats were sacrificed by cervical spinal transection. Tissues and organs were removed for analysis of ^{14}C content.

Radiocarbon Analysis. The urinary radiocarbon was measured by liquid scintillation counting (lsc) using duplicate 0.1- or 0.5-mL aliquots of urine and 5 mL of liquid scintillation fluid. The fluid was prepared according to the following formula: 3 L of toluene, 1 L of Triton X-100 (Rohm and Haas), 16.5 g of 2,5-diphenyloxazole (PPO), and 0.3 g of 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene (dimethyl POPOP). The samples were assayed in a Packard (Downers Grove, Ill.) Model 3375 liquid scintillation counter. The counting efficiency was determined by automatic external standardization.

Samples of tissues, organs, and feces were homogenized in 50% aqueous methanol using a Polytron tissue homogenizer (Brinkmann Instruments, Westbury, N.Y.). The carcasses were ground in a hand-operated meat grinder prior to homogenization in 50% aqueous methanol. Duplicate 0.1- or 0.5-mL aliquots of each homogenate were used for ^{14}C radioassay by lsc in 10 or 20 mL of scintillation fluid. Counting efficiencies were determined by internal standardization with [^{14}C]toluene.

Isolation and Characterization of Metabolites. Urine and fecal samples used for metabolite characterization were collected at 6, 24, 48, 72, and 96 h after dosing. Combined urine samples (approximately 20 mL at pH 6.5) from each collection interval were extracted twice with an equal volume of diethyl ether. The ether was concentrated under a stream of N_2. The aqueous phases were lyophilized and the lyophilizates were reconstituted in anhydrous methanol which solubilized >95% of the total ^{14}C.

Samples of the aqueous phases before and after lyophilization were subjected to enzymatic hydrolysis using β-glucuronidase (GL) (Worthington, Freehold, N.J.) and β-glucuronidase–arylsulfatase, Grade B (Calbiochem, La Jolla, Calif.) in 0.05 M sodium acetate buffer adjusted with HCl to pH 4.5 and 6.5, respectively. A typical incubation had 100 000 dpm of urinary radiocarbon, 0.1 mL of water, 0.3 mL of buffer, and 0.04 IU of β-glucuronidase or 800 Whitehead units of arylsulfatase. Control samples were run in pH 4.5 buffer with no added enzyme. Aliquots of test samples which had been incubated for 18 h at 36 °C were chromatographed directly on 250 μm silica gel TLC plates. Radioactive areas were located by autoradiography and scraped from the supporting plates for direct counting to quantify the distribution of metabolites in the urine. A 50 times scaled-up incubation with β-glucuronidase–arylsulfatase was used to liberate larger quantities of deconjugated metabolites. These were recovered from the incubation mixture by ether extraction and were purified by preparative TLC.

Metabolites were extracted from fecal samples by homogenization twice in 4 mL of methanol per g of sample, and the methanol was removed by decantation. The residues were further extracted twice with acetone. The final solid residues were combusted in a Packard Model 305 sample oxidizer to convert organic residues to $^{14}CO_2$ and water. Trapped $^{14}CO_2$ was counted by lsc to determine the residual radiocarbon. The acetone and methanol extracts were concentrated by rotary vacuum evaporation, and the urinary and fecal metabolites were separated by TLC.

Gas chromatography (GC) was performed on a Finnigan Model 9500 fitted with a hydrogen flame ionization detector (Finnigan Corp., Sunnyvale, Calif.). The separations were performed in a 1.5 m × 2 mm glass "U" column packed with 3% OV-1 on GC-Q (Applied Science Laboratories, State College, Pa.) at 150 °C. Other conditions were: injector and detector, 200 °C; He, 30 mL/min; H_2, 40 mL/min; and air, 260 mL/min. Samples for gas chromatography–mass spectometry (GC–MS) were run on a Finnigan 1015D using the same column used in the Finnigan gas chromatography unit.

