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ANIMAL METABOLISM OF INSECTICIDES

Bovine Metabolism of Organophosphorus Insecticides. Metabolism and Residues Associated with Oral Administration of Dimethoate to Rats and Three Lactating Cows

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Dimethoate is active as a systemic insecticide for cattle. Three lactating cows were treated orally with the radioactive compound and analysis of blood, tissues, excreta, and milk showed Dimethoate to be rapidly metabolized and excreted. Twelve days after treatment, the insecticide was found in trace amounts only in the cow tissues. Hydrolysis of Dimethoate by rats and cows occurred initially at the methyl-phosphate, phosphate-sulfur, sulfur-carbon, and particularly at the carbonyl-nitrogen bonds. Phosphorothioate oxidation occurred with certain of the hydrolysis products and was assumed to occur also with Dimethoate.

DIMETHOATE or Am. Cyanamid 12,880 [*O,O*-dimethyl *S*-(*N*-methylcarbamoylmethyl)phosphorodithioate] has shown promise as a chemotherapeutic agent for the control of cattle grubs. Oral administration to calves of 10 to 40 mg. per kg. yields excellent grub control (*5*). When tested as an animal systemic against *Aedes* mosquitoes, it gave excellent control during the first 24 hours, but decreased rapidly thereafter (*4*). This study concerns the residues and metabolites associated with the proposed use of Dimethoate as an animal systemic insecticide.

Methods and Results

Chemical Studies

Synthesis and Characterization of Radioactive Dimethoate Derivatives. Radioactive *O,O*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate was prepared from phosphorus-32 pentasulfide obtained by neutron irradiation (Atomic Energy Commission, Oak Ridge, Tenn.) or by isotopic exchange with phosphoric-32 acid (*1*). Radioactive *O,O*-dimethyl phosphorodithioic acid was then prepared (*9*) and neutralized with potassium hydroxide to yield the potassium salt in aqueous solution. An amount of *N*-methyl α-chloroacetamide equimolar to the potassium *O,O*-dimethyl phosphorodithioate was dissolved in water, layered with chloroform, and the two-phase system stirred rapidly and refluxed at 85° C. as the potassium *O,O*-dimethyl phosphorodithioate solution was added dropwise over a 30-minute period (*14*). After an additional 15 minutes of refluxing, the chloroform layer was separated, and the aqueous phase extracted three times with an equal volume of chloroform. The product formed in 60% yield had a specific activity of about 5 mc. per gram and it was purified on a silica gel column (Figure 1). *O,O,S-*

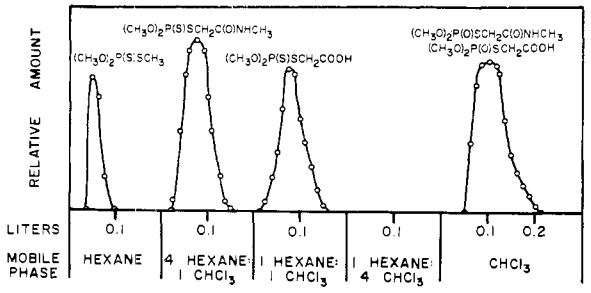

Figure 1. Chromatographic separation on silica gel columns of Dimethoate and certain possible metabolites

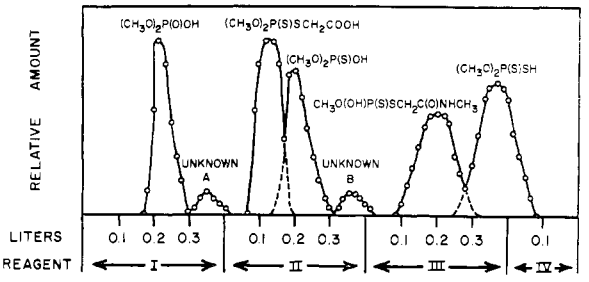

Figure 2. Ion exchange separation on Dowex 1 of metabolites of Dimethoate

Reagents: I, elution gradient pH 2 to pH 1 HCl; II, elution gradient pH 1 HCl plus methanol (1 to 3) to 1N HCl plus methanol (1 to 3); III, elution gradient 1N HCl plus acetone (1 to 3) to concentrated HCl, water, and acetone (1 to 1 to 6); IV, concentrated HCl, water, and acetone (1 to 1 to 6)

Trimethyl phosphorodithioate, present as an impurity of less than 1.0% was removed by this column purification. The radioactive product was identical in infrared spectrum and rate of hydrolysis in 0.1 molar aqueous sodium carbonate to a known sample of highly purified Dimethoate.

