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Metabolism of *O*-Ethyl *O*-[4-(Methylthio)phenyl] *S*-Propyl Phosphorodithioate (BAY NTN 9306) by White Rats

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A 10 mg/kg dose of phenyl-^{14}C-labeled BAY NTN 9306 (*O*-ethyl *O*-[4-(methylthio)phenyl] *S*-propyl phosphorodithioate), administered orally to female white rats, was metabolized rapidly and excreted primarily in the urine (ca. 92% in 24 hr). No $^{14}CO_2$ was detected and essentially all the radioactivity in urine consisted of water-soluble materials that were converted to three substituted free phenolic derivatives by hydrolysis with glucuronidase–aryl sulfatase or acid. Analyses of tissues taken from rats 3 hr after treatment with an oral dose of 70 mg/kg revealed the presence of BAY NTN 9306, five phosphorus-containing metabolites formed by oxidations of thiono and thioether sulfur groups of the molecule, and three substituted phenols, both free and conjugated.

The experimental organophosphorus (OP) insecticide *O*-ethyl *O*-[4-(methylthio)phenyl] *S*-propyl phosphorodithioate (BAY NTN 9306, Mobay Chemical Corp.) appears unusually promising for use in controlling *Heliothis* spp. pests of field crops, including the tobacco budworm, *Heliothis virescens* (F.), which has become resistant to most OP compounds in many areas where it is a major pest of cotton. With the added advantage that the mammalian toxicity is relatively low (acute oral LD_{50} for rats is 227 mg/kg), there is considerable interest in the development of this insecticide for commercial use.

The present report describes the fate of radiolabeled BAY NTN 9306 after oral treatment of white rats.

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EXPERIMENTAL SECTION

Chemicals. The Chemagro Agricultural Division of Mobay Chemical Corp., Kansas City, Mo., provided pure samples of BAY NTN 9306 (hereafter designated 9306), including some that was radiolabeled uniformly with ^{14}C in the phenyl ring of the molecule (>99% radiochemical purity, specific activity 7.06 mCi/mmol), and certain chemicals considered as potential metabolic derivatives (Table I).

Rats and Their Treatment. Female white rats (Sprague-Dawley strain, 150–170 g) were each treated orally with sublethal doses of ^{14}C-labeled 9306 in 0.5 ml of corn oil. The dose was administered with a syringe and stomach tube after light anesthesia of the rats with ether. Animals were fed immediately before treatment but were provided only water through the next 24 hr; those held longer were fed each day.

For most tests the rats were treated with a dose of 10 mg/kg, using ^{14}C-labeled 9306 that was diluted with 4 parts of nonradioactive 9306. Those used for studies of the distribution of radioactive compounds in tissues were treated with a dose of 70 mg/kg, using ^{14}C-labeled 9306

Table I. TLC Properties of BAY-NTN-9306 and Potential Metabolites

Chemical name	Trivial name	R_f in TLC system				
		A	B	C	D	E
O-Ethyl *O*-[4-(methylthio)phenyl] *S*-propyl phosphorodithioate	9306	0.84	1.00	0.93	0.92	0.89
O-Ethyl *O*-[4-(methylsulfinyl)phenyl] *S*-propyl phosphorodithioate	9306 sulfoxide	0.25	0.82	0.79	0.44	0.79
O-Ethyl *O*-[4-methylsulfonyl)phenyl] *S*-propyl phosphorodithioate	9306 sulfone	0.39	0.93	0.89	0.80	0.86
O-Ethyl *O*-[4-(methylthio)phenyl] *S*-propyl phosphorothioate	O-Analog	0.51	0.95	0.89	0.83	0.88
O-Ethyl *O*-[4-(methylsulfinyl)phenyl] *S*-propyl phosphorothioate	O-Analog sulfoxide	0.14	0.62	0.62	0.23	0.77
O-Ethyl *O*-[4-(methylsulfonyl)phenyl] *S*-propyl phosphorothioate	O-Analog sulfone	0.21	0.78	0.74	0.55	0.84
O-[4-(Methylthio)phenyl] *S*-propyl hydrogen phosphorodithioate	Deethyl-9306	0.01	0.22	0.43	0.00	0.74
O-[4-(Methylthio)phenyl] *S*-propyl hydrogen phosphorothioate	Deethyl-O-analog	0.00	0.26	0.25	0.00	0.71
p-(Methylthio)phenol	Phenol sulfide	0.21	0.76	0.87	0.69	0.86
p-(Methylsulfinyl)phenol	Phenol sulfoxide	0.04	0.18	0.54	0.10	0.76
p-(Methylsulfonyl)phenol	Phenol sulfone	0.07	0.27	0.73	0.35	0.82
β-[*p*-(Methylthio)phenyl] D-glucuronide[a]	Phenol glucuronide	0.00	0.00	0.00		0.58
p-(Methylthiophenyl sulfate[a]	Phenol sulfate	0.00	0.03	0.26		0.66

