Bovine Metabolism of Organophosphate Insecticides. Subacute Feeding Studies with *O,O*-Dimethyl 1-Carbomethoxy-1-propen-2-yl Phosphate

J. E. CASIDA, P. E. GATTERDAM, J. B. KNAAK, R. D. LANCE, and R. P. NIEDERMEIER

Departments of Entomology and Dairy Husbandry, University of Wisconsin, Madison, Wis.

O,O-Dimethyl 1-carbomethoxy-1-propen-2-yl phosphate, fed to cows, caused marked blood cholinesterase depression; however, it did not appear in the milk or tissues of the cows. Tests with the radioactive insecticide confirmed the lack of significant residues in the milk and tissues and showed that it is rapidly detoxified and excreted as dimethyl phosphoric acid. Calves showed a similar sensitivity. O,O-Dimethyl 1-carbomethoxy-1-propen-2-yl phosphate was hydrolyzed by cow, calf, and human plasma to yield dimethyl phosphoric acid.

CATTLE may come in contact with organophosphate insecticides through ingestion of residues on forage crops, as dermal or inhalation exposure from spray drifts, or when they are treated with the organophosphate antimyiatic agents. For safe utilization of organophosphates on field crops or as animal systemics, their metabolism and residues in cattle must be understood. Considerable progress in such an understanding has been made with parathion (*6, 7, 14*), malathion (*13*), demeton (*8*), Diazinon (*16*), and Bayer L 13/59 (*17*). Compared with the chlorinated hydrocarbon insecticides (*5*), the phosphates that have been investigated are generally less likely to result in fat accumulation and the appearance of high milk residues.

O,O-Dimethyl 1-carbomethoxy-1-propen-2-yl phosphate (Phosdrin insecticide) is used on several agricultural, horticultural, and fruit crops. It is also very effective against many forage insects. Phosdrin consists of two geometrical isomerides, the alpha or cis isomer being the most toxic (*3, 4*). When applied to plants, the residues are quickly dissipated by volatilization and hydrolysis, the beta or trans isomer persisting the longest (*4*) and dimethyl phosphoric acid being formed within the plant (*9*). Extensive toxicological studies have been made with rats fed on Phosdrin-containing diets (*10–12, 18*).

Experimental

Insecticide. Technical Phosdrin insecticide used in these studies was from a standard sample containing 65% alpha and 34% beta *O,O*-dimethyl 1-carbomethoxy-1-propen-2-yl phosphate and 1% *O,O*-dimethyl 1-carbomethoxy-1-propen-2-chloro-2-yl phosphate based on chromatographic and infrared determinations (*4, 9*). Radioactive Phosdrin insecticide was prepared from both neutron-irradiated phosphorus trichloride and irradiated red phosphorus, but synthesis was unsuccessful from irradiated trimethyl phosphite (irradiations for 4 weeks at 7×10^{11} neutrons per sq. cm. per second flux by AEC at Oak Ridge, Tenn.). The radioactive phosphorus trichloride was diluted with nonradioactive phosphorus trichloride, distilled, and reacted with three molar equivalents of anhydrous methanol in pentane in the presence of three molar equivalents of diethylaniline.

Distillation of the radioactive trimethyl phosphite gave material identical in infrared absorption spectrum with known trimethyl phosphite and its further reaction with α-chloromethylacetoacetate yielded radioactive Phosdrin insecticide (*4*). The radioactive Phosdrin insecticide contained 57.7% alpha isomer based on radioactivity and 54.5% based upon total phosphorus content. The beta isomer constituted only 14.9% of the total radioactivity, but was 38.6% of the total phosphorus. A 27% impurity was therefore present in the radioactive Phosdrin used in these studies. This radioactive impurity was similar to β-Phosdrin in partitioning properties and readily hydrolyzed to yield dimethyl phosphoric acid; it was less than 0.005 times as toxic as Phosdrin to houseflies and may have been a result of a radioactive dimethyl hydrogen phosphite impurity in the synthesis.