Radioactive *O,O*-dimethyl *S*-carboxymethyl phosphorodithioate—designated as the carboxy derivative of Dimethoate—was prepared in 70% yield according to the same procedure as for Dimethoate except that α-chloroacetic acid replaced *N*-methyl α-chloroacetamide (*14*). This radioactive product was identical in chromatographic properties and infrared spectrum to a known nonradioactive sample supplied by American Cyanamid Co.

Radioactive potassium *O*-methyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate—designated as the des-methyl derivative of Dimethoate—was prepared by refluxing 1.0 molar equivalent of radioactive Dimethoate with 1.6 molar equivalents of nonradioactive potassium *O,O*-dimethyl phosphorodithioate in acetone for 2 hours (*14*). The acetone was then evaporated, the residue dissolved in water and extracted first with *n*-hexane and then with chloroform. The *n*-hexane extract contained 0.8 molar equivalent of *O,O,S*-trimethyl phosphorodithioate which was identified by comparison of its infrared spectrum with that of the known derivative. Unreacted Dimethoate was present in the chloroform extract based on an infrared spectrum. The water was removed under reduced pressure to leave the potassium salt of the radioactive desmethyl derivative and nonradioactive potassium *O,O*-dimethyl phosphorodithioate. These salts were insoluble in chloroform but could be readily converted to the free acids by reaction in acetone with hydrochloric acid followed by filtration to remove the potassium chloride. When the radioactive desmethyl derivative was chromatographed on an ion exchange column, it was eluted as a single radioactive compound in a position different from (the other) known phosphorothioic acid esters investigated (Figure 2).

Dimethoate and the carboxy derivative were individually reacted with equimolar anhydrous peracetic acid in chloroform for 12 hours at 28° C. The oxidation products were purified on a silica gel column. The yields from Dimethoate and the carboxy derivative of products chromatographing in a position different from the original phosphorothioates were about 30 and 45%, respectively (Figure 1). When the oxidized carboxy derivative was chromatographed on an ion exchange column, only *O,O*-dimethyl phosphoric acid was recovered indicating instability under these conditions. Infrared spectra on the oxidized Dimethoate (Dimethoate-P=O) and the oxidized carboxy derivative after purification on silica gel were almost identical with the phosphorodithioates with the exception of the loss of an absorption peak at 15.6 microns and the formation of a strong peak at 8.0 microns. These spectral changes are consistent with the formation of phosphorothiolate derivatives through oxidation with peracetic acid.

The infrared spectra were obtained with the compounds as 10% solutions in chloroform using sodium chloride optics in a Baird Model B double beam infrared spectrophotometer. Dimethoate, Dimethoate-P=O, the carboxy derivative, and the carboxy P=O showed carbonyl absorption peaks at 6.0, 6.0, 5.9, and 5.9 microns, respectively. Dimethoate and its oxygen analog had an N—H stretching peak at 3.0 microns, while the latter three compounds had a broad peak at 3.4 microns.

Partition and Ion Exchange Chromatography. Partition chromatography with silica gel columns (*13*) served to separate certain Dimethoate impurities and possible metabolites (Figure 1). Dimethoate, the carboxy derivative and *O,O,S*-trimethyl phosphorodithioate were readily separated with different hexane-chloroform mixtures, while the phosphorothiolate analogs were all eluted with chloroform.

Ion exchange chromatography (*10*) separated several hydrolysis products of Dimethoate when an elution gradient of hydrochloric acid, methanol, and acetone were employed (Figure 2).

Metabolism of Dimethoate and Derivatives in Rats

Rate of Excretion and Nature of Excretory Products. One-hundred-and-fifty-gram male and female rats were treated orally at 100 mg. per kg. with radioactive Dimethoate in corn oil solution *via* a stomach tube. The cumulative per cent excretion was determined separately for the sexes using metabolism cages which allowed separation of the urine and feces (*3*). Four female and 10 male rats were used. The results are shown in Figure 3.