N-2596 and its metabolites were evaluated for anticholinesterase activity against bovine erythrocyte acetylcholinesterase (Winthrop Laboratories, New York, N.Y.). After a 10-min incubation period of the compound and enzyme (200 units/mL), residual enzyme activity was determined by adding 3 mL of a solution containing acetylthiocholine (0.19 mM) and Ellman's reagent [5,5′-dithiobis(2-nitrobenzoic acid)] (1.26 mM in phosphate buffer, 0.05 M, pH 7.0), and measuring the change in absorbance at 415 nm for 2 min (Ellman et al., 1961). All reactions were carried out at 37 °C.

For toxicity determination, female albino rats of the Sprague-Dawley strain weighing 200–250 g were fasted overnight and then treated with the test compound. The test procedure is in accordance with the Code of Federal Regulations (Part 119.1, Chapter I, Title 21) for evaluating highly toxic substances. LD_{50} values were determined from mortality at 14 days.

RESULTS AND DISCUSSION

Balance and Tissue Residue Elimination. None of the animals in the metabolism studies showed overt signs of toxicity from the N-2596. Urine and feces ^{14}C content was very similar for both male and female rats (Figure 1). An average of 80% of the administered radiocarbon was recovered in the urine. The small sex-related difference in distribution between urine and feces is not significant for such a small sample. Analysis of the expired air for $^{14}CO_2$ within a 96-h holding period accounted for an average of 0.2% of the administered radiocarbon, indicating little or no opening of the aromatic ring had taken place.

The results of tissue ^{14}C analysis for rats sacrificed at 1, 2, 4, and 8 days were expressed as parts per million equivalent to the parent compound (Table II). The parts per million values for the tissues at 48 h were all less than half those found at 24 h. Further reductions of radiocarbon content in kidney, liver, and other tissue were observed through 8 days. Residues in tissues as measured by total ^{14}C content are expressed as parts per million equivalent to the parent compound, but the extensive metabolism (see metabolite characterization) and decreased tissue residues

Table II. Total ^{14}C Tissue Residues Expressed as Equivalent Parts per Million of [^{14}C]N-2596 for Rats Given a Single Oral Dose

	Analysis interval							
	24 h		48 h		96 h		8 days	
Sample	Male[a]	Female	Male	Female	Male	Female	Male	Female
Blood	0.09	0.09	0.03	0.03	ND[b]	ND	0.02	0.03
Brain	0.22	0.23	0.07	0.03	ND	ND	ND	ND
Fat	0.47	0.80	0.21	0.11	ND	ND	ND	ND
Gonads	0.43	0.36	0.06	0.07	ND	ND	ND	ND
Hide	0.29	0.33	0.12	0.07	0.14	0.20	0.02	ND
Kidney	0.55	0.54	0.19	0.10	0.04	0.04	0.02	0.02
Liver	0.23	0.20	0.08	0.08	0.005	0.004	0.003	ND
Intestine	0.41	0.35	0.08	0.07	ND	ND	ND	ND
Carcass	0.17	0.22	0.04	0.03	0.03	0.04	0.003	0.002
Av ppm for whole body	0.21	0.23	0.06	0.02	0.03	0.04	0.004	0.005

[a] Each value is the average for two animals. [b] ND, no detectable ^{14}C at 5 cpm above background.

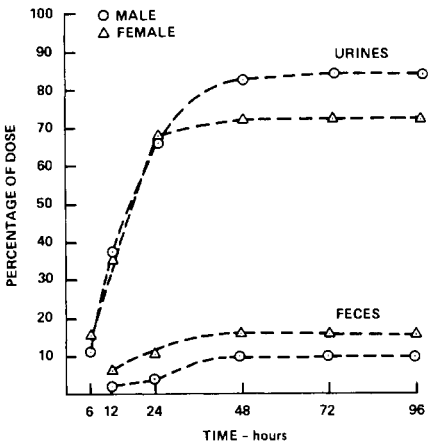


Figure 1. The excretion of ^{14}C in urine and feces of rats given a single oral dose of [^{14}C]N-2596. Each point represents the average of individual determinations from two animals.

with time suggest little or no N-2596 is present in the tissues.