[a] Synthesized as described by Bull and Stokes (1970).

diluted with 239 parts of nonradioactive material. Treatments for all tests were administered at the same time each day, and none caused visible symptoms of OP poisoning.

Analytical Procedures. Studies to detect expired $^{14}CO_2$ were conducted in a glass metabolism cage (Delmar, Maywood, Ill.). Incoming air passed through adsorbents to remove CO_2 and moisture; outgoing air was drawn through gas-washing flasks containing a solution of 2-aminoethanol and 2-methoxyethanol (1:1, v/v) that was changed frequently through 72 hr after treatment. Animals used for all other studies were held in cages equipped only for the separation of urine and feces.

For studies of changes in levels of radioactivity in tissues after treatment, rats were decapitated at specified times, and the blood was collected and heparinized. Triplicate samples of brain, liver, kidney, muscle, and fat were collected immediately, weighed, and frozen. These samples were later air-dried, and radioactive residues were determined by combustion under 1 atm of oxygen. The combustion gases were bubbled through a solution of 2-aminoethanol and 2-methoxyethanol (1:1, v/v); then the trapping solution was radioassayed.

Analyses to characterize the radioactive materials in tissues were done with whole livers (42.3 g), kidneys (8.3 g), and muscle (58.1 g) from four rats. The tissues were taken 3 hr after treatment and homogenized (Virtis Co., Gardiner, N.Y.) immediately with 2–3 vol of water. These samples were homogenized again with 2 vol of ethyl acetate in a Willems Polytron homogenizer (Brinkmann Instruments, Westbury, N.Y.) and then centrifuged to break emulsions and separate different fractions. The organic layer was removed, and the aqueous and solid portions were extracted two more times with ethyl acetate. The combined ethyl acetate extracts were dried over anhydrous sodium sulfate and then concentrated and analyzed by thin-layer chromatography (TLC). Radioactivity remaining in the aqueous-residue phase was analyzed further by enzyme or acid hydrolysis, followed by ethyl acetate extraction and TLC.

Urine samples were collected at definite times, extracted twice with chloroform (1:4, v/v), radioassayed, and then lyophilized and stored in a freezer. Subsamples of dried urine were incubated with a mixture of glucuronidase and aryl sulfatase (50000 Fishman/5000 Whitehead units, Calbiochem, La Jolla, Calif.) in 5 ml of sodium acetate buffer (0.1 *M*, pH 4.5) for 24 hr at 37°C and then extracted with chloroform as described. The aqueous fraction was then made 1 *N* with HCl, heated 30 min in a boiling water bath, and extracted again three times with ethyl acetate (1:4, v/v). Different fractions were radioassayed, and combined organic extracts were held for analyses of radioactive components. Radiocarbon in feces was determined by oxygen combustion as described.

