

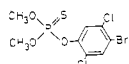
Absorption, Distribution, and Metabolism of *O*-(4-Bromo-2,5-dichlorophenyl)-*O,O*-dimethylphosphorothioate (Bromophos) in the Rat

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O-(4-Bromo-2,5-dichlorophenyl)-*O,O*-dimethylphosphorothioate is absorbed from the intestine of rats. The distribution pattern shows no accumulation. The main excretory route is through the urine. The metabolic detoxification

takes place by the hydrolysis of the methyl phosphate and/or the phenyl phosphate bond. Bromophos or its oxygen analog is not found in the urinary excretion products.

O-(4-Bromo-2,5-dichlorophenyl)-*O,O*-dimethylphosphorothioate, common name Bromophos



(30), is a new phosphorothioate insecticide (24) of extremely low mammalian toxicity (12).

Bromophos is widely used in public health in controlling mosquitoes and flies (2, 5, 15, 16, 22, 26, 31), in crop protection mainly for the control of vegetable root flies (10, 11, 14, 20), and in storage pest control (13, 21) as well as in household insecticides. Since Bromophos is also a very effective agent in the veterinary field (6, 7), its fate in mammals is of interest.

Methods

Phosphorus³²-Labeled Bromophos (Bromophos-P³²) was synthesized according to Sehring and Zeile (24), Radiochemical Centre, Amersham, Bucks., England. The specific activity at the beginning of the experiments was 1.8 mc. per mmole. The purity of the substance was approximately 95% using thin-layer chromatography (solvent system I, Table I). Six impurities could be detected. The crude product was purified to a purity grade more than 99% by column chromatography on silica gel (E. Merck AG., Germany, 0.05 to 0.2 mm.) using carbon tetrachloride. The product was identical in all respects with authentic Bromophos.

Tritium-Labeled Bromophos (Bromophos-T) was synthesized using dimethyl thiophosphoryl chloride and 2,5-dichloro-4-bromophenol-3,6-T (Radiochemisches Laboratorium der Farbwerke Hoechst AG., Frankfurt/Hoechst, Germany). It had a specific activity of 120 mc. per mmole. For the biochemical studies the labeled material was diluted with nonlabeled Bromophos to an activity of 2.5 mc. per mmole. The highly active Bromophos-T had a purity of more than 99% and was identical with authentic Bromophos (29).

Animal Housing and Application. Male rats of the Wistar strain weighing 200 to 250 grams were housed in metabolism cages allowing the separate collection of urine and feces. The animals were fasted for 24 hours before administration. Subsequently the rats were given food (Altromin R) and water *ad libitum*. Bromophos-P³² or Bromophos-T was dissolved in Carbowax 300 at a concentration of 2 mg. per ml. The solution was

administered orally by intubation or intravenously by injection into the tail vein.

The bile liquid experiments were carried out by the usual technique on urethane-narcotized rats (4). The solution of Bromophos-P³² or Bromophos-T was injected intraduodenally. For excretion studies a solution of 2 mg. of dichlorophenol-3,6-T per milliliter of aqueous propylene glycol (10% water) was administered subcutaneously. Its specific activity was 17.5 μ c. per ml. of solution. The nonlabeled 2,5-dichloro-4-bromophenol was dissolved to a 20% (w./v.) solution in aqueous propylene glycol (25% water) and administered subcutaneously.

Measurement of Radioactivity. The radioactivity was determined in the organs, urine, and feces according to the method of Herberg (9). For blood level determination, the blood of the treated animals was taken from the tail vein after different postdosage times and

Table I. *R_f* Values ($\times 100$) of Bromophos, Its Derivatives, and the Urinary Metabolites in Different Solvent Systems, TLC