Experimental Animals and Feeding Procedures. Twelve lactating dairy cows were fed for a 12-week period with Phosdrin insecticide at levels of 0, 1, 5, and 20 p.p.m. on a daily dry matter intake basis. The cows were subdivided into four groups based on body weight in an attempt to minimize differences between the groups. The experimental cows varied in body weight from 900 to 1400 pounds, in age from 4 to 11 years, in average daily milk production from 8 to 20 pounds, and in average butterfat content of the milk from 2.9 to 5.0%. The cows were held in stanchions except for a 2-hour exercise period each day. Feed consisted of alfalfa hay and silage fed on the basis of body weight (3 pounds per 100 pounds of body weight) and grain fed on the basis of milk production (1 pound per 5 pounds of milk). Phosdrin was fed in capsules each morning after milking and the amount fed was derived from the total dry matter intake of the alfalfa hay, silage, and grain. Capsules were formulated within 4 days of use by pipetting the insecticide in acetone onto a few grams of ground grain in the capsule, closing the capsule, and holding it at −10° C. until fed. Average daily dosages for the cows are shown in Table I. All cattle were sacrificed for residue analyses at the end of the 12-week period.

Two lactating Holsteins were fed radioactive Phosdrin insecticide. These cows were catheterized and held in individual metabolism stalls to allow controlled feeding and total separation of urine and feces. One cow was fed a single encapsulated dose of 2.00 mg. per kg. and held 1 week to study the fate of this single dose. The second cow was fed 1.00 mg. per kg. per day for a week, or a dose comparable to about 40 p.p.m. based on the daily dry matter intake. The feeding procedure for these cows was identical to that described above.

Sixteen Holstein bull calves were placed on liberal milk feeding where the Phosdrin insecticide was incorporated into the milk. The calves consumed about 10 pounds of this insecticide-fortified milk each day for 3 weeks, and then the calves were held an additional 4 weeks without further exposure to Phosdrin. At the start of the study, the calves varied from 1.5 to 4 weeks old and between 80 and 100 pounds. Three calves were fed on 0, 0.2, 2.0, and 20 p.p.m. in the milk or about 0, 0.02, 0.2, and 2.0 mg. per kg. per day.

Table I. Effects of Feeding Phosdrin Insecticide to Cows

Phosdrin Level in Diet			Change from Pretreatment Determinations after Weeks Feeding, % Indicated													
			Total Body Wt.[a]			Dry Matter Intake[b]			Av. Milk Prod.[c]				Milk Fat Content as %[d]			
P.p.m.	Mg./kg./day	Breeds[e]	7	10	12	4	6	9	1	4	8	12	1	4	8	12
0	0	H,S,S	+2.3	+3.5	+3.2	+13	+1	−12	−5	−19	−19	−27	−1	+1	+1	+4
1	0.026	H,H,G	+1.0	+3.2	+4.7	−8	0	+4	−9	−23	−38	−46	−1	+10	+6	−8
5	0.13	H,H,G	−1.5	−1.4	−1.6	−5	−7	−17	−13	−24	−26	−27	+5	+8	+9	+3
20	0.52	H,H,G	−1.1	+1.7	+1.2	−1	−2	−9	−14	−19	−23	−31	+7	+3	−1	+3

[a] Based on duplicate pretreatment weights 1 week before administering Phosdrin.
[b] Based on average of determinations 5, 3, and 1 days before treatment.
[c] Compared with an average of the 3 weeks before treatment with the omission of one cow in the 1-p.p.m. group that went dry during the experimental period.
[d] Compared to duplicate pretreatment determinations, and one cow in the 1-p.p.m. group was not included because of peanut oil treatment for mastitis.
[e] H, Holstein; S, Brown Swiss; and G, Guernsey.

A single calf was fed at 4.5 p.p.m. in the milk or about 0.45 mg. per kg. per day. Three other calves were fed for 3 weeks with the milk from the cows that had received 20 p.p.m. of Phosdrin insecticide in their feed for 2 to 8 weeks.