Quantitation and preliminary identifications of radioactive compounds in organic extracts were done with TLC by using precoated silica gel chromatoplates (Silplate F–22, 0.25 mm thick; Brinkman Instruments, Westbury, N.Y.). Samples were routinely chromatographed in two dimensions with different combinations of solvent mixtures: (A) 9:4:1, heptane, chloroform, and methanol; (B) 6:3:2, chloroform, hexane, and glacial acetic acid; (C) 2:2:1, hexane, ethyl acetate, and methanol; (D) 6:3:2, chloroform, hexane, and acetone; and (E) 12:8:6, 1-butanol, pyridine, and water. Identifications were based on the coincidence of radioactive areas located by exposing plates to X-ray films with authentic standards that were detected under ultraviolet light. Radioactive areas on plates were scraped from plates and radioassayed or extracted with acetone and analyzed further.

The identifications of metabolites established by TLC were confirmed with gas–liquid chromatography (GLC)–mass spectral studies. Instrumentation included a Varian-Mat-CH-7 spectrometer coupled with a Varian 2700 gas chromatograph and a 620 L Varian computer (Varian Associates, Palo Alto, Calif.). All compounds were resolved on a 0.6 m × 2 mm i.d. glass column packed with 10% DC-200 + 1.5% QF-1 on 80–100 mesh Chromosorb W-HP (R. R. Gronberg, Mobay Chemical Corp., personal communication). Operating parameters were as follows: injector, 210°C; column, 125°C (phenol sulfide), 175°C (phenol sulfoxide and sulfone); 200°C for all others; detector oven, separator, and inlet, 210°C; ion source, 225°C; helium flow, 50 ml/min; ionizing voltage, 70 eV. None of the compounds required derivatization prior to injection.

Radioactive measurements were made by liquid scin-

Table II. Radiocarbon Residues in Tissues of Rats Treated with ^{14}C-Labeled 9306 at 10 mg/kg[a]

hr after treatment	ppm of ^{14}C-labeled 9306 equivalents in					
	Blood	Brain	Liver	Kidney	Muscle	Fat
2	0.84	0.25	4.27	8.38	0.25	1.20
4	0.37	0.14	1.54	1.27	0.09	0.87
8	0.30	0.02	1.00	0.98	0.03	0.97
24	0.05	0.01	0.41	0.10	0.02	0.38
48	0.01	0.00	0.22	0.04	0.02	0.11
72	0.00	0.00	0.05	0.00	0.01	0.09

[a] Averages: two rats per time, three replicates of each tissue per time and rat.

Table III. Distribution of Excreted Radioactivity from Rats Treated with ^{14}C-Labeled 9306 at 10 mg/kg[a]

Treatment interval, hr	% of dose in			
	Expired as $^{14}CO_2$	Urine		Feces
		$CHCl_3$ sol.	H_2O sol.	
0-2	0.0	0.2	15.7	0.09
0-4	0.0	0.4	41.5	0.08
0-8	0.0	0.4	62.2	0.05
0-24	0.0	0.5	91.2	0.08
0-48	0.0	0.4	94.6	0.07
0-72	0.0	0.5	95.7	0.08

[a] Averages of two to four rats per time.

tillation (Beckman Instruments, Inc., Fullerton, Calif.). Appropriate corrections for quenching were made via internal standardization.

RESULTS

Radioactive residues were maximum in all tissues studied at 2 hr posttreatment (Table II) and then declined to less than 0.1 ppm after 72 hr. The greatest initial radiocarbon concentrations were found in kidney, but liver and fat also contained maximum residues of >1 ppm.

No $^{14}CO_2$ was recovered from treated rats during a 72-hr posttreatment period (Table III). Almost all administered radioactivity was recovered in the urine (ca. 92% after 24 hr and 96% at 72 hr), and essentially all of this was in the form of water-soluble materials. TLC analyses of the small concentrations of organosoluble radioactivity extracted from urine included traces (<0.1%) of 9306 sulfoxide and 9306 sulfone, and the balance was the three substituted phenols. Less than 0.1% of the administered dose was found in the feces at any time.

Direct TLC of the water-soluble fraction of urine indicated that small amounts of the radioactivity cochromatographed with the phenol glucuronide and sulfate, but none corresponded to deethyl 9306 or the deethyl O-analog.