The nature and proportions of the hydrolysis products excreted in the urine by the male rats were analyzed using ion exchange chromatography (Table I). The proportion of *O,O*-dimethyl phosphoric acid increased with time after administration. However, the remaining identified hydrolysis products did not change appreciably in proportion during the same period of time.

Rats were also treated orally with the radioactive Dimethoate-P=O, carboxy and des-methyl derivatives. The urine from two surviving male rats treated orally at 50 mg. per kg. with Dimethoate-P=O was collected at 12, 24, and 48 hours. The cumulative per cent of the administered radioactivity excreted over the indicated times were 16, 19, and 30%, respectively. Utilizing ion exchange chromatography the metabolites found in the 48-hour urine composite were *O,O*-dimethyl phosphoric acid, unknown *A*, *O,O*-dimethyl phosphorothioic acid, and unknown *B* at 34, 52, 9.5, and 4.5%, respectively. Radioactive carboxy derivative was administered orally to three male rats at 100 mg. per kg., and a sample of the 24-hour urine composite was chromatographed. The metabolites found were the carboxy derivative, *O,O*-dimethyl phosphorodithioic acid, *O,O*-dimethyl phosphorothioic acid, and *O,O*-dimethyl phosphoric acid at 65, 23, 9, and 3%, respectively. Radioactive des-methyl derivative was administered to rats at

Table I. Nature of Dimethoate Metabolites in the Urine of Male Rats and a Steer Treated Orally at 100 Mg. per Kg.

Hours after Administration	Various Hydrolysis Products in the Urine, % $(CH_3O)_2P(O)OH$	Carboxy	Carboxy + $(CH_3O)_2P(S)OH$	$(CH_3O)_2P(S)OH$	Des-methyl	$(CH_3O)_2P(S)SH$	Unknowns A + B
			MALE RATS				
2	6.2	32.4	...	20.5	12.0	23.4	5.5
4	19.6	21.1	...	25.8	5.2	18.3	10.0
12	11.4	42.4	...	20.4	1.2	21.4	3.2
18	12.4	...	63.9[a]	...	1.2	21.0	1.5
24	17.6	35.2	...	26.6	1.8	17.2	1.6
48	21.7	22.0	...	32.7	5.5	17.4	0.7
Composite 0–168 hrs.	12 5	...	63.4[a]	...	2.7	19 2	2.2
			STEER				
1.2	2.1	74.0	...	9.0	2.1	11.6	1.2
2.3	4.3	...	84.5[a]	...	2.1	6.2	2.9
4.2	11.9	...	61.0[a]	...	8.1	15.1	3.9
6 1	28.0	22.9	...	28.9	10.3	8.0	1.9
12.3	44.4	11.1	...	25.8	8.3	6.9	3.5
19.6	56.3	...	31.0[a]	...	2.7	7.4	2.6
30.1	45.7	18.3	...	12.5	3.9	17.4	2.2
Composite[b] of 0 to 30.1 hrs.	32.6 ± 2.1	24.8[c]	45.8 ± 0.67	21.0[c]	7.1 ± 1.1	12.6 ± 4.0	1.9 ± 1.7

[a] The resolution of these columns was inadequate to differentiate the carboxy and $(CH_3O)_2P(S)OH$ fractions. [b] Variability from three analyses indicated as standard deviation. [c] Average of two values.

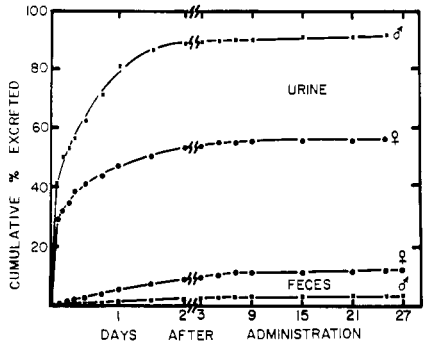

Figure 3. Cumulative per cent excreted by male and female rats treated at 100 mg. per kg. of Dimethoate

Table II. Tissue Residues Expressed as Total Parts per Million Dimethoate Equivalents after Oral Administration of Dimethoate to Male Rats at 100 Mg. per Kg.