Results and Discussion

Toxicological Studies. Blood cholinesterase determinations were made on all experimental animals. The activity was determined manometrically (*2*) in duplicate with 0.20 ml. of whole blood, or the plasma, or red blood cells from 0.20 ml. of blood. As the red blood cells were 18 times more active than the plasma in hydrolyzing acetylcholine, the cells only were assayed from the lactating cows and the whole blood was used for the cows treated with radioactive Phosdrin and for the calves. One or two pretreatment cholinesterase activity determinations were made on all animals, and inhibition figures are calculated from the pretreatment values.

The blood cholinesterase assay times and results are shown in Figure 1 for the lactating cows on subacute Phosdrin feeding, in Figure 2 for the calves on subacute feeding, and in Figure 4 for the lactating cows treated with radioactive Phosdrin. In the 12-week feeding experiment with lactating cows, the threshold level for definite corpuscle cholinesterase depression was between 1 and 5 p.p.m. based on the total dry matter intake or between 0.026 and 0.13 mg. of Phosdrin per kg. of body weight per day (Figure 1). A similar sensitivity was found with the calves where the threshold of definite depression of whole blood cholinesterase occurred between 0.02 and 0.2 mg. per kg. per day (Figure 2). Calves showed no cholinesterase depression when fed on the milk of cows receiving 20 p.p.m. of Phosdrin in their diet, indicating that less than 0.2 p.p.m. of Phosdrin was secreted into the milk of these cows.

The corpuscle cholinesterase of the cows on the 0.52 mg.-per-kg.-per-day level was maintained at below 20% of pretreatment values for over 6 weeks, and the whole blood cholinesterase activity of the calves on the 2.0 mg.-per-kg.-per-day level was below 5% of pretreatment values for 2 weeks. When the calves were no longer fed Phosdrin, they recovered their blood cholinesterase activity, so that in 4 to 6 weeks they were again near pretreatment values (Figure 2). A single dose of 2.0 mg. per kg. of Phosdrin for a cow resulted in about 40% depression and seven daily doses of 1.0 mg. per kg. resulted in about 75% depression of the whole blood cholinesterase (Figure 4). When the cattle on the 12-week subacute feeding experiment were sacrificed, the brain and spinal cord cholinesterase were assayed using 0.20 ml. of 20% homogenates. Less than 50% inhibition had occurred with either tissue at the 20-p.p.m. level in the diet, but the results were too variable for other interpretations because of inability to obtain uniform samples of the tissues for assay.

Total body weights were determined for the lactating cattle at 0, 7, 10, and 12 weeks after treatment and for the calves every 3 days during the experimental period. Total feed intake studies were made at 0, 4, 6, and 9 weeks with the cows, and daily total milk intake was determined for the calves. Butterfat percentages were determined on all milk samples used for residue analysis (*1*). Complete milk production records were kept. The Phosdrin insecticide had very little effect on the production of the cows. Comparisons can best be made with the data from the 12-week feeding experiment (Table I) and between the cows on the 0- and 1-p.p.m. levels, where little cholinesterase depression resulted, *vs.* the cows on the 5- and 20-p.p.m. levels, where there was marked corpuscle cholinesterase depression. The cows with marked cholinesterase inhibition appeared to gain less weight and drop slightly in their daily dry matter intake. This effect was not evident in the milk production or milk fat content (Table I). A

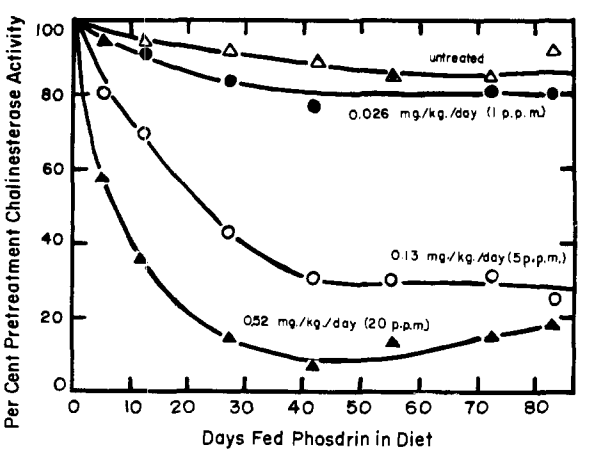

Figure 1. Blood cholinesterase (corpuscle) depression curves for cows fed varying dietary levels of Phosdrin