	Solvent Systems ^a					
	I	II	III	IV	V	IV
Bromophos	54	70	72	87	81	...
Bromoxon ^b	20	64	29	84	79	...
Dichlorobromophenol	35	52	31	81	84	7
Monodesmethylbromophos	0	0	0	62	58	28
Monodesmethylbromoxon	0	0	0	62	57	41
Dimethyl thionophosphate	0	0	0	29	30	...
Urine metabolite A (P ³²)	0	0	0	4	4	...
Urine metabolite B (P ³²)	0	0	0	10	10	...
Urine metabolite C (P ³²)	0	0	0	27	30	...
Urine metabolite D (P ³²)	0	0	0	51	50	...
Urine metabolite D (T)	0	0	0	49	51	...
Urine metabolite E (P ³²)	0	0	0	62	59	...
Urine metabolite E (T)	0	0	0	62	58	28
Urine metabolite F (T)	35	52	31	81	84	7

^a Solvent systems used
Silica gel G (Merck, Darmstadt, Germany) + fluorescent dye ZS Super

I. Hexane-acetone 4 to 1
II. Benzene-methanol 95 to 5
III. Gasoline-benzene-ethanol 30:10:3
IV. Butanol-acetic acid-water 4:1:5
V. Butanol-formic acid-water (upper phase) 50:5:25
(upper phase)
Cellulose powder 300 G (Macherey & Nagel, Düren, Germany) + fluorescent dye ZS Super
VI. Saturated (NH₄)₂SO₄ solution-water-1M Na-acetate solution-2-propanol 52:28:18:2 (25).

^b *O,O*-Dimethyl-*O*-(2,5-dichloro-4-bromophenyl)phosphate.

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the radioactivity determined by liquid scintillation counting (9). The bile samples were taken every 20 minutes from each animal separately and weighed 100 to 400 mg. These samples were diluted with liquid scintillator and measured directly.

Samples with P³²-labeled material were measured in a Packard liquid scintillation counter Tri-Carb 314 A—high voltage 670 volts, discriminator setting 100 to 1000. The counting efficiency was about 74%. Tritium samples were measured in a Packard liquid scintillation counter Tri-Carb 3000—gain 50%, discriminator setting 50 to 1000. The counting efficiency was about 23%. The quench-effect correction was carried out by the internal standard technique.

Thin-Layer Chromatography (TLC). TLC was used to trace and identify the metabolites. For composition of the solvent systems see Table I. The TLC was carried out according to the usual methods (28).

The compounds containing the phenyl group can be located in ultraviolet light at 254 m μ . They can also be stained after hydrolysis *in situ* with NaOH (10 minutes, 100° C.) by spraying with diazotized sulfanilic acid or Echtblausalz B (28). Compounds with a thiono bond are detected with HCl-PdCl₂ solution (1).

P³²-labeled substances were preferably identified by autoradiography using Osray-Roentgen film (Gevaert, Antwerpen, Belgium). The exposure time was varied according to the activity of the material to be used. Under the used conditions, 10⁵ decays per sq. cm. induce a slight blackening which is just visible. Tritium-labeled compounds were detected by means of the thin-layer scanner (Berthold, Wildbad, Germany).

Determination of Dichlorobromophenol. The enzymatic breakdown of the dichlorobromophenol-glucuronide-sulfate complex was carried out by incubating the urine for 24 hours at 37° C., with glucuronidase-aryl-

sulfatase enzyme preparation (C. F. Boehringer & Soehne, Mannheim, Germany) in 0.1N acetate buffer, pH 4.6. The dichlorobromophenol was extracted with chloroform and determined by the method of Smith and Thiess (27) using the 4-aminoantipyrine complex.

Results and Discussion

Distribution of Bromophos in the Rat. Male rats were administered orally 10 mg. per kg. of Bromophos-P³² and Bromophos-T, respectively. After different postdosage periods, two animals each were sacrificed. Table II shows the distribution of radioactivity in organs, urine, and feces. After intravenous and intraperitoneal application of Bromophos-P³² the distribution of radioactivity was roughly the same.

These studies show that the material is well absorbed from the gastrointestinal tract. In the first 12 hours after administration, larger quantities of radioactivity are found in the stomach, intestine, and liver, as well as in the kidneys. After 24 hours the elimination, however, is practically complete. There was no accumulation of the insecticide in any of the examined organs.