Rats dosed with [^{14}C]N-2596 for tissue residue analysis had been preconditioned with unlabeled N-2596. The effect of this preconditioning on tissue residues was not established in the present study. However, in studies with the nonchlorinated analogue, Dyfonate, the effect of such preconditioning was found to be negligible (Hoffman et al., 1971). Expressed as a whole body residue, the ^{14}C content of the rats decreased from 18.8% of the dose at 24 h to 0.3% at 8 days. The average total recovery after 4 days was 80.3% in urine, 13.7% in feces, 0.2% as $^{14}CO_2$, and 2.8% in the tissues for an overall average recovery of 97.0%.

Metabolite Characterization. *Organosoluble Urinary Metabolites.* Urinary aliquots from four time intervals were chromatographed directly in the benzene–ether 7:3 (BE), chloroform–ethyl acetate 1:1 (CEA), 1-butanol–acetic acid–water 4:1:1 (BAW), and BEW solvent systems. The autoradiographs showed no significant differences between the urinary metabolites of the male and female rats. Further analyses were therefore conducted on pooled samples. In a separate test, male rats were treated with [^{14}C]N-2596 without pretreatment with unlabeled N-2596. The urinary metabolite distribution was identical based on TLC in solvent systems CEA and BEW to those found for the preconditioned rats, indicating the small preconditioning doses, equal to 4% of the final dose, had no significant effect on N-2596 metabolite distribution.

Table III. Distribution and Identity of Rat Urinary and Fecal Metabolites of [^{14}C]N-2596 24 h after Treatment

	Metabolites as a percent of the total ^{14}C	
Metabolites	Urine	Feces
Organosoluble		
N-2596	ND[a]	4.2
4-ClPhSOMe	0.06	1.9
4-ClPhSO$_2$Me	7.3	27.8
4-Cl-3-OHPhSO$_2$Me	1.3	15.2
Unknowns	1.0	
Total organo-soluble	9.7	49.1
Polar		
4-Cl-3-OHPhSO$_2$Me		
-*o*-glucuronide	37.4	ND
-*o*-sulfate	28.9	ND
4-ClPhSO$_3$H	2.0	1.3
4-ClPhS-gluc	5.5	ND
Unknowns		
Metabolite 5	10.2	
Metabolite 7	4.7	
Metabolite 8	1.6	4.5
Total water soluble	90.3	5.8
Unextracted residues	ND	45.1
Total	(100.0)	(100.0)

[a] ND, none detectable.

Table IV. Distribution of Ether Extractable Rat Urinary Metabolites of [^{14}C]N-2596 at Various Intervals after Dosing

	Percent of total urinary ^{14}C at each interval		
Compound	0-6 h	6-24 h	24-48 h
4-ClPhSOMe	0.3	ND	ND
4-ClPhSO$_2$Me	18.1	4.6	3.4
4-Cl-3-OHPhSO$_2$Me	1.1	1.4	3.6
Unknown	2.4	0.7	0.6
Total ether extractable ^{14}C	21.9	6.7	7.6

The organosoluble metabolites were extracted into ether and cochromatographed with standard N-2596, N-2596 oxon, 4-ClPhSH, and 4-ClPhSMe. None of these compounds were detected in any of the urine samples. Three metabolites, 4-ClPhSOMe, 4-ClPhSO$_2$Me, and 4-Cl-3-OHPhSO$_2$Me (Table III), were identified by thin-layer cochromatography with authentic reference standards in the following solvent systems: BE, CEA, benzene–acetonitrile 3:1 (BA), and chloroform–ether 1:1 (CE).