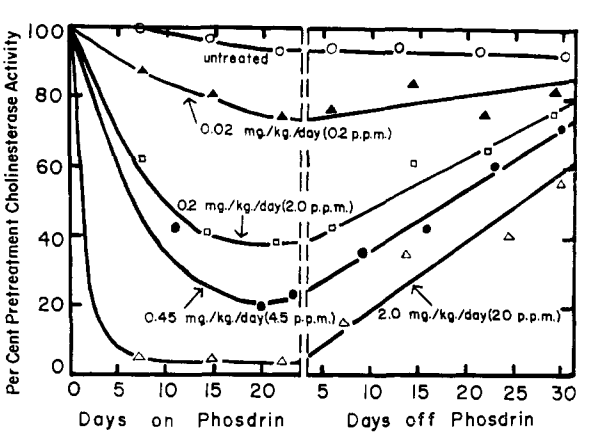

Figure 2. Blood cholinesterase depression and recovery curves for calves fed varying dietary levels of Phosdrin

Table II. Anticholinesterase Analyses Made on Milk and Tissues from Cows Fed Encapsulated Phosdrin for 12 Weeks

Sample[a]	Phosdrin in Diet, P.P.M.	Feeding before Sampling, Wk.
Milk	0	0,1, 4, 8, 12
	1	0, 1, 4, 8, 12
	5	0, 1, 4, 8, 12
	20	0, 1, 2, 4, 6, 8, 10, 12
Fat biopsy	20	0, 1, 4, 6, 8
Fat	0, 1, 5, 20	12
Liver	0, 20	12
Kidney	0, 1, 20	12
Muscle	0, 20	12
Heart	0, 1, 5, 20	12
Brain	0, 20	12

[a] All samples showed less than 0.03 p.p.m. residue of Phosdrin.

veterinarian noted no abnormalities which might be associated with organophosphate poisoning on any of the animals nor were any gross pathological effects noted when the animals were sacrificed. Calves on 20 p.p.m. of Phosdrin in their milk (about 2.0 mg. of Phosdrin per kg. per day) could be kept on the diet for only about 3 days at a time or their milk consumption and body weight were affected; however, they could be put back on the Phosdrin diet after a single day on normal milk. Excess salivation, muscular weakness, and lack of appetite were evident with the calves where the cholinesterase was depressed to below 5%. Lower levels of Phosdrin in the milk (up to 4.5 p.p.m.) had no similar effect on the calves other than blood cholinesterase depression.

Milk and Tissue Residues Associated with Subacute Feeding of Phosdrin Insecticide to Cattle. Composite A.M. and P.M. milk samples were taken for analysis at the times indicated in Table II and six tissue samples were taken for analysis on sacrifice of the animals after 12 weeks.

For extraction of Phosdrin insecticide, 100 ml. of fresh whole milk, or 20 grams of tissue, and 80 ml. of water were homogenized with 200 ml. of chloroform in a Waring Blendor. Centrifugation of the resulting emulsion yielded an upper aqueous layer which was discarded, and an intermediate protein and lower chloroform layer which were filtered through a Celite pad on a Büchner funnel. The chloroform filtrate was concentrated to a small volume, dried with anhydrous sodium sulfate, and stored at −10° C. The Celite-protein mixture from the funnel was then extracted in a Waring Blendor with 200 ml. of acetone, the acetone extract was filtered and dried with sodium sulfate, the acetone was removed, and the extract was dissolved in chloroform and added to the original chloroform extract for storage at −10° C. For cleanup of the extracts just prior to analysis, the chloroform was removed and the lipoidal material extracted twice with 10 ml. of 90% ethyl alcohol by shaking the melted lipides with the ethyl alcohol for 2 to 3 minutes, then cooling to −10° C. to solidify the liquids so that the ethyl alcohol could be poured off through a filter. The total ethyl alcohol extracts were then evaporated to dryness, 5.00 ml. of distilled water was added, and the mixture was shaken thoroughly. Up to 3.00 ml. of this aqueous extract was then added to 1.50 ml. of citrated whole human blood and the anticholinesterase activity was determined by a described method (*4*).