Determination of Excretion Rates. Two groups of two rats were administered 10 mg. per kg. of Bromophos-P³² by oral intubation. Two other groups of two rats were given the same dose of Bromophos-T. Subsequently, the radioactivity in the excretion products was determined, and the percentages of dose excreted were calculated (Table III). The excretion of dichlorobromophenol was determined in separate experiments after subcutaneous administration of 10 mg. per kg. of dichlorobromophenol-3,6-T and 520 mg. per kg. of non-labeled dichlorobromophenol, respectively. Dichlorobromophenol is excreted conjugated with glucuronic and/or sulfuric acid. In the nonlabeled study, these conjugates broke down enzymatically (Table IV).

Table II. Distribution of Radioactivity after Oral Administration of 10 Mg. per Kg. of Bromophos, Mean Value of Two Animals

Substance Animals sacrificed after hours	Bromophos-P ³²								Bromophos-T							
	6		12		24		48		8		24		48			
	% ^a	%/g. ^b	%	%/g.	%	%/g.	%	%/g.	%	%/g.	%	%/g.	%	%/g.		
Liver	6.6	0.8	2.9	0.4	2.0	0.3	1.6	0.2	1.6	0.2	0.2	0.02	<0.1	...		
Kidney	2.5	2.1	0.9	0.9	0.5	0.4	0.5	0.4	0.5	0.3	<0.1	...	<0.1	...		
Stomach	4.6	...	2.3	...	1.2	...	0.8	...	21.3	1.7	0.2	0.02	<0.1	...		
Small intestine									5.8	0.6	0.1	0.01	<0.1	...		
Large intestine	4.6	...	3.2	...	2.0	...	0.7	...	4.5	0.6	0.2	0.04	0.1	0.1		
Heart	0.3	0.4	<0.1	...	<0.1	...		
Spleen	<0.1	...	<0.1	...	<0.1	...		
Lungs	0.1	...	<0.1	...	<0.1	...		
Mesenteric fat	0.1	...	<0.1	...	<0.1	...	<0.1	...	<0.1	...	<0.1	...	<0.1	...		
Muscle (leg)	0.1	0.1	<0.1	...	<0.1	...	<0.1	...	<0.1	...	<0.1	...	<0.1	...		
Brain	<0.1	...	<0.1	...	<0.1	...		
Feces	6.8	...	6.2	...	13.5	...	0.8	0.5	3.1	0.76	7.8	0.65		
Urine	40.0	...	66.1	...	66.8	...	64.3	...	56.8	7.5	96.0	8.40	91.5	4.65		
Residual rate	42.6	...	9.9	...	6.8	...	8.4	...	5.4	...	1.7	...	1.3	...		
Total	101.0	...	92.1	...	85.5	...	89.8	...	97.1	...	101.5	...	100.7	...		

^a Radioactivity in organs (% of applied dose).

^b Per cent applied dose per gram of organ.

Table III. Excretion of Radioactivity in Per Cent of Applied Dose after Oral Administration of 10 Mg. per Kg. of Bromophos-P³² or Bromophos-T

Time, Hours	Bromophos-P ³²			Bromophos-T		
	Urine, %	Feces, %	Urine + feces, %	Urine, %	Feces, %	Urine + feces, %
24	57.6	13.7	71.3	95.9	1.0	96.9
48	2.7	2.1	4.8	4.1	0.9	5.0
72	0.9	0.4	1.3	1.0	0.1	1.1
96	0.7	...	0.7	1.6	...	1.6
Cage	0.6	...	0.6	0.6	...	0.6
Total	62.5	16.2	78.7	103.2	2.0	105.2

Table IV. Excretion of Tritium-Labeled and Nonlabeled Dichlorobromophenol after Subcutaneous Administration of Different Doses