Tissue	Dimethoate Equivalents, P.P.M., after Hours Indicated 24	72	168	336	672
Liver	10.1	2.0	1.2	0.58	0.53
Heart	2.1	0.08	0.12	0.22	0.07
Kidney	5.6	0.61	0.38	0.20	0.03
Brain	1.1	0.26	0.49	0.14	0.16
Fat	2.0	0.32	0.30	0.11	<0.01
Muscle	1.6	0.25	0.29	0.08	0.09
Skin	5.9	4.1	1.8	2.1	0.32
Blood	2.8	0.11	0.14	...	0.02
Bone	2.8	1.80	1.8	...	...

10 mg. per kg., and the 12-hour urine sample was chromatographed. Eighty-five per cent of the radioactivity which was excreted in the urine chromatographed with nonradioactive des-methyl carrier. The remaining 15% was eluted, with no definite peak, before and after the des-methyl peak.

Tissue Residues. Samples of 21 tissues were taken at the time of sacrifice of the Dimethoate-treated rats and the total parts per million of Dimethoate equivalents were determined. A portion of the data from the male rats is presented in Table II. The highest levels of total radioactivity persisted in the skin, liver, and bone. The dissipation rate of the radioactivity from the kidney was similar to that from the spleen, pancreas, lung thymus, adrenals reproductive tract, submaxillary glands, and lymph nodes. At 24 hours the stomach wall, caecum wall, large intestine, and small intestine, with their respective contents, were the highest of the tissues in total radioactivity. However, the radioactivity in these tissues diminished to below the level in the liver by 72 hours. At 168 hours after treatment, the total Dimethoate equivalents in the tissues from the female rats were 2 to 5 times the level found in the male rats, but this difference was less consistent after 672 hours.

Metabolism of Dimethoate by Lactating Cows

Treatment and Handling of the Cows. Radioactive Dimethoate mixed with bran, encapsulated in gelatin capsules was fed to three lactating cows. One Holstein cow was treated at 40 mg. per kg. while two Jersey cows were treated at 10 and 9 mg. per kg. The three cows were catheterized and held in metabolism stalls to allow for the total separation and collection of urine and feces. Samples of jugular blood, subcutaneous fat, urine, feces, and milk were periodically taken for analysis. The Holstein cow and one Jersey cow were sacrificed 144 hours after administration of Dimethoate; the remaining cow was sacrificed after 288 hours.

Rate of Excretion and Nature of Excretory Products. The percentages of the 9, 10, and 40 mg. per kg. doses excreted during the first 24 hours were 73.0, 72.0, and 55.3, respectively in the urine, and 2.4, 3.2, and 2.4, respectively, in the feces.

Urine samples from a steer treated with 10 mg. per kg. radioactive Dimethoate were analyzed by ion exchange chromatography for the nature and proportions of the hydrolysis products present (Table I). Three milligrams of a radioactive metabolite were recovered from a composite sample of the total cow urine excreted within 288 hours after treatment of a lactating cow at 9 mg. per kg. Recovery was effected by adjusting a portion of the cow urine to pH 1 and extracting with chloroform (*12*) and chromatographing the chloroform-soluble radioactive compound on a silica gel column. This radioactive metabolite was identical in infrared spectrum to a known sample of *O,O*-dimethyl *S*-carboxymethyl phosphorodithioate, and when added to this known nonradioactive sample as a carrier for ion exchange chromatography, the elution peaks of radioactivity and total phosphorus were coincidental. The remaining metabolites, *O,O*-dimethyl phosphoric acid, *O,O*-dimethyl phosphorothioic acid, *O,O*-dimethyl phosphorodithioic acid, and *O*-methyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithioic acid were identified by chromatography with known nonradioactive carrier compounds.

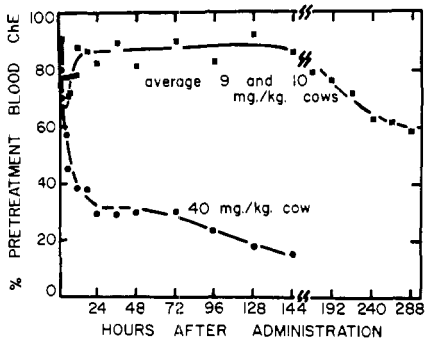

Figure 4. Blood cholinesterase (corpuscle) depression curves for cows fed encapsulated Dimethoate