Table III. Phosdrin Insecticide in Milk from Radioactive Phosdrin Feeding Study

Milk Sample, Hours after Administration	P.P.M. of Phosdrin-P^{32} in Milk[a] 2.0 mg./kg. single dose	1.0 mg./kg./day for 7 days
6	0.062[b]	0.036
24	0.040[b]	0.049[b]
48 + 60	0.019[b]	0.061
72 + 84	0.022[b]	0.045
96 + 108	<0.007	0.047
120 + 132	<0.007	0.053
144 + 156	<0.007	0.047
168	<0.007	0.047

[a] Total content of Phosdrin plus hydrolysis products was less than 0.3 p.p.m. in all samples from both cows. Figures for part per million of Phosdrin are based on chloroform plus acetone extracts, three replicates, and are corrected for the 74% extraction efficiency.

[b] Only these samples had detectable anticholinesterase agents so the parts per million figures given represent less active anticholinesterase agents than Phosdrin per se and may be primarily the beta isomer.

The per cent cholinesterase inhibition resulting from this procedure with Phosdrin in water and Phosdrin recovered after addition to milk is shown in Figure 3. When the same sample of blood was used as the cholinesterase source, the results could be reproduced almost exactly from day to day in different runs. The per cent recovery of 0.005 to 5.0 p.p.m. of Phosdrin from milk and tissues varied from about 40 to 50%. No cholinesterase inhibition resulted from control milk or tissue samples carried through the described cleanup procedures. As little as 0.3 γ of Phosdrin can easily be detected in an extract representing an original milk volume of 60 ml. so that this method is sufficiently sensitive to detect as little as 0.005 p.p.m. of Phosdrin contaminating milk. A sensitivity of 0.01 p.p.m. could also be achieved for analyses of liver, kidney, heart, brain, and muscle. Analyses of milk and tissues from treated cows were always compared with samples assayed at the same time where 0.10 and 0.03 p.p.m. of Phosdrin were added to control milk and tissues (Table III).

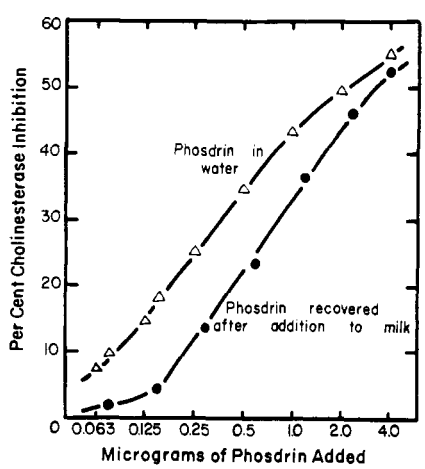

Figure 3. Recovery of Phosdrin from milk after addition of 0.005 to 5 p.p.m.

A slight modification of this procedure was used with the fat samples. After combining the chloroform and acetone extracts, the solvents were removed, and the material was dissolved in 100 ml. of hexane. This hexane was extracted twice with 100-ml. amounts of acetonitrile, and the pooled acetonitrile extracts were evaporated to dryness. By adding this cleanup step with hexane-acetonitrile partitioning to remove most of the liquid material, analyses were sensitive to 0.03 p.p.m. of Phosdrin for fat samples and the ethyl alcohol extraction was not necessary.

The efficiency of the extraction procedures for residue analyses was also determined with radioactive Phosdrin. Known amounts of radioactive Phosdrin were added to milk, liver, and fat samples and then extracted by the previously described procedures. Recovery samples on the milk showed 74% in the chloroform plus acetone extracts, but only 44% after extraction into ethyl alcohol. Recoveries with liver were 66% in the chloroform plus acetone and 37% in the ethyl alcohol, and with fat, 68% in the chloroform plus acetone, and 56% in the acetonitrile. Acetonitrile partitioning was more efficient in cleanup and recovery of Phosdrin than ethyl alcohol extraction.