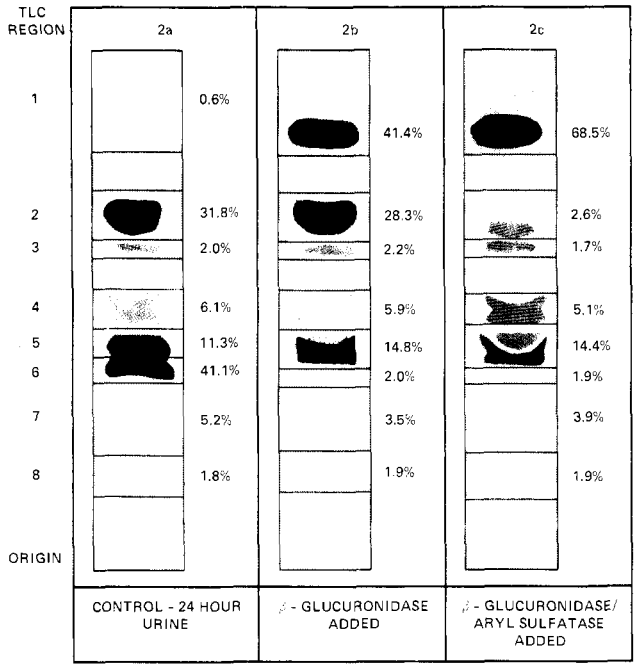


Figure 2. TLC autoradiograph and distribution of polar urinary metabolites with and without enzymatic hydrolysis. TLC solvent system, BEW. Distribution is given as a percent of the total in each sample.

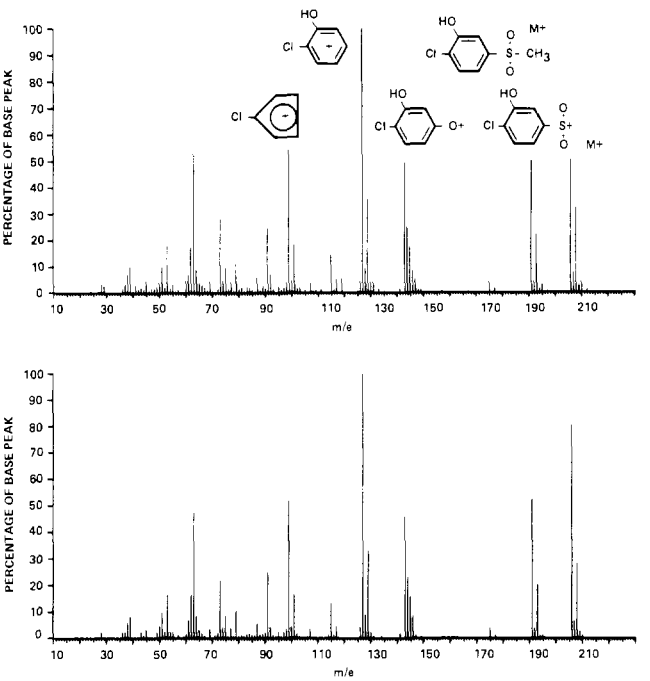


Figure 3. Mass spectrum of the isolated urinary metabolite (top) and the standard compound, 4-Cl-3-OHPhSO_2Me.

These metabolites are formed from 4-ClPhSH via the S-methylation reaction, followed by oxidation of the sulfur and hydroxylation of the ring. Table IV shows the distribution of the metabolites and total percent of radiocarbon in the ether extracts of urine at three different time intervals. The results show the facile nature of the reactions leading to the S-methylation products observed in the 6-h urine samples.