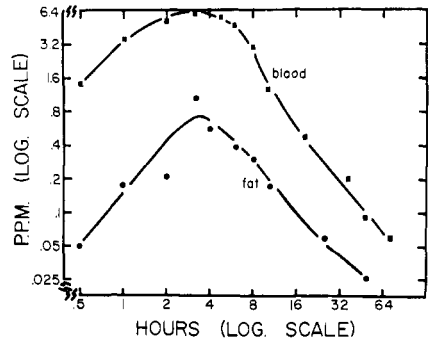

Figure 5. Average parts per million of total Dimethoate equivalents in the blood and subcutaneous fat from the 9.0- and 10-mg.-per-kg. cows

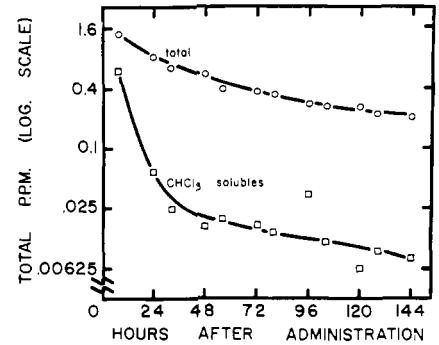

Figure 6. Secretion of Dimethoate and derivatives in milk of a cow after oral administration of 9.0 mg. per kg.

The carboxy derivative of Dimethoate was the predominant hydrolysis product shortly after treatment of the steer. As the proportion of carboxy derivative and dimethyl phosphorothioic acid decreased, there was an almost corresponding increase in the proportion of dimethyl phosphoric acid. The other hydrolysis products were excreted in more uniform proportions throughout the 30.1-hour period. Fractionation of the urine from the three lactating cows at various times after treatment showed the same general relationship in the proportion of the various hydrolysis products.

A composite sample of the total feces excreted by the cow treated with 9 mg. per kg. of Dimethoate and held 288 hours was extracted with water and chloroform by a similar method to that described later for the tissue extractions. The chloroform-soluble radioactivity was chromatographed with nonradioactive Dimethoate carrier on a silica column. Of the administered dose, 3.7% of the total radioactivity appeared in the feces, with 0.015% as chloroform-soluble radioactive compound(s) and only 0.0015% as radioactive compound chromatographing in a similar manner to Dimethoate.

Dimethoate Equivalents in Blood and Cholinesterase Depression. The cholinesterase activity of the jugular blood was determined manometrically (*2*). A sharp depression in blood cholinesterase activity occurred for the cow treated with 40 mg. per kg. within 4 hours after treatment (Figure 4). Symptoms of phosphate poisoning occurred at 24 hours at which time the treated animal went off feed and developed diarrhea. The cow appeared to have fully recovered 96 hours after administration of the compound. No symptoms of phosphate poisoning were observed in the cows treated with 9.0 and 10.0 mg. per kg. nor were any gross pathological abnormalities observed in any of the organs and tissues recorded in Table IV when the three cows were sacrificed.

The total parts per million of Dimethoate equivalents reached a maximum in the blood 3 hours after treatment (Figure 5). The blood was fractionated by diluting 3 ml. of blood to 25 ml. with water and extracting three times with an equal volume of chloroform. The chloroform-soluble radioactivity in the blood reached a maximum of 1.5 p.p.m. at 2 hours for the 9.0- and 10.0-mg.-per-kg. cows; after 48 hours, less than 0.05 p.p.m. of chloroform-soluble radioactivity was found.

Dimethoate Equivalents in Milk. Dimethoate plus its metabolites were secreted in the 0- to 8-hour milk to the extent of 1.5 p.p.m. (Figure 6). For the fractionation of the milk, 100-ml. samples were mixed in a Waring Blendor with 200 ml. of chloroform. After centrifugation, the chloroform and protein layers were filtered through Celite on a Büchner funnel. The mixed Celite and protein layer were then mixed with 200 ml. of acetone in a Waring Blendor, chilled and filtered. The acetone and chloroform extracts were combined and evaporated. The milk fat was then dissolved in hexane and extracted three times with an equal volume of acetonitrile to recover the Dimethoate in the acetonitrile fraction. The acetonitrile was evaporated, the radioactivity determined and further fractionated by chromatography on silica gel column with nonradioactive carrier Dimethoate. The per cent recovery of Dimethoate in milk was determined by adding 4 p.p.m. of radioactive Dimethoate initially to a control milk sample. Immediately after the addition of Dimethoate, the milk sample was fractionated as described previously. Recovery of Dimethoate was only 65%.