The major loss in preparing residue samples for analysis appeared to be in the volatilization of Phosdrin in evaporating solvents rather than through inefficient extraction. In all milk, fat biopsy, and tissue samples from the cows on the 12-week subacute feeding experiment, there was less than 0.03 p.p.m. of Phosdrin based on anticholinesterase analyses (Table II). Phosdrin had no tendency to accumulate in any tissue, but was rapidly detoxified, and only hydrolysis products were present

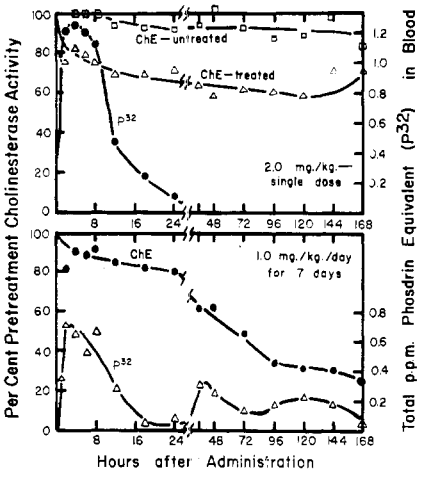

Figure 4. Blood levels of radioactive Phosdrin metabolites and blood cholinesterase activity after treatment of cows

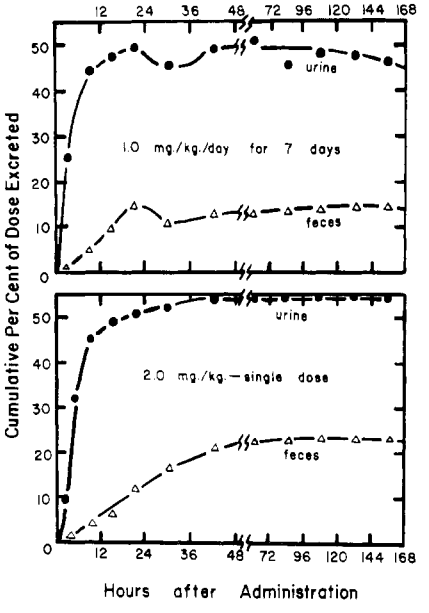

Figure 5. Rate of excretion of radioactive Phosdrin metabolites after treatment of cows

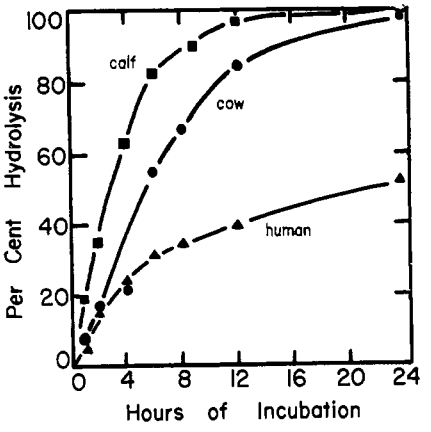

Figure 6. Rate of hydrolysis of α-Phosdrin by various mammalian plasma

in the milk and tissues after 12 weeks of feeding with dietary levels as high as 20 p.p.m., based on the total daily dry matter intake.

Metabolic Fate of Radioactive Phosdrin Insecticide Ingested by Lactating Cows. Blood samples from the cows treated with radioactive Phosdrin were assayed for total radioactivity and then 3.00-ml. samples were extracted twice with 6.00-ml. amounts of chloroform to determine the chloroform-soluble radioactivity. Blood levels of Phosdrin metabolites reached a peak between 2 and 4 hours after oral administration and the radiation from this initial dose was essentially gone from the blood within 24 hours (Figure 4). With the cow receiving a single oral dose of 2.0 mg. per kg. of Phosdrin, the radioactivity in all the blood samples was 0 to 8% extractable into chloroform and, with the repeated oral dose of 1.0 mg. per kg. per day, there was never more than 30% of the radioactivity extracting into chloroform as Phosdrin. Repeated daily dosages of 1.0 mg. per kg. resulted in an equilibrium level of about 0.15 to 0.30 p.p.m. of Phosdrin equivalents circulating in the blood (Figure 4), and this was largely hydrolysis products.