Enzyme hydrolysis of the water-soluble fraction of urine converted ca. 60% of the radioactivity associated with each sample to organosoluble materials, almost all in the form of the three substituted phenols (Table IV). Subsequent treatment with acid converted most of the remaining radioactivity in the water-soluble fraction to an organosoluble form, and most of this fraction was also accounted for by the same phenolic products. When each of the suspected substituted phenols was isolated via preparative TLC and then analyzed further by GLC–mass spectrometry and compared with similar analyses of authentic standards, the respective identifications were confirmed (Table V).

Concentrations of radioactivity that remained at the origins of TLC plates [unknown(s) A] were not identified. These may represent water-soluble material carried over during partitioning or radioactivity trapped by impurities.

Table IV. Metabolism of ^{14}C-Labeled 9306 after Oral Treatment of Rats at 10 mg/kg; Radioactive Compounds in Urine

Nature of radioact.	% of dose at indicated time interval (hr) posttreatment				
	0-2	0-4	0-8	0-24	0-48
After Enzyme Hydrolysis[a]					
Unknown A	0.4	0.4	0.4	0.6	0.6
Phenol sulfide	1.9	3.0	6.9	11.0	11.2
Phenol sulfoxide	2.8	6.7	8.1	10.8	11.1
Phenol sulfone	1.8	5.9	10.6	16.5	17.2
9306 sulfoxide	<0.1	<0.1	<0.1	<0.1	<0.1
9306 sulfone	<0.1	<0.1	<0.1	<0.1	<0.1
H_2O soluble	9.0	25.9	36.6	52.8	54.9
After Further Hydrolysis with Acid					
Unknown A	0.5	1.1	2.1	2.7	2.9
Phenol sulfide	0.3	2.1	3.0	5.3	5.5
Phenol sulfoxide	5.0	13.0	18.1	26.1	27.0
Phenol sulfone	1.5	5.1	7.6	11.0	11.5
H_2O soluble	1.7	4.6	5.8	7.7	8.0

[a] Data include small amounts of free phenols and known phosphorus-containing metabolites recovered in initial chloroform extract of urine.

Results of the analyses of radioactivity extracted from kidney, liver, and muscle of four rats at 3 hr after treatment with a dose of 70 mg/kg ^{14}C-labeled 9306 are shown in Table VI. The largest concentrations of radioactivity were recovered from the liver, and this included 9306 and all five possible toxic oxidation products as well as the three substituted phenols. The principal products found in kidney and muscle were 9306 and its sulfoxide and sulfone derivatives. Acid hydrolysis of the water and tissue slurry that remained after initial extraction with ethyl acetate converted the balance of the radioactivity to a form that could be recovered by further extraction with ethyl acetate. Most of this was accounted for by the three substituted phenols (Table VI). In the muscle, the final volume was so large that failure to measure any radioactivity may have been experimental error due to the great dilution factor.

GLC–mass spectral evidence to support the TLC identifications of 9306 and its metabolites in tissues is shown in Table V. The presence and identity of 9306 and its sulfoxide and sulfone derivatives in all tissues were confirmed, as were O-analog sulfoxide and phenol sulfoxide in liver, and phenol sulfone in kidney and liver. Analyses of the O-analog and O-analog sulfone found in liver extracts did not lead to definitive mass spectral characterizations; therefore, their identifications are supported only by two-dimensional TLC.

DISCUSSION

An examination of the array of radioactive products identified in the studies with ^{14}C-labeled 9306 suggests that the pathway for its metabolism in rats is fully comparable to those elucidated for other organophosphorus compounds with comparable structural constituents (see review by Bull, 1972). In other words, the molecule is involved in a series of oxidation reactions that result in replacement of the thiono sulfur atom with oxygen and the addition of first one and then two oxygen atoms to the thioether sulfur. This leads to the formation of five potentially toxic oxidation products. However, the toxic materials are rapidly detoxified apparently by enzymes that cleave the molecules at P–O–phenyl linkages and by others that mediate formation of conjugates with the liberated substituted phenols. Indeed, essentially all of an administered dose of 9306 is eliminated in the urine within 24 hr; this includes only traces of intact organophosphorus compounds or free