Time, Hours	Dichlorobromophenol-3,6-T, 10 Mg./Kg.			Dichlorobromophenol, ^a 520 Mg./Kg.		
	Urine, %	Feces, %	Urine + feces, %	Urine, %	Feces, %	Urine + feces, %
24	84.2	3.7	87.9	73.5	6.6	80.1
48	1.4	6.0	7.4	13.9	2.9	16.8
72	0.6	0.2	0.8	3.1	1.2	4.3
96	0.3	2.0	2.3	2.6	1.0	3.6
120	0.4	0.6	1.0
Cage	3.3	...	3.3
Total	89.8	11.9	101.7	93.5	12.3	105.8

^a Determined as free plus conjugated dichlorobromophenol.

Table III shows that the excretion of the total amount of the administered radioactivity depends on whether Bromophos-P³² or Bromophos-T is used. In the first case, only about 80% of the administered radioactivity could be found in the urine and feces. This finding is reasonable because the final step of the metabolism of Bromophos will be (radioactive-labeled) inorganic phosphate, which will be incorporated into the phosphate pool of the organism. The phenolic part of Bromophos (dichlorobromophenyl moiety), however, which could be of toxicological interest, is excreted rapidly; 24 hours after application of Bromophos-T, the radioactivity is almost quantitative in the urine. Since administered dichlorobromophenol at the same and even at a higher dosage is eliminated quantitatively in a short period, there is no accumulation of the phenolic part of Bromophos in rats after a single dose.

Excretion of Bromophos via the Bile. Three rats each were administered intraduodenally 10 mg. per kg. of Bromophos-P³² and 5 mg. per kg. of Bromophos-T, respectively. The bile liquid was collected in fractions and the radioactivity determined (Table V). After administration of Bromophos-P³², only about 1% of the radioactivity was in the bile within 8 hours. The excretion rate of Bromophos-T amounted to about 25% within the same time. In the latter case the highest level of radioactivity was reached after about 1.5 to 2 hours. These studies show that neither unchanged

Table V. Excretion of Radioactivity via the Bile after Intraduodenal Administration of 10 Mg. per Kg. of Bromophos-P³² and 5 Mg. per Kg. of Bromophos-T, Respectively, Mean Value of Three Animals

Time	Administered Activity, %	Bromophos-P ³²		Bromophos-T	
		Hours	Minutes	Hours	Minutes
			20		
1			40	0.023	0.48
		00		0.035	1.33
		20		0.044	1.77
2			40	0.043	2.25
		00		0.044	1.57
		20		0.047	1.51
3			40	0.044	1.32
		00		0.043	1.16
		20		0.054	1.19
4			40	0.042	1.17
		00		0.050	1.02
		20		0.042	0.94
5			40	0.038	1.13
		00		0.038	1.21
		20		0.038	1.13
6			40	0.035	1.10
		00		0.032	1.01
		20		0.032	0.99
7			40	0.034	0.96
		00		0.034	0.79
		20		...	0.81
8			40	0.032	0.75
		00		0.021	0.61
Total				0.845	26.20

Bromophos nor its phosphorus-containing metabolites follow the enterohepatic cycle. The tritium activity in the bile must, therefore, be due to dichlorobromophenol and its metabolites. The biliary-excreted tritium activity is again absorbed from the intestine, however, as shown by excretion experiments with Bromophos-T when only about 2% of the orally administered tritium activity appeared in the feces (Table III). The relatively high P³² content in the feces obviously results from the metabolism of Bromophos in the intestine or from the reabsorption of phosphate-containing metabolites in the intestine.

Blood Level Determination. Ten rats were administered Bromophos-P³² perorally at a dose of 10 mg per kg. Another 10 rats were administered Bromophos-P³² intravenously at a dose of 5.5 mg. per kg. The blood was taken from the tail vein of each rat after different postdosage times, and the radioactivity of each of the samples was measured separately according to Herberg (9). The blood level at different post-dosage times is given in Figure 1.