With the cow treated at 9.0 mg. per kg., the chloroform-soluble Dimethoate equivalents in the milk were less than 0.02 p.p.m. after 48 hours (Figure 6). By extracting a composite sample representing the total milk secreted over a 288-hour period, only 20% of the chloroform-soluble radioactivity chromatographed coincidently with nonradioactive carrier Dimethoate. This corresponded to 0.0043 p.p.m. of actual Dimethoate secreted in the milk from the composite or 0.0068% of the administered dose of Dimethoate. The cow treated with 40 mg. per kg. secreted 9.5 p.p.m. of total Dimethoate equivalents or 4.0 p.p.m. chloroform-soluble equivalents in the 0- to 8-hour milk sample. Forty-eight hours later, only 0.04 p.p.m. of chloroform-soluble Dimethoate equivalents were secreted in the milk.

Partitioning of Dimethoate into and out of Fat in Vivo. Biopsies of subcutaneous back fat were taken from the cows treated at 9.0 and 10.0 mg. per kg. at various times after the administration of the radioactive Dimethoate. The total p.p.m. of Dimethoate equivalents in the fat compared with Dimethoate equivalents in the blood are shown in Figure 5. The total Dimethoate equivalents reached a maximum in the fat and the blood 3 hours after treatment. The fat was fractionated by mincing the tissue with 25 ml. of water and 100 ml. of chloroform in a Waring Blendor. The remainder of the extraction procedure was identical to that utilized for the milk fractionation. The chloroform-soluble radioactivity reached a maximum in the fat at 3 hours with 0.95 p.p.m. and dropped below 0.1 p.p.m. at the 8-hour period.

Tissue Residues. Samples of 34 tissues were taken on sacrifice of the cows and the total parts per million of Dimethoate equivalents were determined (Table III and IV). Liver, heart, kidney, brain, subcutaneous fat, and loin muscle samples were fractionated. Fifty-gram tissue samples were minced with 100 ml. of water and 200 ml. of chloroform in a Waring Blendor. After centrifugation, the chloroform and protein layers were filtered through Celite on a Büchner funnel. The mixed Celite and protein layer was then mixed with 200 ml. of acetone in a Waring Blendor, chilled, and filtered. The remainder of the fractionation procedure was identical to the milk extraction. Table III presents the parts per million of total Dimethoate equivalents and chloroform soluble equivalents for the tissues fractionated. The chloroform-soluble radioactivity was chromatographed with nonradioactive carrier Dimethoate to deter-

Table III. Nature of Tissue Residues in Dimethoate-Treated Cows

Tissue	% Recovery[a] ± Std. Dev. Used for Correction	Dimethoate Equivalent, P.P.M., in Fraction Indicated							
		9.0 Mg./Kg. — 288 Hours			10.0 Mg./Kg. — 144 Hours			40.0 Mg./Kg. — 144 Hours	
		Total	$CHCl_3$ solubles	Dimethoate	Total	$CHCl_3$ solubles	Dimethoate	Total	$CHCl_3$ solubles
Liver	69.8 ± 2.9	2.1	0.23	0.026	4.4	0.45	0.075	5.0	0.62
Heart	59.6 ± 6.2	0.17	0.052	0.003	0.24	0.084	0.006	0.93	0.35
Kidney	63.5 ± 4.6	0.26	0.14	0.013	0.55	0.19	0.10	1.7	0.25
Brain	51.6 ± 6.5	0.13	0.083	0.004	0.15	0.097	0.009	1.8	0.91
Fat subcutaneous	74.4 ± 5.0	0.007	0.0052	<0.001	0.007	0.005	0.002	0.22	0.0
Muscle loin	65.3 ± 4.4	0.10	0.011	<0.001	0.22	0.006	0.002	0.35	0.046

[a] The per cent recovery was determined by adding 4 p.p.m. of radioactive Dimethoate initially to the tissues and immediately proceeding with the normal extraction procedure.