Milk samples were assayed for total radioactivity and extracted as for anticholinesterase residue analysis to determine the Phosdrin content by both radioactivity and cholinesterase inhibition. The cow fed 1.0 mg. per kg. of radioactive Phosdrin daily for 7 days never excreted more than 0.062 p.p.m. of Phosdrin in the milk, and this appeared to be mostly the beta isomer because of its low anticholinesterase activity (Table III). Fat and tissue samples were extracted as indicated for anticholinesterase determinations with the omission of the ethyl alcohol or acetonitrile cleanup extractions. The radioactivity in the fat, heart, muscle, brain, and spinal cord at sacrifice of the cow were too low for fractionation. Fractionation was possible with the fat biopsy, liver, and kidney samples. On sacrifice at the end of the 7-day period, Phosdrin metabolites could be detected in many tissues (Table IV), but this radioactivity was mostly hydrolysis products with only 0.30 p.p.m. of Phosdrin in the liver, 0.04 p.p.m. in the kidney, and lower levels in other tissues. Fractionation of fat biopsy samples taken from this cow at 1, 3, and 7 days after treatment yielded less than 0.1 p.p.m. of Phosdrin based on total radioactivity and less than 0.03 p.p.m. based on anticholinesterase activity, indicating that some of the persisting chloroform-soluble radiation may have been the beta isomer of Phosdrin.

Excretion accounted for 77% of the administered dose for the 2.0-mg.-per-kg. level within 3 days (Figure 5). Over half this amount was excreted in the urine within the first 12 hours after treatment. A similar initial excretion curve was found for a cow receiving 1.0 mg. per kg.; this resulted in an equilibrium where 45 to 50% of the daily dose was excreted in the urine and 12 to 15% in the feces (Figure 5). Feces samples from 0.5 and 6 days for the cow receiving 1.0 mg. per kg. per day and from 0.75 day for the cow receiving the single dose of 2.0 mg. per kg. were extracted in the same way as with the tissues, and the radioactivity in the combined chloroform and acetone extracts were determined. All the radioactivity in the feces was present as hydrolysis products of Phosdrin. All urine samples were partitioned with chloroform and only Phosdrin hydrolysis products were present. A urine sample taken 4 hours after treatment of the cow with 2.0 mg. per kg. of Phosdrin and another taken after the second cow had received six daily dosages of 1.0 mg. per kg. of Phosdrin were chromatographed on an anion exchange resin (*15*) with carrier phosphoric, methyl phosphoric, and dimethyl phosphoric acids; the only Phosdrin hydrolysis product present was dimethyl phosphoric acid.

Calf plasma was found to be more active than cow and human plasma in catalyzing the hydrolysis of α-Phosdrin (chromatographically pure). Fresh oxalated whole blood for these studies was centrifuged at 5° C., and 0.30-ml. portions of the plasma were incubated with 600 γ of Phosdrin, and, after incubation times up to 24 hours, 2.70 ml. of water were added and the plasma was partitioned with 3.00 ml. of chloroform. The radioactivity in the water phase was considered to be hydrolysis products (Figure 6). The hydrolysis products from 24 hours incubation of Phosdrin with human plasma were further studied by precipitating the plasma proteins with acetone and heat, and the radioactivity was found to chromatograph

Table IV. Phosdrin Plus Metabolites in Tissues of Holstein Cow after Daily Administration of 1.0 Mg. per Kg. of Phosdrin for 7 Days

P.P.M.	Tissues
1.17	Liver
0.40–0.43	Kidney, udder gland
0.32–0.34	Reticulum, supermammary lymph node, mesenteric lymph node
0.21–0.23	Lung, spleen, ovaries, rumen contents, small intestine contents, subiliac lymph node, submaxillary lymph node
0.13–0.19	Bladder wall, gall bladder fluid, tongue, heart, rib, submaxillary gland, parotid gland
0.01–0.06	Ventral rumen pouch, rumen fat
Less than 0.01	Brain, spinal cord, hide, bone marrow, uterine horns, loin muscle, body fat, renal fat, subcutaneous fat, small intestine fat

entirely as dimethyl phosphoric acid on an anion exchange resin (*15*).