The slopes of the blood level curves demonstrate that Bromophos is eliminated with a half lifetime of about 14 hours corresponding to an eliminating constant $K_2 = 0.05 h^{-1}$. From this value and the time of reaching the maximum blood level at about 7 hours after oral intubation the absorption constant [Dost (3) and Rescigno (19)] is calculated as $K_1 = 0.31 h^{-1}$.

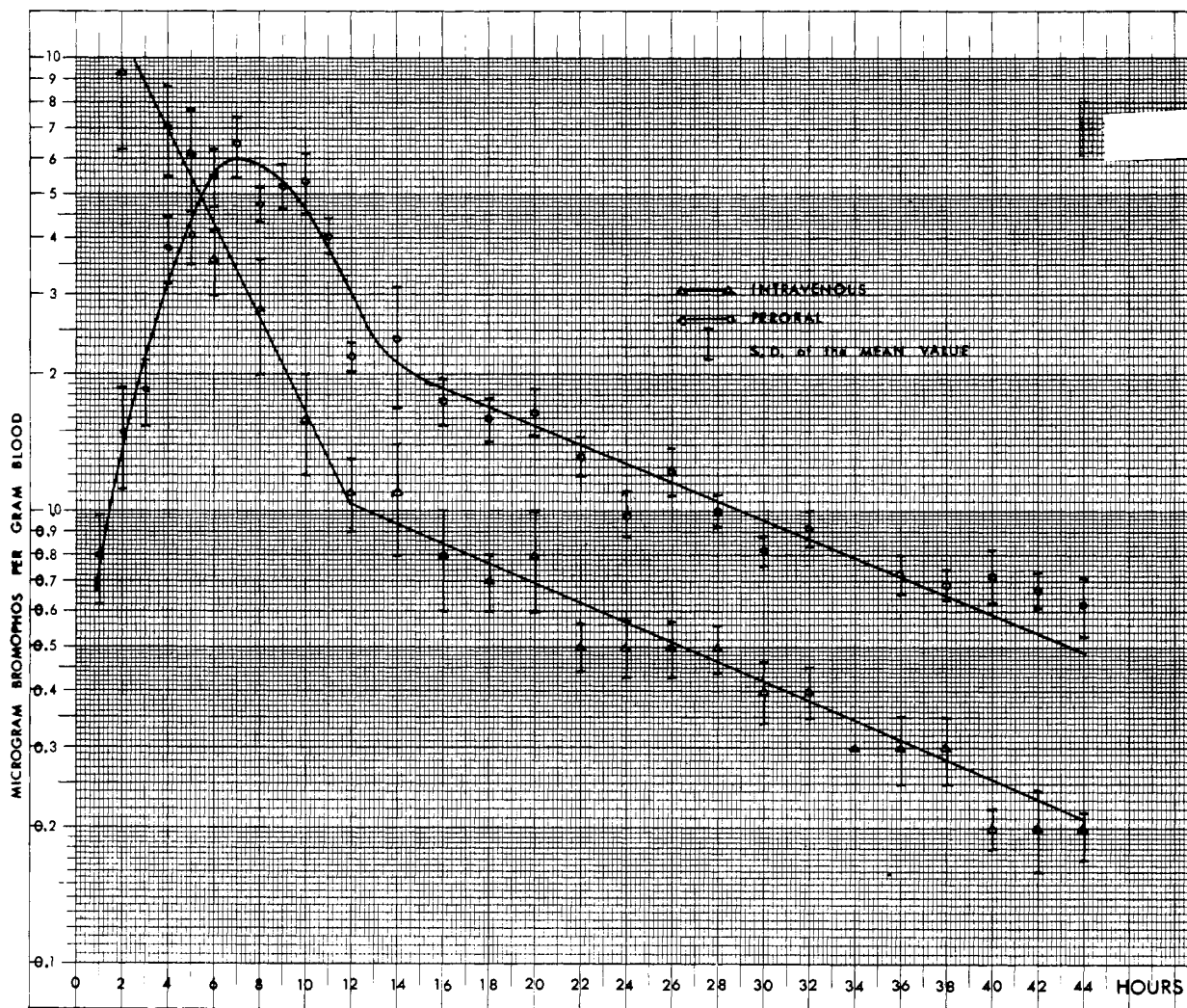


Figure 1. Blood level ($\mu\text{g./g.}$) after oral intubation of 10 mg. per kg. and intravenous administration of 5.5 mg per kg. of Bromophos- P^{32}