Table IV. Tissue Residues Expressed as Total Parts per Million of Dimethoate Equivalents after Oral Administration to Dairy Cattle

Tissue	9.0 Mg. per Kg. 288 Hours	10.0 Mg. per Kg. 144 Hours	40.0 Mg. per Kg. 144 Hours
Bile	0.07	0.12	8.82
Bone marrow	0.00	0.05	0.16
Fat			
Kidney	<0.01	0.02	0.09
Mesenteric	<0.01	0.01	0.22
Gall bladder	0.10	0.01	...
Gland			
Adrenal	...	0.58	2.16
Mammary	0.17	0.32	1.15
Parotid	0.14	0.12	0.92
Thyroid	0.13	0.40	1.38
Intestine, small	0.16	0.30	2.16
Lung	0.29	0.86	1.40
Lymph node			
Mammary	0.25	...	0.68
Mesenteric	0.15	0.26	0.86
Subiliac	0.26	0.30	0.92
Submaxillary	0.13	0.55	2.00
Muscle, rear leg	0.10	0.14	0.37
Pancreas	0.34	0.43	1.20
Spinal cord	0.07	0.07	0.41
Spleen	0.24	0.23	1.10
Stomach			
Abomasum	0.20	0.22	0.62
Omasum	0.11	0.19	0.72
Reticulum	0.09	0.21	1.34
Rumen	0.15	0.15	2.50
Sublingual parotid	0.52	2.30	0.92
Skin	0.02	0.06	0.33
Tongue	0.11	0.22	0.26
Urinary bladder	0.09	0.11	0.33
Uterine horn	0.08	0.20	0.57

Average of three replicates ±15% variation.

Table V. Biological Activity of Dimethoate and Derivatives

Compound	LD_{50} Housefly (topical, mg./kg.)	LD_{50} White rat (oral, mg./kg.)
Dimethoate	0.43	600
Dimethoate-P=O	0.10	55
Des-methyl[a]	152	1500–2000
Carboxy	1700	2500–3000
O,O,S-trimethyl phosphoro-dithioate	45	900–1100

[a] An impure sample containing some potassium *O,O*-dimethyl phosphoro-dithioate.

mine the amount of actual insecticide. Of all the tissues fractionated, the liver and kidney had the greatest amount of total Dimethoate equivalents and of actual Dimethoate.

Toxicity of Dimethoate and Its Metabolites. Oral LD_{50} values were determined using male albino rats. The compounds were fed *via* a stomach tube as 15% solutions in propylene glycol. Initially, two rats were treated at each concentration tested, and when the LD_{50} region was identified, five replicates were used. The mortality counts were taken 24 hours after treatment and the approximate LD_{50}'s are given in Table V.

LD_{50} determinations were also made with the adult female housefly *Musca domestica* (L.). Contact insecticidal toxicity was based on 24-hour mortality counts following application of the insecticidal solutions in 1.3 μl. of acetone to individual flies (*6*). Dosage-mortality plots (*8*) yielded the LD_{50} values and the average of three LD_{50} determinations are reported in Table V.

Dimethoate-P=O was the most toxic of all the compounds tested. Whereas Dimethoate and Dimethoate-P=O produced death with typical phosphate symptoms to rats the carboxy derivative *O,O,S*-trimethyl phosphorodithioate and the des-methyl derivative caused death without these symptoms.

Discussion

The rat and cow showed similarities in the types of metabolites and the amounts excreted in the urine, hence the metabolic pathway of Dimethoate in cows and rats can be assumed to be the same.

Dimethoate was susceptible to in vivo degradation through the pathway shown in Figure 7. Evidence obtained on the metabolites indicates that Dimethoate was attacked hydrolytically at five sites on the molecule: P—S, S—C, and the C—N bonds and also at the alkoxy group to yield a stable phosphorodiester. No Dimethoate-P=O was isolated in the extracts of urine, feces, milk, blood, and tissues, but this phosphorothioate oxidation apparently occurred in view of other evidence such as the in vivo cholinesterase depression. After an initial hydrolytic attack, further oxidation of the phosphorothioic acid esters would follow (*11*). Hydrolysis of Dimethoate-P=O and the carboxy derivative was observed in experiments in which these compounds were fed. Two hydrolytic metabolites of Dimethoate which were designated as unknowns *A* and *B* were not identified.