Table V. GLC-Mass Spectral Properties of 9306 Analogs from Synthesis and of Metabolites from Rats Treated with ^{14}C-Labeled 9306

Compound	GLC retention, min[a]	Diagnostic ions (*m/e*) obsd in compounds from indicated source[b]				
		Synthetic[c]	Kidney	Liver	Muscle	Urine[d]
9306	2.4	322 (P, B), 280, 156, 155, 140, 139	322, 280, 156, 155, 140, 139	322, 280, 156, 155, 140, 139	322, 280, 156, 155, 140, 139	-
9306 sulfoxide	6.2	338 (P), 322, 296, 281, 141 (B)	338, 322, 296, 281, 141	338, 322, 296, 281, 141	338, 322, 296, 281, 141	-
9306 sulfone	7.1	354 (P), 312, 188 (B)	354, 312, 188	354, 312, 188	354, 312, 188	-
O-Analog	1.8	306 (P, B), 140, 139	-	-	-	-
O-Analog sulfoxide	4.8	322 (P), 307 (B), 306, 140, 139	- -	322, 307, 306, 140, 139	307, 306, 140, 139	- -
O-Analog sulfone	5.9	338 (P), 296, 172 (B)	-	-	-	-
Phenol sulfide	1.0	140 (P, B), 125	-	-	-	140, 125
Phenol sulfoxide	1.1	156 (P), 141 (B)	-	156, 141	-	156, 141
Phenol sulfone	1.5	172 (P), 157, 93 (B)	172, 157, 93	172, 157, 93	-	172, 157, 93

[a] Column temperature 125°C (phenol sulfide), 175°C (phenol sulfoxide, phenol sulfone), all others 200°C. [b] Dashes indicate that the indicated compounds were either not observed (see Table VI) or that GLC-mass spectral analysis did not result in definitive characterization. [c] Ions followed by letters in parentheses indicate parent (P) and base peak (B) ions. [d] Compounds obtained by acid hydrolysis of urinary conjugates.

Table VI. Metabolism of ^{14}C-Labeled 9306 after Oral Treatment of Rats at 70 mg/kg; Radioactive Compounds in Tissues at 3 hr Posttreatment

Nature of radioact.	% of each product in indicated tissue		
	Kidney	Liver	Muscle
Organosoluble			
9306	11.3	23.2	32.4
9306 sulfoxide	21.1	13.4	41.2
9306 sulfone	12.4	14.7	21.1
O-Analog	0.0	5.8	0.0
O-Analog sulfoxide	0.0	2.0	4.7
O-Analog sulfone	0.0	2.4	0.0
Phenol sulfide	0.0	2.7	0.0
Phenol sulfoxide	0.0	2.1	0.0
Phenol sulfone	5.8	15.4	0.6
Water Soluble[a]			
Phenol sulfide	4.9	4.9	0.0
Phenol sulfoxide	14.8	1.8	0.0
Phenol sulfone	23.3	7.0	0.0
Unknown(s) A	6.4	4.6	0.0
Total μg-equiv extracted	900	1339	281

[a] Compounds in ethyl acetate extract after hydrolysis of the aqueous fraction with acid.

phenols, and the balance is represented by water-soluble materials. Based on the results of a similar study (Bull and Stokes, 1970) of Allied Chemical ACD-6506 [dimethyl *p*-(methylthio)phenyl phosphate], which contains an identical substituted phenyl group, it is likely that the water-soluble materials included conjugates of the respective intact phenols with glucuronic and sulfuric acids. In neither study was there any evidence of rupture or other alteration of the substituted phenyl group. Of the radioactivity associated with different samples of the urine of rats treated with ^{14}C-labeled 9306, a maximum of only 3.5% of the administered dose was not identified and, in kidney and liver tissues, all but 6.4 and 4.6%, respectively, was identified.

Thus, these studies have shown that radioactivity associated with ^{14}C-labeled 9306 is rapidly metabolized and eliminated from rats following oral treatment, and that there are no apparent problems with respect to lingering residues in tissues.

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