Metabolism of Bromophos in the Rat. Table V shows that five metabolites of Bromophos could be detected in the urine of rats after application of P^{32} -labeled material, whereas three radioactive excretion products were found after giving Bromophos tritiated in the phenyl ring. These metabolites are more hydrophilic than Bromophos itself.

The urinary metabolites A to E were estimated by TLC and the per cent of each metabolite (0 to 48 hours) excreted is given in Figure 2. Metabolites A, B, and C contain phosphorus but no dichlorobromophenol; compounds D and E contain both phosphorus and the phenolic component; and metabolite F contains dichlorobromophenol but no phosphorus. From the chromatographic behavior, there is good evidence that compound A is identical with phosphate and C with dimethyl thionophosphate. Compounds B and D have not yet been identified. The metabolic product E was identified as monodesmethylbromophos [*O*-methyl-*O*-(2,5-dichloro-4-bromophenyl)-phosphorothioate] by cochromatography as well as by isolating radioactive dichlorobromophenol after alkaline hydrolysis. Compound F was identified as dichlorobromophenol by

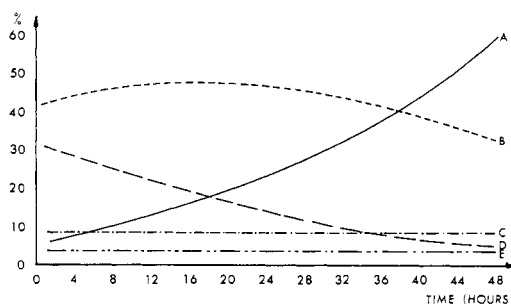


Figure 2. Excretion of metabolites A to E after oral administration of 10 mg. of Bromophos- P^{32} per kg. from rat urine

cocrystallization together with authentic nonlabeled dichlorobromophenol to constant specific activity. The identification of metabolite F was also carried out by cochromatography.

Bromophos *per se*, its *O*-analog Bromoxon, and monodesmethyl-Bromoxon were not found in the urine. This may be interpreted that either Bromophos is metabolized—i.e., oxidation of Bromophos to Bro-

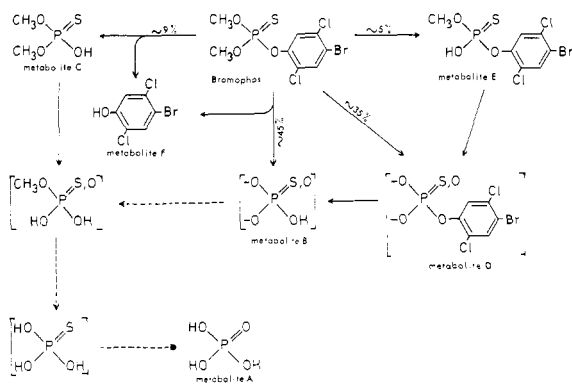


Figure 3. Metabolic pathway as suggested for Bromophos

moxon and its hydrolytic splitting—so rapidly that only small amounts of the oxygen intermediates are present to be identified—or that this route is not followed. Since it was demonstrated on the other hand by *in vitro* and *in vivo* studies that Bromophos is a weak indirect cholinesterase inhibitor (18), the oxidative metabolic pathway cannot be excluded completely.

From the metabolic products found in the rat the suggested pathway of metabolism of Bromophos is shown in Figure 3. This scheme is in good accordance with the metabolism proposed for the related *O,O*-dimethyl-*O*-(2,4,5-trichlorophenyl)phosphorothioate (ronnel) (17) and corresponds to other dimethyl thiophosphate insecticides (8, 23).

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