Probably, the major site of initial attack is on the amide C—N bond to produce the carboxy derivative, because: 20 to 40% of the metabolites in the urine are as the carboxy derivative; the carboxy derivative is itself further degraded as shown in independent experiments and therefore the amount observed in the urine is probably less than that actually produced; and in the case of Dimethoate-P=O treatment, 34% of the metabolites were excreted as *O,O*-dimethyl phosphoric acid in the urine, while following Dimethoate treatment, only about 20% appeared as *O,O*-dimethyl phosphoric acid. Yet total

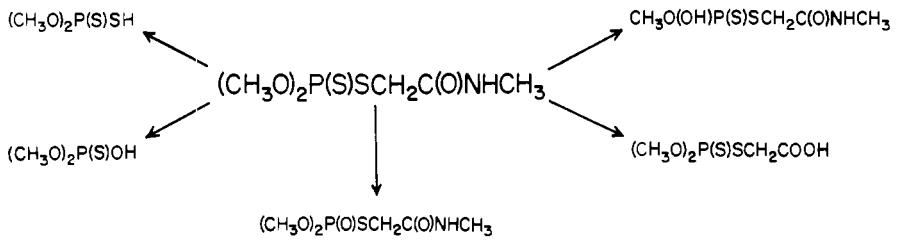

Figure 7. Proposed initial sites of metabolic attack of the Dimethoate molecule

degradation was more extensive with Dimethoate than with the Dimethoate–P=O (with male rats, 81% of the administered dose was excreted in the first 24 hours following Dimethoate treatment compared with 19% for the Dimethoate-P=O treatment). Therefore Dimethoate is more readily attacked at the amide C—N bond than is the Dimethoate-P=O, and this must be the principal route of degradation of Dimethoate.

The selective toxicity of Dimethoate may be dependent on the ability of the mammal to attack the C—N bond more vigorously than can the insect.

As the des-methyl derivative was excreted intact after administration to rats and initially excreted in the urine of rats and cows in small amounts, the hydrolytic attack at the alkoxy group must be a minor pathway in the detoxification of Dimethoate in mammals.

Of the identified metabolites, the two hitherto undescribed are the carboxy and des-methyl derivatives. Hydrolytic attack at the alkoxy group has been reported for several compounds (*7, 11*). However, this is the first example of the hydrolysis of a carbamoyl phosphate to yield an identified carboxy phosphate metabolite.

Dimethoate is of short persistence in cows after oral administration. It appears to partition readily from the blood into and out of tissues and is rapidly metabolized and excreted.

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LABORATORY TESTING OF RAT REPELLENTS

Quantitative Evaluation of Repellency of Chemical Coatings on Paperboard

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THE SEARCH FOR EFFECTIVE REPELLENTS for rodent-proofing corrugated paperboard boxes and other containers has been handicapped by lack of a simple, quantitative laboratory test for repellency. Existing methods of testing rodent repellents, both in the laboratory and in the field, have been reviewed by Welch (*12*). Laboratory testing has been done by food acceptance tests (*1*) and barrier tests (*2*). In food acceptance tests, a candidate repellent was mixed with food, and its effectiveness was measured by a comparison of the amount of treated and untreated food eaten by a rat within a given period of time. In barrier tests, hungry rats were trained to gnaw through laminated paper barriers coated with repellent to obtain food, and the effectiveness was determined by a comparison of the time required for rats to penetrate coated and plain barriers. Unfortunately, some chemicals, although highly effective in making food unacceptable, were not repellent to rats gnawing on treated barriers. Therefore, food acceptance tests were useful only as preliminaries (*13*). A possible explanation was, that rats, when gnawing paper, did not eat or chew it, but tore and shredded it with their incisors (*13*).

To express repellency of a chemical quantitatively, any test used becomes a biological assay, and must meet certain requirements (*4*). Especially germane to this problem is that the results obtained in the test parallel field effectiveness when the repellent is applied as a coating to a paperboard box, and express activity in terms of an effective repellent as a reference standard. The values obtained must be reproducible on repeat tests. Lastly, the precision of results must be analyzed by suitable statistical methods. Other desirable features would be minimal training of experimental animals and a simple method of applying coatings using small amounts of chemicals.

For these studies, the highly repellent antibiotic substance cycloheximide Actidione, Upjohn, [3-2-(3,5-dimethyl-2-oxo-cyclohexyl)-2-hydroxyethyl] glu-

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