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ANIMAL METABOLISM OF INSECTICIDES

Bovine Metabolism of Organophosphorus Insecticides. Metabolic Fate of *O,O*-Dimethyl *O*-(2,4,5-trichlorophenyl) Phosphorothioate in Rats and a Cow

F. W. PLAPP and J. E. CASIDA

Department of Entomology, University of Wisconsin, Madison, Wis.

O,O-dimethyl O-(2,4,5-trichlorophenyl) phosphorothioate is susceptible to hydrolysis at either the methyl-phosphate or phenyl-phosphate bond. Both sites of hydrolysis have been demonstrated with alkali and bovine rumen juice and in rats, houseflies, and a cow. The oxygen analog of this insecticide undergoes similar hydrolic cleavage. The excretory metabolites of O,O-dimethyl O-(2,4,5-trichlorophenyl) phosphorothioate and three derivatives were established for rats. A slower detoxification and excretion of the insecticide metabolites occurred with the cow compared to rats, but the same metabolic pathway was demonstrated for each.

SEVERAL ORGANOPHOSPHATE INSECTICIDES are effective chemotherapeutic agents for cattle grubs following oral, subcutaneous, or epidermal administration (*17, 18*). Robbins, Hopkins, and Eddy have published on the distribution and metabolic fate of two of these, Diazinon and Dipterex, in lactating cows (*24, 25*) and have presented a preliminary report on the fate of a third, *O,O*-dimethyl *O*-(2,4,5-trichlorophenyl) phos phorothioate (Trolene), in a calf (*23*). Trolene administered orally at 100 mg. per kg. not only kills larvae present in the backs of cattle, but also completely prevents the encystment of new larvae (*17, 26*). At the recommended dose, the insecticide rarely produces symptoms and these are of a mild transient nature (*22*).

In this study, Trolene was administered orally to rats and a lactating cow at 100 mg. per kg., and the distribution and metabolic pathway were determined.

Methods and Results

Chemical Studies

Radioactive Syntheses. Phosphorus-32 trichloride was obtained through neutron irradiation of phosphorus trichloride or by chlorination (*10*) of red phosphorus-32 from AEC service irradiation at Oak Ridge, Tenn. (both irradiations at 7×10^{11} neutrons per sq. cm. per second flux for 4 weeks). The phosphorus trichloride was converted to thiophosphoryl trichloride (*14*) and then to dimethyl thiophosphoryl chloride (*9*). *O,O*-Dimethyl *O*-(2,4,5-trichlorophenyl) phosphorothioate was formed by reaction in acetone of the dimethyl thiophosphoryl chloride with equimolar 2,4,5-trichlorophenol (purified sample from Hooker Electrochemical Co.) in the presence of equimolar sodium carbonate (*9*). The product was dissolved in hexane, washed three times with 10% sodium carbonate, and further purified by partition chromatography on a Celite–*iso*-octane–methanol column (*2*). The purified organophosphate represented a 20% yield from phosphorus trichloride and was identical in infrared spectrum to known *O,O*-dimethyl *O*-(2,4,5-trichlorophenyl) phosphorothioate.

O,O-Dimethyl *O*-(2,4,5-trichlorophenyl) phosphate (the oxygen analog of Trolene) was prepared in 70% yield from phosphorus-32 trichloride by the method of Fukuto and Metcalf (*11*) and purified as above yielding an infrared spectrum identical with the known compound. The compound was also prepared (nonlabeled) by nitric acid oxidation of the corresponding phosphorothioate after the method of Johnson (*13*).

O-Methyl *O*-hydrogen *O*-(2,4,5-trichlorophenyl) phosphorothioate was prepared from radioactive Trolene by alkaline hydrolysis with 2 molar equivalents of alcoholic potassium hydroxide and purified by ion exchange chromatography. The radioactive sample was