Polar Urinary Metabolites. Polar urinary metabolites, that is those not extracted with ether, increased from 78% of the urinary radiocarbon in the 6-h samples to 96% in the 72-h sample. Seventy percent of the polar urinary radiocarbon extracted with ether after the incubation of the urine with the β-glucuronidase–arylsulfatase preparation, thus indicating the enzymes had hydrolyzed conjugated metabolites. The incubated samples from a second deconjugation experiment were chromatographed directly (Figure 2) and were compared with the control incubation (Figure 2a). Metabolites in regions 2 and 6 were hydrolyzed by the β-glucuronidase–arylsulfatase preparation (Figure 2c), while β-glucuronidase preparation hydrolyzed only the metabolite in region 6 (Figure 2b). Therefore, both a sulfate conjugate (in region 2) and a glucuronide conjugate (in region 6) were present in the urine.

The hydrolysis product from both enzymatic incubations migrated to region 1 and cochromatographed with standard 4-Cl-3-OHPhSO_2Me in the BE, CEA, BA, and CE solvent systems. Two other hydroxyphenyl methyl sulfones, 4-OHPhSO_2Me and 3-Cl-4-OHPhSO_2Me, which are potential metabolites, did not cochromatograph with the deconjugated metabolite. Thus, no evidence for the 3-chloro-4-hydroxy isomer, the so called "NIH shift product", was found in our studies (Jerina and Daly, 1974). The identity of the isolated 4-Cl-3-OHPhSO_2Me was also confirmed by GC analysis and by GC–MS of the sample from a preparative scale enzymatic hydrolysis of the conjugated urinary metabolites. After preparative TLC cleanup, the aglycone was chromatographed on the 3% OV-1 column with reference compounds. These compounds and their retention times were: 3-Cl-4-OHPhSO_2Me (2.10 min), the metabolite (2.20 min), 4-Cl-3-OHPhSO_2Me (2.25 min), and 4-OHPhSO_2Me (3.05 min). Based on cochromatography and similar retention times, the metabolite appeared to be the 4-chloro-3-hydroxy isomer. Its structure was further confirmed by GC–MS which gave a mass spectrum identical with that of the authentic 4-Cl-3-OHPhSO_2Me compound (Figure 3).

The metabolite in region 3 was identified by cochromatography with a reference standard as 4-ClPhSO_3H in TLC solvent systems BEW, BAW, ethyl acetate–methanol–water 12:3:1 (EAMW), and ethyl acetate–formic acid 30:1 (EAFA). It constitutes 2.0% of the total urinary radiocarbon. A standard 4-ClPhSO_2H sample did not cochromatograph with any of the urinary metabolites, although it is a minor metabolite of 4-ClPhSH based on other work done in our laboratory (Menn et al., 1975). The metabolite in region 4 was identified by cochromatography with a reference standard in the above solvent systems as 4-ClPhS-gluc. It represents 5.5% of the total urinary radiocarbon.

Fecal Metabolites. The methanol and acetone fecal extracts contained metabolites identical with those found in urine, except that 4.2% of the fecal radiocarbon was identified as N-2596 (Table III). Its identity was established by cochromatography with authentic N-2596 in the HC and BH solvent systems. None of the fecal radioactive metabolites cochromatographed with standard N-2596 oxon, 4-ClPhSH, and 4-ClPhSMe. The unextracted radiocarbon in the feces represented a large portion of the fecal ^{14}C (45%) but accounted for a relatively small percentage (4.0%) of the total excreted radioactivity. This unextracted ^{14}C was not liberated by usual extraction techniques from the solid matter; however, no attempts were made to free the ^{14}C using reflux extraction or hydrolytic procedures. This amount of bound material was relatively constant for fecal samples from 1 to 4 days after dosing.

The proposed metabolic pathway of [^{14}C]N-2596 in the rat is shown in Figure 4. Although 4-ClPhSH was not

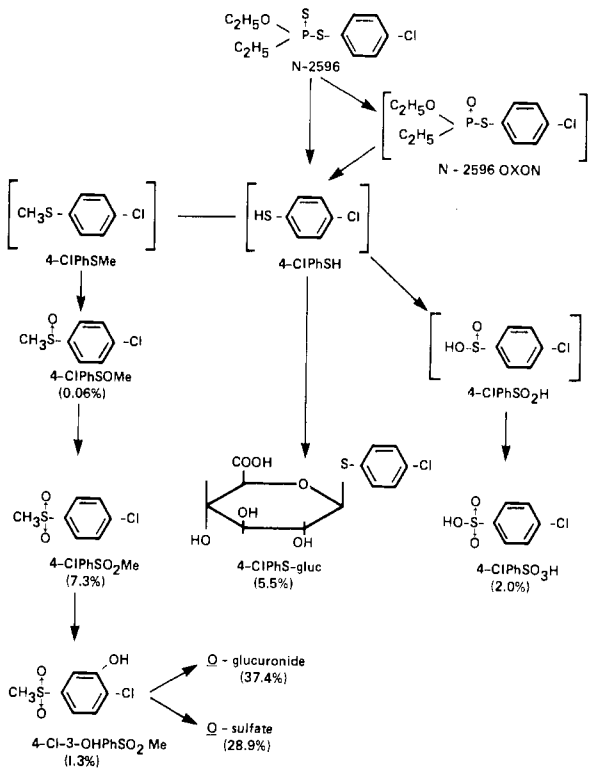


Figure 4. Proposed metabolic pathway for N-2596 in the rat. Compounds in brackets were not observed in urine or feces. Percent values are for distribution in urine at 24 h.

found as a metabolite, it presumably is the intermediate in the formation of the excreted metabolites. Metabolism of 4-chlorothio[U-^{14}C]phenol yielded each of the metabolites shown, as reported by Menn et al. (1975). No evidence is available from the present study to indicate whether the 4-ClPhSH is formed directly from N-2596 or partially from its oxygen analogue, N-2596 oxon.

The S-methylation of 4-ClPhSH and the subsequent oxidation to the corresponding sulfoxide and sulfone is analogous to that observed with the thiophenyl moiety of Dyfonate (McBain et al., 1971). Hydroxylation yields 4-Cl-3-OHPhSO$_2$Me, which is the precursor of the glucuronide and sulfate conjugates, the two major urinary metabolites of N-2596. Although the major urinary metabolites were formed by S-methylation of 4-ClPhSH, the identified metabolites indicate that N-2596 can be metabolized by two other biotransformation pathways. One involves the oxidation of 4-ClPhSH to 4-ClPhSO$_3$H acid, probably through the intermediate sulfinic acid. Formation of the *S*-glucuronide of 4-ClPhSH provides evidence for yet another metabolic pathway.

The female rat oral LD$_{50}$ and the anticholinesterase activity of the metabolites of N-2596 identified in this study are summarized in Table V. With the exception of the oxygen analogue of N-2596, the compounds show less acute toxicity and are less potent inhibitors of the bovine erythrocyte acetylcholinesterase than the parent insecticide. Thus, the metabolism of N-2596 leads to the formation of metabolites which are readily excreted and are significantly less toxic than N-2596 based on the aforementioned data.

Table V. Toxicity and Acetylcholinesterase Inhibition Values for N-2596, Metabolites, and Related Compounds

Compound	Rat oral LD$_{50}$, Female (95% confidence range), mg/kg	Bovine erythrocyte acetylcholinesterase inhibition I_{50}[a], M
N-2596	3.23 (2.2-4.6)	2×10^{-5}
N-2596 oxon	5.8 (4.3-7.9)	2×10^{-9}
4-ClPhSH	316 (216-463)	$>1 \times 10^{-3}$
4-ClPhSMe	>500	$>1 \times 10^{-3}$
4-ClPhSOMe	>500	$>1 \times 10^{-3}$
4-ClPhSO$_2$Me	>500	$>1 \times 10^{-3}$
4-Cl-3-OHPhSO$_2$Me	>500	$>1 \times 10^{-3}$
4-ClPhSO$_2$H	>500	
4-ClPhSO$_3$H	>500	

[a] I_{50}, molar concentration inhibiting 50% of the enzyme in 10 min at 37 °C.

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